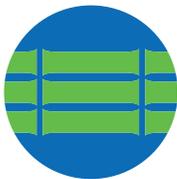


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World Bamboo

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Letter from the WBO President Bamboo - a new global opportunity

Twenty years ago, in Minamata (Japan), three hundred dedicated people gathered together for the third World Bamboo Congress. It was to be a very unique one. At the wounded spot, where many individuals had suffered from foolish industrial development, they decided to build a bamboo bridge. A strong symbol for the future, it also showed the will to promote the use of a natural material: bamboo. They were dreaming, they were full of hope, they were conscious. Without any arrogance or strategy, they started a movement with a great sense of love and faith in bamboo. It was a timeless moment and a fundamental boost.

After the Congress, back to their everyday lives, full of energy, they became the breeze that sets things into motion. Researchers, architects, designers, economists, planters, businessmen or simply bamboo lovers, added to their daily work their « commitment to bamboo », to build a new global opportunity. Year after year, they planted, they invested, they developed the knowledge to transform the breeze into wind, a wind to force a change. With time, that force has become a strong movement.

Twenty years later, the intention of the beginning has become a powerful will. Today, bamboo is a booming market. From 5.5 billion euros in 2010, it will reach 15 billion euros by 2017. A testimony for all creative people to acknowledge the potential change bamboo can bring.

That will - to innovate- drives this 9th World Bamboo Congress in Antwerp (Belgium), taking place 10-15 April 2012 under the lead of the World Bamboo Organization (WBO) and the efforts of the IKEBANA team.*

Like an open shell this congress will be in two parts : « Bamboo Biosciences, Bioengineering and Agroforestry Potentials » and « Architecture, Design and Development». A forum to stimulate the dialogue, a bridge to cross the gap between supply and demand, need and fulfillment, producer and consumer, problem and solution. Anyone working with bamboo, from the materials engineer to the land-use developer, the farm manager to the forest researcher in the field, the student to the bamboo enthusiast - many topics relating to bamboo will be revealed and discussed.

Today we are grateful to the people who have shared the efforts to put everything on line, especially the IKEBANA team with Ms Frances Schutte of the University of Antwerp as Project Manager and WBC Secretariat, Dr. Geert Potters of the University of Antwerp as Promoter, Dr. Ing. Johan Gielis of Oprins Plant NV as Program Chair and Co-promoter.

We are thankful to those behind the scenes who support all year round the energy of WBO, Ms Susanne Lucas, Organizing Chair Person of this Congress. We are also deeply grateful to Jan Oprins, the president of Oprins Plant NV, who is materially supporting the event and who accepted with a great sense of sharing the offer of De Kolonie to welcome the architectural manifestation. I also want

to thank Dr. Jean-Luc Koujoumji, from « bambou, science et innovation », who has been a scientific adviser and a great help for the second part of the congress. A lot of work has been done before and after the Toulouse collapse, and the best choices led us to concentrate our efforts together with Antwerp team.

And to all the friends of bamboo who are coming to enjoy the forum, the exchange and the feast, I have the pleasure to announce his excellency ambassador to bamboo, Mister Flyboo, the bamboo airplane sharing his message : “let the forest breathe, use bamboo”.

With my best wish of great success to the 9th World Bamboo Congress, and the hope it will become a milestone for the continuation of the global bamboo opportunity.



Michel Abadie
President, WBO

In addition, we are grateful to...

> Our Keynote Speakers, including American bamboo taxonomist Dr. Lynn Clark, bamboo preservation specialist Professor Walter Liese, Gunther Pauli of Emissions Zero (The Blue Economy), bamboo builder David Sands of BambooLiving, Pablo Van der Lugt , Aart Willem Van Vuure, Colombian architect Carolina Salazar, Professor Goshrow Ghavami of Rio de Janeiro, and many more.

> The Bamboo Pioneers Award recipients, renowned experts who have dedicated their life work to bamboo: Jules Janssen (the Netherlands), Oscar Hidalgo-Lopez (Colombia), Masatoshi Watanabe (Japan), Hsiung Wenyu (China), and Shuen Chao Wu (Taiwan).

> Bamboo builders and artists by BeBamboo (Nic Geereart and team), Cru!, Bamboost, Georges Cuvillier, Luk Vermeerbergen, Frans Demedts and Carla Feijen, as well as the display of Michel Abadie's bamboo airplane, FLYBOO.

> Ornamental Bamboo Session speakers Jos Van der Palen of Kimmei Nursery (Holland), Ned Jaquith of Bamboo Garden (USA), Ray Townsend (Kew), Helder Carvalho (Portugal), and the exhibitors and vendors. Special thanks go to our guest musicians, including John Kaizan Neptune and his band, TAKE DAKE, from Japan!

* IKEBANA = International Knowledge and Expertise Bamboo Centre (<http://www.ikebana-bamboo.eu/>)

The World Bamboo Congress in Antwerp: a dream come true

Johan Gielis
Department of Bioscience Engineering
University of Antwerp

Welcome to the IXth World Bamboo Congress, in Antwerp.

On behalf of IKEBANA and the University of Antwerp, on behalf of Geert Potters, Frances Schutte and myself, I wish all of you a warm welcome to the IXth World Bamboo Congress in Belgium, for the first part at the University of Antwerp, and for the second part in Merksplas.

The history

Almost exactly three years ago we had a meeting at Oprins Plant with Jan Oprins and Corneel Dewindt and they asked me to explore the possibility of EFRO-funding. EFRO stands for European Fund for Regional Development, funding. Deadline was exactly six days later. My answer was yes, on one condition: Geert Potters' time schedule. Fortunately, this was no problem and after one week of hard but enjoyable work, the project was submitted and granted. The project is called IKEBANA, International Knowledge Center for Bamboo, Northern Campinas-Antwerp. Antwerp because of the University, and Northern Campinas because the main co-financing came from Oprins Plant, engaged already a long time in bamboo research.

The goal of IKEBANA is to promote the use of bamboo in agriculture, in a broad sense. Frances Schutte was appointed as program manager. Our two-year project involved two major meetings on biomass, inviting competitors from *Miscanthus* and poplar/willow/SRC sides to create biomass platform, focusing on real data for farmers. Energy from biomass is a hot topic, but in great need of real data, for all energy crops. The step from research institute to farmer's adoption is a difficult one.

The final or ultimate goal of the EFRO-IKEBANA project was to organize a World Bamboo Congress, focusing on research. The organization of a World Bamboo Congress required the approval of the World Bamboo Organization WBO, and to this goal a bid book was prepared. Being at a University and having funding of EFRO, we had all pieces together to achieve our goal. So in fact, the basic planning was already done two years ago. And we got permission to organize WBC, at least the part on Bioscience and Bioengineering. Architecture and Design was going to be organized in Toulouse in September 2012.

Belgium, a hothouse for bamboo research

In some sense, bamboo research is not cutting edge research. The genome is more complicated than *Arabidopsis*, controlled hybridization and genetic transformation is not yet possible, and so on, so there is not much glory to be gained with bamboo research. On the other hand, bamboo is a REAL plant, and a wild, natural resource, with many possible uses. Both from a biological point of view (Bamboo as centerpiece of the grass family, a giant grass with gargantuan inflorescences..) and from a technological point of view, bamboo is interesting and has in the past led to some nice or even great advances. Square bamboo even entered into mathematics as the archetype of Lamé curves, or anisotropic metrics.

There is much to be studied. And we find research in almost every university and every university college today in Flanders and Belgium. Much of it started more than 15 years ago when we applied for research funding with IWT and Europe. One year later we could organize a workshop in Meise to

explore the potential of using bamboo in Western Europe. Later this resulted in the Bamboo for Europe project, coordinated by Joris Devos of Cobelgal, combining 9 partners from Belgium, France, Germany, Spain and Portugal. This successful project then was carried over in the Bamboo Thematic Network. In one decade bamboo research was carried out at all universities and the level of research has steadily been increasing. This can be witnessed in the program today with the talks of Laura Van Hoyweghen (University of Gent), Suzanne Van den Akker, Davina Van Goethem and Litsa Bogaerts (research at University of Antwerp) and the crew from Aart Willem Van Vuure at the University of Louvain.

The congress Part A: Bamboo Bioscience, Bioengineering and Agroforestry Potentials

Belgium has become a real hothouse of bamboo research, and it seemed very logical to us to have the next bamboo congress at the University, symbolizing the research focus. This could increase the level of research in the future with a platform that is dedicated to the communication of scientific results, and at the same time, show to other fields that bamboo, either as a plant or as a material is really worth trying. Previous WBC's have been held at private estates (Linda Garland, Bali & Prafrance for example) or in hotels or resorts. It is the first time that it is really organised at a university, with the explicit aim and hope that future WBC's will follow the same path.

The National Organizing Committee was chaired by Geert Potters, and the scientific committee by Johan Gielis. Frances Schutte was appointed for organizational & financial work, with a lot of help from Susanne Lucas on the promotional side. We built a strong scientific committee:

Walter Liese, Germany
Lynn Clark, USA
Azmy Mohammed, Malaysia
Rajani Nadgauda, India
Amita Pal, India
Tesfaye Hunde, Ethiopia
David Midmore, UK/Australia
Shozo Shibata, Japan
Chris Stapleton, UK
Jinhe Fu, China
Ximena Londono, Colombia
Pablo Vanderlugt, The Netherlands
Geert Potters, Belgium
Johan Gielis, Belgium

All scientific papers have been reviewed by two scientists. More than 2/3 of the reviewers is from within the field of bamboo, and about 20% had no prior research in this field. 45% of the reviewers were Belgian, 55% from outside Belgium. Here we could use our own extensive academic network for specific papers or topics including special statistics or molecular biology. The list:

Amita Pal, Rajani Nadgauda, Azmy Mohamed, Walter Liese, Pablo Van der Lugt, Tesfaye Hunde, Jinhe Fu, Geert Potters, Shozo Shibata, Chris Stapleton, David Midmore, Roeland Samson, Laura Van Hoyweghen, Aart Willem Van Vuure, Joris Van Acker, Raf Dewil, Mike Temmerman, Marcel Jansen, Roland Valcke, Victor Brias, Evelyn Rottke, Jules Janssen, Andry Widyowijatnoko, Frances Schutte, Sarah Lebeer, Roland Caubergs, Davina Van Goethem, Richard Murphy, Khosrow Ghavami, Elizabeth Magel, L.B. Singha, Anil Sood,

Birger Hauchecorne, Jean-Luc Kouyoumji, Othman Sulaiman, Margaret Stern, Andrea Melnychenko and myself.

It has been an open review process, from which many papers have benefitted. The final level of papers is good in general, with some really excellent papers of young researchers, although initially we had hoped to receive more papers on molecular biology and on taxonomy. Special thanks to Geert Potters for his patient work on the Proceedings, planning to hold a session on how to write and format papers decently, according to the guidelines. We will publish a selection of the best papers in a book, later this year.

We look forward to an inspiring congress, where all presenters have 25 minutes or more. This will allow good presentations, detailing on materials & methods, results and discussion, and time for questions and answers. Our goal was not merely to bring people together to report, but to have in-depth interactions between scientists.

In the current economic crisis that prevented many to come to Belgium, live streaming will be provided. To benefit from modern technology live streaming of most presentations will be available. This will allow researchers worldwide to experience the WBC presentations anywhere in the world. Our special thanks go to the EFRD, the European Fund for Regional Development that made this World Congress possible without registration fees, thereby facilitating the participation of researchers from all over the world.

The EFRD project was co-sponsored by the Flemish government (Agentschap Ondernemen / Vlaanderen in Actie VIA), the University of Antwerp, the province of Antwerp, Oprins Plant and Genicap. We are also very proud that our congress has been backed by various international organisations like International Network for Bamboo and Rattan (with the organization of a special INBAR session), Food and Agricultural Organization FAO, and the International Union of Forestry Research Organizations IUFRO. Unfortunately a planned FAO session had to be cancelled because of personal reasons of the planned speakers.

We should also mention the presence of many bamboo pioneers, made possible by Susanne Lucas and EcoPlanet, and two travel grants of 1000\$ for young researchers by the American Bamboo Society. These are awarded to Francisca Ely and Andrea Melnychenko. Also Kamesh Salam (South Asian Bamboo Foundation) helped with travel costs for Indian scientists.

Part B: Bamboo Design Innovations, Architecture and Modern Technologies

On September 19 we learned that Toulouse could not host the Architecture and Design due to financial difficulties. We then decided that this was an opportunity to have the whole WBC in Belgium. We also decided not to do it at the university, but at a dedicated location. This location was De Kolonie in Merksplas. Not a university or a hotel, but a unique setting in a magnificent landscape, worthy of hosting this Part B.

The organization of this event, with main focus on Architecture, Development and Design, required a complete different organization, with many groups involved.

Jan Oprins & De Kolonie

Litsa Bogaerts, Corneel Dewindt, Wim Reniers & NemeC vzw

Nic Geeraert, Filip Van Lierde, Julie De Rouck & Philippe Lecompte, Bebamboo

Frances Schutte, Davina Van Goethem, Geert Potters, Johan Gielis (IKEBANA vzw)

Susanne Lucas (World Bamboo Organization)

Sven Mouton, CRU! Architects

Simcha Nyssen (Puntoverde Bamboe Toepassingen),

Artists Georges Cuvillier (BAMBOOST), Francesco Fransera, Carla Feijen, and Luk Vermeerbergen and musicians like John Kaizan Neptune (TAKE DAKE)

Luc Boeraeve (Belgian Bamboo Society).

50 years ago, 50 years ahead

The bamboo pioneers here today started their career in the 1950's. Prof. Liese will speak on his sixty years involvement in bamboo research. On the other hand, in our congress we will have a workshop Bamboo: a 50 year perspective. In the 21st century, the biotech century a lot will be possible. Goals to achieve are, amongst others: breaking the code of flowering, and methods for genetic improvement of bamboo, including genetic transformation methods. It is my great pleasure that Prof. Van Montagu accepted to do the opening speech.

Fifty years ahead is a long time, but so is 50 years back in time. I was born in 1962, exactly 50 years ago. My bamboo career is half my life: 25 years ago I started to work with Oprins Plant. This congress is for me a dream come true. A special thanks to Walter Liese, Geert Potters, Frances Schutte, Jan Oprins and Susanne Lucas, for helping to make this congress my dream come true!



Our History:

2009 World Bamboo Congress
Thailand / Host: WBO and the Thai Royal Forest Department

2004 World Bamboo Congress
India / Host: Government of India

1998 International Bamboo Congress & Workshop
Costa Rica / Host: Fundacion Bambu

1995 International Bamboo Congress & Workshop
Indonesia / Host: Environmental Bamboo Foundation

1992 International Bamboo Congress
Japan / Host: National Government of Japan

1991 International Bamboo Workshop
Thailand / Host: INBAR

1988 International Bamboo Congress
France / Host: European Bamboo Society

1984 International Bamboo Congress
Puerto Rico / Host: American Bamboo Society

The WBO is a non-profit trade association (501-c6) formed to facilitate the exchange of information from around the world on the environmental, socioeconomic, biological, and cultural aspects of bamboo. By bringing together local and regional bamboo people and creating mechanisms for global communications, the WBO's goal is to facilitate the development of new partnerships and alliances to advance the causes of bamboo and furthering the efforts of bamboo practitioners worldwide.

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VOLUME 1

BAMBOO BIOSCIENCE, BIOENGINEERING AND AGROFORESTRY POTENTIALS

Keynote Speakers – Bamboo Pioneers – UEDA Lecture

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KEYNOTE LECTURE

An Updated Tribal and Subtribal Classification of the Bamboos (Poaceae: Bambusoideae)

Bamboo Phylogeny Group¹

Presented by Lynn Clark

Abstract

The Bambusoideae (bamboos), comprising 1,439 described species in 115 genera, is one of 12 subfamilies of Poaceae (grass family), and it is the only major lineage of the family to diversify in forests. The bamboos are ecologically and economically important, but they remain understudied relative to other grasses, especially with respect to morphology, and an updated, phylogeny-based classification for the Bambusoideae has not previously been available. In this paper, a compilation of described bamboo diversity by tribe and subtribe is presented, phylogenetic studies of bamboos are reviewed, and the basis for the revised classification presented here is discussed, with putative synapomorphies indicated in the text and descriptions. The taxonomic treatment includes descriptions of the subfamily, the three tribes, and all accepted subtribes, and included genera are listed as appropriate.

Keywords

Arundinarieae, bamboo classification, Bambuseae, Bambusoideae classification, Olyreae.

List of Abbreviations

AFLPs – amplified fragment length polymorphisms; ESTs – expressed sequence tags; GBSSI – granule-bound starch synthase subunit I; g – genera; in prep. – in preparation; ITS – internal transcribed spacer; pers. comm. – personal communication; RAPDs – random amplification of polymorphic DNA; RFLPs – random fragment length polymorphisms; spp – species; SSRs – simple sequence repeats; unpubl. data – unpublished data; # – number.

Introduction

The Bambusoideae (bamboos) is one of 12 currently recognized subfamilies of Poaceae (grasses), receiving strong bootstrap support in comprehensive molecular analyses of the family [Grass Phylogeny Working Group (GPWG) 2001; Duvall et al. 2007; Bouchenak-Khelladi et al. 2008; GPWG II 2012; Wu and Ge 2012]. A putative structural synapomorphy for the subfamily is the presence of strongly asymmetrically invaginated arm cells in the foliage leaf chlorophyll (Zhang and Clark 2000). The bamboos are notably the only major lineage of grasses to diversify in forests (Zhang and Clark 2000; Judziewicz and Clark 2007; Sungkaew et al. 2009) and the complex morphology and unusual flowering behavior of most bamboos are likely the result of adaptations to this habitat or the retention of ancestral states, as is the case with their broad, pseudopetiolate leaves with fusoid cells in the mesophyll (Clark 1997; Judziewicz et al. 1999).

Bambusoideae are worldwide in distribution (see Maps, Bamboo Biodiversity), occurring between 46° N and 47° S latitude, with an altitudinal range from sea level to 4,300 m (Judziewicz et al. 1999). Estimates of total diversity vary from source to source, but our compilation reveals 1,439 described species in 115 genera (Table 1). Three tribes reflecting the three main lineages of Bambusoideae are currently recognized (Sungkaew et al. 2009): Arundinarieae (temperate woody bamboos, 533 species), Bambuseae (tropical woody bamboos, 784 species) and Olyreae (herbaceous bamboos, 122 species). New species and new genera in all of these tribes continue to be discovered and described and phylogenetic analyses in some cases support generic recircumscriptions (e.g., Fisher et al. 2009).

Table 1. Diversity of Bambusoideae by tribe and subtribe and by region for Bambuseae.

Taxon	Number of genera	Number of species
Arundinarieae	28	533
Bambuseae	66	784
Neotropical	19	377
Arthrostylidiinae	13	172
Chusqueinae	1	160
Guaduinae	5	45
Paleotropical	47	407
Bambusinae	28	264
Hickeliinae	8	33
Melocanninae	10	88
Racemobambosinae	1	22
Olyreae	21	122
Buergersiochloinae	1	1
Parianinae	2	36
Olyrinae	18	85
Total for subfamily	115	1,439

Woody bamboos possess culm leaves (leaves modified for the protection and support of the tender young shoots), complex vegetative branching, an outer ligule (contraligule) on the foliage leaves, usually gregarious monocarpy (with flowering cycles ranging from a few years to 120 years), and bisexual flowers (Judziewicz et al. 1999; GPWG 2001; Judziewicz and Clark 2007). Herbaceous bamboos usually lack differentiated culm leaves and outer ligules and have restricted vegetative branching, usually nearly continuous or seasonal flowering, and unisexual spikelets (Judziewicz et al. 1999; Judziewicz and Clark 2007). All Olyreae, except for the New Guinea endemic *Buergersiochloa*, also have crenate (olyroid) silica bodies (Soderstrom and Ellis 1987; Zhang and Clark 2000; Clark et al. 2007).

Woody bamboos, which colonize forest gaps and edges, are an important element in tropical and temperate forests, especially in mountainous regions (Soderstrom and Calderón 1979; Li and Xue 1997; Judziewicz et al. 1999; Zhou et al. 2011). These bamboos may be canopy or understory dominants, and they may form nearly mono-dominant stands that can cover extensive areas (Numata 1979; Wong 1991, 1995, 2004; Judziewicz et al. 1999; Franklin and Bowman 2004). Woody bamboos also are important and sometimes dominant in high altitude grasslands in both tropical and subtemperate montane systems (Soderstrom and Calderón 1979; Soderstrom et al. 1988; Judziewicz et al. 1999; Judziewicz and Clark 2007). As a function of their colonizing ability or status as understory dominants, woody bamboos appear to play a key role in the ebb and flow of forest dynamics, while over the long term, the gregarious, monocarpic flowering of woody bamboos permits the forest to reoccupy previously disturbed sites (Wong 1991; Widmer 1997; Judziewicz et al. 1999; Martins et al. 2004). Flowering and death of woody bamboos may also affect nutrient uptake, soil fertility and water relations in forests (e.g., Takahashi et al. 2007; Ishii et al. 2008; Marchesini et al. 2009) and these bamboos affect the regeneration of other forest species in various ways (e.g., Oliveira-Filho et al. 1994; Caccia et al. 2009; Larpkern et al. 2010; Montti et al. 2011). Bamboos can play an important role in carbon sequestration, especially as the extent of bamboo forests is increasing in some parts of the world (Zhou et al. 2011 and references cited therein).

Although much remains to be learned about the ecology of woody bamboos, the ecology of herbaceous bamboos is even more poorly studied. Olyreae usually occupy the forest understory and may even become dominant (Poulsen and Balslev 1991; Judziewicz et al. 1999); some occur in savannas or wet cliff faces associated with waterfalls (Zuloaga and Judziewicz 1991; Judziewicz et al. 1999; Judziewicz and Sepsenwol 2007) and *Ekmanochloa* is a serpentine endemic (Zuloaga et al. 1993). A few species, especially of *Lithachne* or *Olyra*, may become weedy (Judziewicz et al. 1999; Judziewicz and Clark 2007). The association of gall midges and other insects with the showy inflorescences of *Pariana* species suggests the possibility of insect pollination (Soderstrom and Calderón 1971), but this remains to be confirmed. The hardened female floret characteristic of Olyreae may be an adaptation for dispersal by granivorous birds; in some taxa, a swollen, oil-containing internode attached to the female floret presumably functions as an elaiosome for ant dispersal (Davidse 1987; Judziewicz et al. 1999). Olyreae exhibit their greatest species diversity from 7–10° N and 12–18° S, with minimal diversity near the equator and their greatest endemism in the Atlantic forests of Brazil (Soderstrom et al. 1988). Many species of Olyreae are endangered due to the continuing loss of these forests (Oliveira and Clark 2009).

The often extensive biomass of woody bamboos, coupled with their unique life form, not surprisingly provide an important resource for many other organisms. The giant panda is by far the best known animal that depends on bamboo (Schaller 1994; Dierenfeld 1997), but there are also bamboo lemurs (Mutschler and Tan 2003; Ravaloharimanitra et al. 2011), bamboo rats (Medway 1964, 1969; Emmons

1997), bamboo birds (Judziewicz et al. 1999; Lentino and Restall 2003; Areta and Bodrati 2008), and even a bamboo bat (Ridley 1908; Medway 1969; Ades 1999). Many invertebrates take advantage of the sheaths and hollow stems for shelter (Brailovsky 1988; Kovac 1993; Conover 1994; Louton et al. 1996; Hidalgo et al. 2012), thus attracting larger animals to this food source. Fungi also use bamboo as a host (Judziewicz et al. 1999; Higgins et al. 2010). And the periodic gregarious flowering of woody bamboos can produce massive amounts of fruit that are particularly attractive to birds and small mammals (Judziewicz et al. 1999; Areta et al. 2009), especially rodents (Jaksic and Lima 2003; Singleton et al. 2010).

The increasing economic importance of woody bamboos in housing construction (especially in earthquake-prone regions) but also as a material for flooring, furniture and other household items, food (new shoots), fencing, scaffolding, and as ornamentals is well known and growing (McClure 1966; Bahadur 1979; Wong 1989; Judziewicz et al. 1999; Yang et al. 2004). Woody bamboos are also increasingly viewed as a sustainable resource for developing countries, where the bulk of bamboo diversity is found (Bystriakova et al. 2003, 2004). Technology for *in vitro* propagation of woody bamboos has been commercially developed, allowing mass production for use in reforestation or other projects (Agro Vitro 2012; Oprins Plant 2012). And despite their reputation for invasiveness, many bamboos are of conservation concern due to destruction of their forest habitats (Bystriakova et al. 2003, 2004). The massive gregarious flowering and fruiting of certain bamboos can have negative effects on local human populations through rodent outbreaks and subsequent crop losses and increased incidence of rodent-borne diseases (Jaksic and Lima 2003; Sage et al. 2007; Singleton et al. 2010).

The Bamboo Phylogeny Group was formed in 2005 to address the need for a robust, global phylogeny of the Bambusoideae and an updated tribal, subtribal, and generic classification based on the phylogenetic results (BPG 2006). A comprehensive analysis across the subfamily with extensive sampling has yet to be achieved, but much has been learned through intermediate phylogenetic analyses of various lineages based on both plastid and nuclear data (Table 2). We here present a compilation and review of the phylogenetic findings to date and a revised and updated tribal and subtribal classification of the Bambusoideae based on a synthesis of these results. A separate manuscript by the BPG is in preparation, in which a rigorously tested phylogenetic analysis of plastid sequences is presented for representatives of all tribes and subtribes of Bambusoideae.

Review of Bamboo Phylogeny and Classification

Table 2. Prior published molecular/morphological phylogenetic analyses focused on Bambusoideae. These are not included and the number of taxa does not include outgroups.

Authors	Year	# Taxa (Focus)	Data Used	Findings
Friar and Kochert	1994	1 g, 20 spp (<i>Phyllostachys</i>)	nuclear RFLPs	Support for division of <i>Phyllostachys</i> into two major groups; utility of RFLPs for species-level problems.
Takahashi et al.	1994	4 g, 21 spp (<i>Sasa</i> and allies)	allozymes	Support for intersectional hybrids between species of <i>Sasa</i> and intergeneric hybrids between species of <i>Sasa</i> and <i>Sasamorpha</i> or <i>Sasa</i> and <i>Pleioblastus</i> .
Watanabe et al.	1994	16 g, 19 spp (Asiatic woody)	plastid RFLPs	Support for monophyly of each of the temperate and paleotropical

		bamboos)		woody bamboos; aspects of morphological evolution.
Gielis et al.	1997	1 g, 42 spp (<i>Phyllostachys</i>)	RAPDs	Support for <i>Phyllostachys</i> section <i>Heteroclada</i> ; utility and reliability of RAPDs in identifying genotypes within <i>Phyllostachys</i> .
Kelchner and Clark	1997	13 g, 35 spp (Bambusoideae)	<i>rpl16</i> intron, indels	Support for monophyly of Olyreae, temperate Bambuseae, tropical Bambuseae, and Chusqueinae.
Kobayashi	1997	31 g, 32 spp (Bambusoideae)	plastid RFLPs	Support for monophyly of the temperate bamboos.
Guala et al.	2000	19 g, 21 spp (woody bamboos)	<i>ndhF</i> (3' end)	Polyphyly of <i>Apoclada</i> confirmed, with <i>A. simplex</i> (type species) in the Guaduinae and the other two species forming a clade in the Arthrostylidiinae.
Hodkinson et al.	2000	4 g, 15 spp/1 g, 23 spp (<i>Phyllostachys</i>)	ITS, AFLPs	Support for monophyly of <i>Phyllostachys</i> and its major subgeneric groups; utility of AFLPs for phylogenetic reconstruction among closely related species.
Loh et al.	2000	4 g, 15 spp (Bambusinae)	AFLPs	Most species with unique bands; possible polyphyly of <i>Bambusa</i> and distinctness of <i>Thyrsochloa</i> .
Zhang and Clark	2000	24 g, 24 spp (Bambusoideae)	<i>ndhF</i> , indels, morphology	Asymmetrically invaginated arm cells as a synapomorphy for Bambusoideae; molecular support for monophyly of the subfamily and 3 major clades (Olyreae, temperate woody and tropical woody).
Guo et al.	2001	3 g, 23 spp (temperate woody)	ITS	Non-monophyly of <i>Yushania</i> , <i>Fargesia</i> ; support for some species groupings within alpine bamboos.
Guo et al.	2002	7 g, 31 spp (temperate woody)	ITS	Putative support for monophyly of the <i>Thamnocalamus</i> group of the temperate woody bamboos and position of <i>Chimonocalamus</i> as sister to the remainder of this group; support for monophyly of <i>Ampelocalamus</i> .
Nayak et al.	2003	6 g, 12 spp	RAPDs	Two clusters recovered but no correspondence to taxonomic groupings.
Guo and Li	2004	8 g, 31/33 spp (temperate	ITS, GBSSI	Support for monophyly of <i>Ampelocalamus</i> and

		woody)		<i>Chimonocalamus</i> ; polyphyly of <i>Thamnocalamus</i> and the <i>Thamnocalamus</i> group.
Barkley et al.	2005	11 g, 42 spp (mainly temperate woody)	EST-SSRs	Used transfer markers from maize, wheat, sorghum and rice to assess genetic diversity of the USDA bamboo germplasm collection; accessions clustered \pm according to taxonomy; utility in identification of contaminated plots.
Sun et al.	2005	3 g, 21 spp	ITS	Polyphyly of <i>Bambusa</i> .
Zhuge et al.	2005	3 g, 17 spp (<i>Arundinaria</i> and related genera)	ITS, <i>trnL-trnF</i>	Moderate support for what are now recognized as the <i>Arundinaria</i> and <i>Phyllostachys</i> clades within the temperate woody bamboos.
Sun et al.	2006	2 g, 15 spp (<i>Bambusa</i> s.l.)	RAPDs	Recovery of a core of thorny <i>Bambusa</i> species; subgenus <i>Leleba</i> polyphyletic.
Clark et al.	2007	27 g, 46 spp (Chusqueinae, Hickeliinae)	<i>rpl16</i> intron, morphology	Support for monophyly of Bambusoideae, Bambuseae (moderate), Olyreae, Chusqueinae, and Madagascan Hickeliinae; possible paraphyly of <i>Neurolepis</i> ; aspects of morphological evolution.
Das et al.	2007	4 g, 15 spp (<i>Bambusa</i> , <i>Dendrocalamus</i> , <i>Pseudobambusa</i> , <i>Gigantochloa</i>)	RAPDs, vegetative morphology	Relationships among the 15 species based on allelic polymorphism data consistent with Gamble (1896); demonstration of potential use of RAPDs for evaluation of phylogenetic relationships.
Ramanayake et al.	2007	4 g, 9 spp (<i>Bambusa</i> , <i>Dendrocalamus</i> , <i>Gigantochloa</i> , <i>Arundinaria</i>)	RAPDs	RAPDs useful in determining the genetic diversity among species, even among putatively closely related species; <i>Arundinaria</i> (temperate) distant from the remainder (paleotropical).
Yang et al.	2007	8 g, 26 spp (paleotropical woody)	GBSSI, <i>trnL-F</i> spacer	Support for monophyly of an alliance of <i>Melocanna</i> , <i>Schizostachyum</i> , <i>Cephalostachyum</i> and <i>Pseudostachyum</i> (= Melocanninae); generic realignments of some species.
Bouchenak-Khelladi et al.	2008	25 g, 25 spp (Bambusoideae) + 80 g, 80 spp (other Poaceae)	<i>rbcL</i> , <i>matK</i> , <i>trnL-F</i>	Sister relationship of Pooideae + Bambusoideae; paraphyly of Bambuseae (woody bamboos).
Hisamoto et al.	2008	9 g, 20 spp	FT homolog	Support for monophyly of

		(Bambusoideae, mainly temperate woody)		Bambuseae (woody bamboos), the <i>Phyllostachys</i> and <i>Arundinaria</i> clades within temperate bamboos, neotropical and paleotropical clades within tropical woody bamboos.
Peng et al.	2008	25 g, 43 spp (temperate woody)	ITS, GBSSI	Support for monophyly of the temperate woody bamboos; non-monophyly of traditional subtribes and many genera; support for inclusion of <i>Menstruocalamus</i> and <i>Qiongzhueta</i> in <i>Chimonobambusa</i> .
Ruiz-Sanchez et al.	2008	12 g, 25 spp (neotropical woody)	<i>rpl16</i> intron, <i>trnH-psbA</i> spacer, morphology	Support for monophyly of Guaduinae including 2 spp of <i>Aulonemia</i> and support for monophyly of constituent genera; diagnostic features for Guaduinae.
Sharma et al.	2008	6 g, 21 spp (1° Bambusinae + <i>Phyllostachys</i> , <i>Sasa</i>)	SSRs	Support for monophyly of <i>Phyllostachys</i> and groupings of <i>Bambusa</i> + <i>Dendrocalamus</i> and <i>Melocanna</i> + <i>Ochlandra</i> ; rice genomic SSRs and sugarcane EST-SSRs can be transferable to bamboo (to 44.8% and 75% respectively).
Yang et al.	2008	17 g, 53 spp (paleotropical woody)	ITS, GBSSI, <i>trnL-F</i> , indels	Support for monophyly of Bambusinae and Melocanninae, but Hickeliinae not sampled; <i>Dinochloa</i> sister to remaining Bambusinae; fruit evolution.
Fisher et al.	2009	2g, 22 spp (Chusqueinae)	<i>ndhF</i> , <i>trnD-T</i> , <i>trnC-rpoB</i> , <i>rps16-trnQ</i> , <i>rpl16</i> intron, indels	Confirmed monophyly of Chusqueinae, <i>Chusquea</i> s.s., <i>Euchusquea</i> clade and <i>Chusquea</i> subg. <i>Rettbergia</i> ; confirmed paraphyly of <i>Neurolepis</i> ; submerged <i>Neurolepis</i> into <i>Chusquea</i> .
Sungkaew et al.	2009	33 g, 52 spp (Bambusoideae)	<i>matK</i> , <i>rps16</i> intron, <i>trnL</i> spacer, <i>trnL-F</i> spacer, <i>atpB-rbcL</i> spacer	Confirmed paraphyly of woody bamboos; Melocanninae as sister to the remaining paleotropical woody bamboos; recognition of three tribes: Arundinarieae, Bambuseae, Olyreae.
Goh et al.	2010	9 g, 24 spp (Bambusinae)	GBSSI, <i>rps16-trnQ</i> , <i>trnC-rpoB</i> , <i>trnH-psbA</i> and <i>trnD-T</i>	Non-monophyly of <i>Bambusa</i> ; climbing Southeast Asian genera are distinct from the core <i>Bambusa</i> group.

Hodkinson et al.	2010	27 g, 41 spp (Arundinarieae)	<i>trnL-F</i> , ITS	Support for monophyly of Arundinarieae and polyphyly of its subtribes; lack of internal resolution due to recent, rapid radiation.
Lewis et al.	2010	4 g, 15 spp (temperate and paleotropical woody)	Vegetative morphological characters	Four clusters detected, with only Cluster IV (<i>Dendrocalamus strictus</i> and <i>D. membranaceus</i>) corresponding to any current phylogenetic groupings.
Ruiz-Sanchez and Sosa	2010	1 g, 7 spp (<i>Otatea</i>)	ITS, <i>atpF-atpH</i> , <i>psbKpsbI</i> , <i>trnL-rpl32</i>	Molecular data showed non-monophyly of most entities and conflicted with morphological data; species delimitations based on total evidence.
Triplett and Clark	2010	28 g, 82 spp (1 st 4 markers)/16 g, 21 spp (all 12 markers) (temperate woody)	<i>rps16-trnQ</i> , <i>trnC-rpoB</i> , <i>trnD-trnT</i> , <i>trnT-trnL</i> + <i>ndhF</i> (3' end), <i>atpI-atpH</i> , <i>psaA-ORF170</i> , <i>rpl32-trnL</i> , <i>trnH-psbA</i> , <i>trnK-rps16</i> , <i>trnV-ndhC</i> , <i>trnG</i> intron	Confirmed monophyly of Arundinarieae and support for 6 major clades within the tribe; polyphyly of morphologically-based subtribes; restriction of <i>Arundinaria</i> to N. Am. taxa; evidence for possible role of hybridization within the tribe.
Triplett et al.	2010	1 g, 3 spp (<i>Arundinaria</i> s.s.)	<i>trnT-trnL</i> spacer, AFLPs	Support for three entities in <i>Arundinaria</i> s.s. and a sister relationship between <i>A. tecta</i> and <i>A. appalachiana</i> ; natural, reciprocal hybridization among the three species.
Zeng and Zhang et al.	2010	26 g, 148 spp (temperate woody)	<i>atpI-H</i> , <i>psaA-ORF170</i> , <i>rpl32-trnL</i> , <i>rpoB-trnC</i> , <i>rps16-trnQ</i> , <i>trnD-T</i> , <i>trnS-G</i> , <i>trnT-L</i>	Confirmed monophyly of Arundinarieae and polyphyly of traditional subtribes; support for 10 major clades within the tribe; low molecular divergence within the tribe.
Yang et al.	2010	8 g, 62 spp (core Bambusinae)	GBSSI, <i>psbA-trnH</i> , <i>rpl32-trnL</i> , <i>rps16</i> intron	Monophyly of Bambusinae, <i>Melocalamus</i> , <i>Thyrsostachys</i> supported; 2 main clades (<i>Bambusa</i> s.l. and <i>Dendrocalamus</i> + <i>Gigantochloa</i> + <i>Oxytenanthera</i> + <i>Neosinocalamus</i>) recovered.
Zhou et al.	2010	38 g, 44 spp (woody bamboos)	Ty1- <i>copia rt</i> , ITS	ITS tree largely congruent with other analyses; Ty1- <i>copia rt</i> tree

				incongruent with this ITS tree; <i>Ty1-copia</i> retroelements widespread and abundant in Bambusoideae and present in multiple copies.
Pattanaik and Hall	2011	1 g, 10 spp (<i>Dendrocalamus</i>)	AFLPs	Evidence for polyphyly of <i>Dendrocalamus</i> ; no support for previous infrageneric classifications of the genus.
Wu and Ge	2012	3 g, 3 spp Bambusoideae, 19 spp other grasses	76 chloroplast protein-encoding genes	Support for (Bambusoideae + Pooideae) Ehrhartoideae.

Subfamily Bambusoideae

The bamboo subfamily (Bambusoideae) traditionally included what are now recognized as the “true” bamboos as well as a number of other taxa now classified as subfamilies or tribes within other subfamilies of grasses (e.g., Clayton and Renvoize 1986; Soderstrom and Ellis 1987). A number of molecular analyses (e.g., Clark et al. 1995; GPWG 2001) unequivocally demonstrate the polyphyly of the traditionally circumscribed Bambusoideae and Zhang and Clark (2000) and the GPWG (2001) provide the narrower circumscription of the subfamily that is currently accepted and consistently supported in all analyses that have sufficient taxon sampling (e.g., Bouchenak-Khelladi et al. 2009; Sungkaew et al. 2009; GPWG II 2012). Detailed reviews of the classification history of the Bambusoideae prior to GPWG (2001) are available in Clark et al. (1995), GPWG (2001) and Judziewicz and Clark (2007).

Tribe Olyreae

The more narrowly drawn Bambusoideae are considered to include two main groups, the woody bamboos (as one or more tribes) and the herbaceous bamboos (Olyreae) (GPWG 2001; Clark et al. 2007; Sungkaew et al. 2009). In all analyses, the olyroid lineage is well supported with molecular data but no unequivocal morphological synapomorphies for it have been identified (Zhang and Clark 2000; GPWG 2001). Herbaceous bamboos have often been classified into three tribes (Buergersiochloaeae, Parianeae, Olyreae) (e.g., Soderstrom and Ellis 1987) based primarily on morphological data but these are now usually treated as three subtribes (Buergersiochloinae, Parianinae and Olyrinae) within the Olyreae (Judziewicz and Clark 2007). To date, phylogenetic relationships within the Olyreae have not been rigorously examined, although preliminary data support these three lineages and indicate the probable paraphyly of *Pariana* and probable polyphyly of *Olyra* (GPWG 2001; de Oliveira et al., in prep.).

Woody bamboos

In earlier phylogenetic analyses, the woody bamboos (as the tribe Bambuseae) are well supported as monophyletic based on morphological characters but receive moderate support at best from molecular data (Kelchner and Clark 1997; Zhang and Clark 2000; Clark et al. 2007). Within woody bamboos, three or four moderately to well supported clades (North Temperate clade, Paleotropical clade, and one or two Neotropical clades) forming a polytomy have usually been recovered (Watanabe et al. 1994; Kelchner and Clark 1997; Kobayashi 1997; Zhang and Clark 2000; Clark et al. 2007; Ramanayake et al. 2007). The most recent analyses based on multiple molecular data sets and more thorough sampling across the subfamily strongly support two lineages of woody bamboos that are

paraphyletic to the Olyreae or form a trichotomy with it (Bouchenak-Khelladi et al. 2008; Sungkaew et al. 2009; BPG, in prep.). Although alternate hypothesis testing is unable to reject monophyly of the woody bamboos (BPG, in prep.), it is likely that temperate and tropical bamboos will each continue to be recognized as a tribe whatever the ultimate resolution of branching order. Sungkaew et al. (2009, Table 1) summarize the subtribal classifications in use for woody bamboos.

Tribe Arundinarieae

The temperate woody bamboos (North Temperate clade) or Arundinarieae exhibit a more or less typical Laurasian distribution pattern (Judziewicz and Clark, 2007; Map 5, Bamboo Biodiversity). The longest known flowering cycles in bamboos, up to 120 years, are found in members of this clade. All Arundinarieae for which data are available are tetraploids with a chromosome complement of $2n = 48$ (Soderstrom 1981). Many also have leptomorph (running) rhizomes, but no consistent morphological synapomorphy has been identified for this clade. Two or three subtribes have traditionally been recognized (e.g., Dransfield and Widjaja, 1995; Ohrnberger 1999; Li 1997; Guo and Li 2004) to accommodate the morphological diversity of the temperate bamboos: Arundinarieae, Shibataeinae and Thamnocalaminae. The temperate bamboo clade is consistently robustly supported in all phylogenetic analyses to date (Kelchner and Clark, 1997; Kobayashi, 1997; Zhang and Clark, 2000; Ní Chonghaile 2002; Guo et al., 2001, 2002; Guo and Li 2004; Zhuge et al. 2005; Bouchenak-Khelladi et al. 2008; Hisamoto et al. 2008; Peng et al. 2008; Sungkaew et al. 2009; Hodkinson et al. 2010; BPG, in prep.), but with relatively little internal resolution. Two recent studies, Triplett and Clark (2010) and Zeng and Zhang et al. (2010), both with extensive sampling across the clade and based on multiple plastid markers, recover six and ten well supported lineages, respectively, within the temperate woody bamboos. The three subtribes are highly polyphyletic, and the two most diverse lineages in both analyses are the *Arundinaria* clade and the *Phyllostachys* clade. Triplett et al. (2010) present evidence that hybridization within the temperate bamboos may be more widespread than previously thought.

Tribe Bambuseae

The tropical woody bamboos or Bambuseae are widespread in both the Paleo- and Neotropics (Judziewicz and Clark, 2007; Maps 3 and 4, Bamboo Biodiversity). All Bambuseae, with one apparent diploid exception in the neotropical genus *Chusquea*, are either tetraploid or hexaploid with base chromosome numbers of mainly $x = 10$ or $x = 12$ (Soderstrom 1981; Judziewicz et al. 1999; Li et al. 2001). All exhibit pachymorph culm bases (= pachymorph rhizomes) although some members of *Chusquea* also have leptomorph rhizomes (Judziewicz et al. 1999). Two well supported neotropical lineages, the Chusqueinae clade (Clark et al. 2007; Fisher et al. 2009) and the Arthrotyliidiinae + Guaduinae clade (Ruiz-Sanchez et al. 2008), may associate in a weakly to moderately supported neotropical clade sister to the paleotropical clade (Bouchenak-Khelladi et al. 2008; Sungkaew et al. 2009; BPG, in prep.), but the two neotropical clades are paraphyletic to the paleotropical clade in other trees (Zhang and Clark 2000; Clark et al. 2007). The relatively strongly supported paleotropical clade includes four currently recognized subtribes (Bambusinae, Melocanninae, Hickeliinae, and Racemobambosinae) distributed throughout Southeast Asia, northern Australia, India, Africa, and Madagascar (Soderstrom and Ellis 1987; Dransfield and Widjaja 1995; Ohrnberger 1999). Evidence of hybridization has been documented in both neotropical (Clark et al. 1989) and paleotropical taxa (Goh et al. 2011; Wong and Low 2011) but how prevalent this might be in the Bambuseae is not yet known.

Neotropical woody bamboos

Chusqueinae is strongly supported as a monophyletic lineage and is diagnosed by the apparent synapomorphies of bi-papillate subsidiary cells in the foliar stomatal apparatus and a uniform spikelet structure of four glumes and one female-fertile floret with no rachilla extension (Clark et al. 2007; Fisher et al. 2009; BPG, in prep.). Within the *Chusqueinae*, four major lineages are robustly supported with these sister relationships based on plastid sequence data (Fisher et al. 2009): *Neurolepis* II + [*Neurolepis* I + (*Euchusquea* clade + *Rettbergia*)]. Non-monophyly of *Neurolepis* led Fisher et al. (2009) to submerge this genus under *Chusquea*.

Analyses of *Arthrostylidiinae* and *Guaduinae*, the other two neotropical subtribes of *Bambuseae*, always recover taxa of each in one moderately to robustly supported clade (Zhang and Clark 2000; Clark et al. 2007; Ruiz-Sanchez et al. 2008; Sungkaew et al. 2009), but only more recent analyses with more extensive sampling support the monophyly of each subtribe (Ruiz-Sanchez et al. 2008; Tyrrell et al., in review; BPG, in prep.). Although a formal morphological analyses remains to be completed, the *Arthrostylidiinae* + *Guaduinae* clade may be diagnosed by the presence of refractive papillae, although these also occur in *Melocanninae* (Soderstrom and Ellis 1987; Ruiz-Sanchez et al. 2008). The *Arthrostylidiinae* is robustly supported as monophyletic based on plastid sequence data (Tyrrell et al., in review) and is also supported by the presence of intercostal sclerenchyma and simple midribs in the foliage leaf blades (Soderstrom and Ellis 1987). A green, waxless stripe along one margin of the abaxial leaf blade surface is characteristic of *Arthrostylidiinae* but not unique to it (Tyrrell et al., in review). The *Guaduinae* have an unusual foliar micromorphology in which the blades are amphistomatic and with papillae overarching the stomates, especially on the adaxial surface (Soderstrom and Ellis 1987; Ruiz-Sanchez et al. 2008). In contrast to the *Arthrostylidiinae*, foliage leaf blades of *Guaduinae* lack intercostal sclerenchyma and have the complex midribs typical of most woody bamboos (Soderstrom and Ellis 1987).

Paleotropical woody bamboos

The paleotropical woody bamboos receive strong support in analyses of plastid sequence data as a monophyletic lineage (Bouchenak-Khelladi et al. 2008; Sungkaew et al. 2009; BPG, in prep.), but no morphological synapomorphy has been identified for this clade. The *Melocanninae* are consistently robustly resolved as sister to the remaining paleotropical woody bamboos in recent analyses (Sungkaew et al. 2009; BPG, in prep.; Chokthaweeapanich et al., unpubl. data) and the subtribe is supported by the possible synapomorphies of a glabrous ovary with a long, slender, hollow style and an S-shaped keel in the foliage leaf blade (Soderstrom and Ellis 1987). The results of Yang et al. (2007, 2008) suggest that some generic realignments within *Melocanninae* will be necessary, but further analyses are required. Within the remaining paleotropical woody bamboos, *Hickeliinae* is supported as monophyletic based on plastid sequence data and the putative synapomorphy of adaxially projecting midribs (Soderstrom and Ellis 1987; Clark et al. 2007; BPG, in prep.) but its position relative to *Racemobambos*, *Bambusinae*, and sometimes other clades (e.g., *Neololeba* + *Cyrtochloa* or *Dinochloa* + *Sphaerobambos*) remains ambiguous due to lack of resolution or incomplete sampling (Yang et al. 2007, 2008; Goh et al. 2010; BPG, in prep.). Molecular data also do not support the placement of *Greslania* in *Hickeliinae* (Clark et al. 2007; Chokthaweeapanich et al., unpubl. data). Monophyly of *Bambusinae*, the most diverse of the four currently recognized subtribes of paleotropical woody bamboos, has not been rigorously tested but *Vietnamosasa* and *Neomicrocalamus* (both previously classified in *Racemobambosinae*) are supported as members of core *Bambusinae* (Yang et al. 2008; Sungkaew et al. 2009). Not surprisingly, given the economic importance of these taxa, most phylogenetic studies of *Bambusinae* have focused on *Bambusa*, *Dendrocalamus* and *Gigantochloa*, the core genera of the subtribe (e.g., Nayak et al. 2003; Sun et al. 2005, 2006; Das et al.

2007; Goh et al. 2010; Yang et al. 2010; Pattanaik and Hall 2011). Relationships among these genera and other putative members of the Bambusinae, as well as circumscriptions of a number of constituent genera, are controversial and require much additional work before these issues can be resolved (Goh et al. 2010).

Basis for an Updated Classification

The recognition of three tribes within the Bambusoideae is clearly supported by the molecular phylogenetic results (Bouchenak-Khelladi et al. 2008; Sungkaew et al. 2009; BPG, in prep.). Although a formal morphological analysis is not yet available, putative synapomorphies have now been identified for the three tribes. These need to be further tested, but for now, Arundinarieae is diagnosed by basipetal branch development and a chromosome number of $2n = 48$, Bambuseae by acropetal or bidirectional branch development, and Olyreae by unisexual, often strongly dimorphic, 1-flowered spikelets with no rachilla extension, although all but the earliest diverging lineage (*Buergersiochloa*) also share cross-shaped silica bodies in the costal zone and crenate (olyroid) silica bodies in the intercostal zone.

Members of what is now recognized as the Arundinarieae were traditionally classified in up to three subtribes, the Arundinariinae, Shibateinae and Thamnocalaminae, based on the presence or absence of pseudospikelets and rhizome structure. The evident polyphyly of all three subtribes has caused them to be abandoned in favor of the recognition of numbered lineages (Triplett and Clark 2010; Zeng and Zhang et al. 2010). Branching order among the 10 current lineages is largely unresolved, so until more data are available, we simply list the genera for the tribe without reference to subtribes or lineages.

Within the Bambuseae, the three neotropical subtribes as delimited by Judziewicz et al. (1999) are supported by molecular phylogenetic analyses, and each has at least one morphological synapomorphy, so we continue to recognize these three. Among the paleotropical subtribes, the Melocanninae, Hickeliinae and Bambusinae remain largely as circumscribed by Soderstrom and Ellis (1987), with the addition of a number of more recently described genera mainly in the Bambusinae and Hickeliinae and the placement of *Greslania* in the Bambusinae. The Racemobambosinae here is restricted to *Racemobambos*, based on recent molecular results indicating that *Neomicrocalamus* and *Vietnamosasa* fall within the Bambusinae (Yang et al. 2008; Sungkaew et al. 2009). Morphological synapomorphies have not yet been identified for either the Bambusinae or the Racemobambosinae. As Goh et al. (in prep. and pers. comm.) suggest, it may be necessary to recognize one or more additional subtribes segregated from the Bambusinae once the major lineages of paleotropical woody bamboos are more fully understood.

Our subtribal treatment of Olyreae is consistent with Judziewicz and Clark (2007) and the few phylogenetic analyses including sampling across the diversity of this tribe (BPG, in prep.). A more comprehensive phylogenetic analysis is in progress (de Oliveira et al., in prep.) and will provide more insight into the evolution of the herbaceous bamboos.

Taxonomic Treatment

The subfamily description is modified from GPWG (2001). Potential synapomorphies for tribes or subtribes are underlined within the descriptions. Genera are listed alphabetically within each tribe or subtribe, and the number of species for each genus is given in parentheses after the genus name. Two

electronic databases are available with more detailed information on bamboo genera: GrassBase (www.kew.org/data/grasses-db/) and Grass Genera of the World. (www.delta-intkey.com/grass).

Bambusoideae Luerss., Grundz. Bot., ed. 5: 451. 1893. Type: *Bambusa* Schreb.

Plants perennial (possibly rarely annual in Olyreae), rhizomes (leptomorph) present or absent, herbaceous or woody, of temperate and tropical forests, tropical high montane grasslands, riverbanks, and sometimes savannas or swamps. Culms hollow or solid; aerial branching often present. Leaves distichous; outer (abaxial) ligule absent (Olyreae) or present (Arundinarieae, Bambuseae); adaxial ligule membranous or chartaceous, fringed or unfringed; sheaths often auriculate or fimbriate or both; blades usually relatively broad, pseudopetiolate, venation parallel; mesophyll non-radiate, an adaxial palisade layer absent, fusoid cells large and well developed in at least shade leaves, arm cells usually well developed and strongly asymmetrically invaginated; Kranz anatomy absent, photosynthetic pathway C_3 ; midrib complex or simple; adaxial bulliform cells present; stomates with dome-shaped, triangular or parallel-sided subsidiary cells; bicellular microhairs present, panicoid-type; papillae common and abundant. Synflorescences spicate, racemose or paniculate, completing development of all spikelets in one period of growth and subtending bracts and prophylls usually absent, or pseudospikelets with basal bud-bearing bracts producing two or more orders of spikelets with different phases of maturity and subtending bracts and prophylls usually present. Spikelets (or spikelets proper of the pseudospikelets) bisexual (Arundinarieae, Bambuseae) or unisexual (Olyreae), consisting of 0, 1, 2 or several glumes and 1 to many florets; lemma lacking uncinata macrohairs, if awned, the awns single; palea well developed; lodicules usually 3 (rarely 0 to 6 or many), membranous, vascularized, often ciliate; stamens usually 2, 3 or 6 (2 to 40 in *Pariana*, 6 to 120 in *Ochlandra*); ovary glabrous or hairy, sometimes with an apical appendage, haustorial synergids absent, styles 2 or 3, sometimes very short but close, stigmas 2 or 3. Caryopsis with hilum linear (rarely punctate), extending its full length (rarely less than full length); endosperm hard, without lipid, containing compound starch grains; embryo small, epiblast present, scutellar cleft present, mesocotyl internode absent, embryonic leaf margins overlapping. First seedling leaf blade absent. Base chromosome numbers: $x = 7, 9, 10, 11$, and 12.

Included Tribes: Arundinarieae, Bambuseae, Olyreae.

Arundinarieae Nees ex Asch. & Graebn., Syn. Mitteleurop. Fl. 2, 1: 770. 1902. Type: *Arundinaria* Michx.

Rhizomes (leptomorph) and culm bases well developed, some taxa lacking leptomorph rhizomes. Culms woody, usually hollow; culm development occurring in two phases, first, new, unbranched shoots bearing culm leaves elongate to full height, second, culm lignification and branch development with production of foliage leaves occur; branch development basipetal; aerial vegetative branching complex, usually derived from a single bud per node (multiple, subequal buds per node in *Chimonocalamus* and *Chimonobambusa*). Culm leaves usually well developed with expanded sheaths and well developed to reduced blades. Foliage leaves with an outer ligule; sheaths often bearing fimbriae and/or auricular appendages at the summit; blades pseudopetiolate, articulated, deciduous; epidermal silica cells lacking cross-shaped or crenate silica bodies. Flowering usually cyclical, gregarious and monocarpic. Synflorescences bracteate or not, determinate (spikelets) or indeterminate (pseudospikelets). Spikelets (or spikelets proper of the pseudospikelets) bisexual with 1 to many bisexual florets; glumes (0-1) 2-4; lemmas multinerved, similar in texture to the glumes; paleas several-nerved with an even number of nerves, bicarinate. Caryopsis basic, uncommonly baccate (e.g., *Ferrocalamus*); hilum linear. Base chromosome number $x = 12$; $2n = 48$.

Included genera: *Acidosasa* C. D. Chu & C. S. Chao ex P. C. Keng (11) (including *Metasasa* W. T. Lin), *Ampelocalamus* S. L. Chen, T. H. Wen & G. Y. Sheng (13), *Arundinaria* Michx. (3), *Bashania* P. C. Keng & Yi (2), *Chimonobambusa* Makino (37) (including *Menstruocalamus* T. P. Yi, *Oreocalamus* Keng, *Qiongzhusa* Hsueh & Yi), *Chimonocalamus* Hsueh & Yi (11), *Drepanostachyum* P. C. Keng (10), *Fargesia* Franchet (90) (including *Borinda* Stapleton, *Sinarundinaria* Nakai), *Ferocalamus* Hsueh & P. C. Keng (2), *Gaoligongshania* D. Z. Li, Hsueh & N. H. Xia (1), *Gelidocalamus* T. H. Wen (9), *Himalayacalamus* P. C. Keng (8), *Indocalamus* Nakai (23), *Indosasa* McClure (15), *Oligostachyum* Z. P. Wang & G. H. Ye (15) (including *Clavinodum* T. H. Wen), *XPhyllosasa* Demoly (1), *Phyllostachys* Sieb. & Zucc. (51), *Pleioblastus* Nakai (40) (including *Nipponocalamus* Nakai, *Polyanthus* C. H. Hu), *Pseudosasa* Makino ex Nakai (19), *Sarocalamus* Stapleton (3), *Sasa* Makino & Shibata (40), *Sasaella* Makino (13), *Sasamorpha* Nakai (5), *Semiarundinaria* Makino ex Nakai (10) (including *Brachystachyum* Keng), *Shibataea* Makino ex Nakai (7), *Sinobambusa* Makino ex Nakai (10), *Thamnocalamus* Munro (4), *Yushania* P. C. Keng (80) (including *Burmabambus* P. C. Keng, *Butania* P. C. Keng, *Monospatha* W. T. Lin).

Bambuseae Kunth ex Dumort., Anal. Fam. Pl.: 63. 1829. Type: *Bambusa* Schreb.

Rhizomes (leptomorph) and culm bases well developed, leptomorph rhizomes occurring only within *Chusquea*. Culms woody, usually hollow (solid in most *Chusquea* and a few species of other genera); culm development occurring in two phases, first, new, unbranched shoots bearing culm leaves elongate to full height, second, culm lignification and branch development with production of foliage leaves occur; branch development acropetal or bidirectional; aerial vegetative branching complex (but absent in *Glaziophyton*, *Greslania* and two clades within *Chusquea*), usually derived from a single bud per node (multiple, subequal buds per node in *Apoclada*, *Filgueirasia*, *Holttumochloa*; multiple, dimorphic buds in most of *Chusquea*). Culm leaves usually well developed with expanded sheaths and well developed to reduced blades, sometimes poorly differentiated from foliage leaves (e.g., *Aulonemia*, two clades within *Chusquea*) or absent. Foliage leaves with an outer ligule; sheaths often bearing fimbriae and/or auricular appendages at the summit; blades usually pseudopetiolate, articulate, deciduous; epidermal silica cells lacking cross-shaped or crenate silica bodies. Flowering usually cyclical, gregarious and monocarpic. Synflorescences bracteate or not, determinate (spikelets) or indeterminate (pseudospikelets). Spikelets (or spikelets proper of the pseudospikelets) bisexual with 1 to many bisexual florets; glumes (0-) 1-4 (-6), sometimes very reduced; lemmas multinerved, similar in texture to the glumes; paleas several-nerved with an even number of nerves, bicarinate. Caryopsis usually basic, sometimes baccate (e.g., *Alvimia*, *Dinochloa*, *Melocanna*, *Ochlandra*, *Olmecca*, at least one species of *Guadua*) or nuroid (e.g., *Actinocladum*, *Merostachys*); hilum linear. Base chromosome numbers $x = 10, (11), \text{ and } 12; 2n = (20) 40, (44), 46, 48, 70, 72$.

Neotropical Woody Bamboo Subtribes

Arthrostylidiinae Bews, World's Grasses: 96. 1929. Type: *Arthrostylidium* Rupr.

Rhizomes (leptomorph) absent. Culm bases sympodial, pachymorph, necks short to somewhat elongated; internodes of the aerial culms usually hollow, sometimes thick-walled, rarely septate (*Glaziophyton*), all subequal or sometimes very short internodes alternating in various combinations with elongated internodes; nodes of aerial culms without a patella. Aerial branching usually well developed and derived from a single bud per node; thorns absent. Culm leaves usually well developed (absent in *Glaziophyton*); margins of the sheath and blade more or less continuous or distinct; sheaths usually bearing fimbriae or fimbriate auricles; oral setae absent; blades erect or reflexed. Foliage leaf

sheaths usually bearing fimbriae or fimbriate auricles at the summit, oral setae absent; blades with a simple, abaxially projecting midrib; intercostal sclerenchyma usually present; adaxial epidermis lacking stomates and papillae or these infrequent and poorly developed; abaxial epidermis usually with a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences usually ebracteate, indeterminate (pseudospikelets) or determinate (spikelets), paniculate or racemose; prophylls present or absent. Spikelets (or spikelets proper of the pseudospikelets) consisting of 2-3 glumes, 1 to many female-fertile florets, and a rachilla extension bearing a rudimentary floret; palea keels wingless. Stamens (2) 3 (6), filaments free. Ovary glabrous, with a short style; stigmas 2 (3). Caryopsis basic, uncommonly baccate (*Alvimia*) or nuroid (*Actinocladum*, *Merostachys*). Base chromosome number $x = 10$; $2n = 40$ (but only 2 counts available for the subtribe).

Included genera: *Actinocladum* Soderstr. (1), *Alvimia* Soderstr. & Londoño (3), *Arthrostylidium* Rupr. (32), *Athroostachys* Benth (1), *Atractantha* McClure (6), *Aulonemia* Goudot (40) (including *Matudacalamus* F. Maekawa), *Colantheria* McClure & E. W. Sm. (7), *Elytostachys* McClure (2), *Filgueirasia* Guala (2), *Glaziophyton* Franch. (1), *Merostachys* Spreng. (48), *Myriocladus* Swallen (12), *Rhipidocladum* McClure (17).

Chusqueinae Bews, World's Grasses: 96. 1929. Type: *Chusquea* Kunth.

Neurolepidinae Soderstr. & R. P. Ellis in Soderstr. et al., Grass Syst. Evol.: 238. 1987. Type: *Neurolepis* Meisner.

Rhizomes (leptomorph) sometimes present. Culm bases sympodial, pachymorph, necks short; internodes of the aerial culms usually solid, all subequal; nodes of the aerial culms without a patella. Aerial branching usually well developed and derived from a multiple, dimorphic bud complement, absent in two clades (= *Neurolepis*) but a single bud per node often present in these taxa; thorns absent. Culm leaves usually well developed (sometimes not well differentiated in the *Neurolepis* clades); margins of the sheath and blade usually distinct; fimbriae or fimbriate auricles absent; oral setae absent; blades usually erect, rarely reflexed. Foliage leaf sheaths usually bearing cilia at the summit, rarely well developed fimbriae present, oral setae absent, auricles absent; blades with a complex, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis lacking stomates and papillae or these infrequent and poorly developed; abaxial epidermis usually lacking a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus bearing two or more papillae per subsidiary cell and also often overarched by papillae from adjacent long cells. Synflorescences usually ebracteate, determinate (spikelets), paniculate or rarely racemose; prophylls absent. Spikelets consisting of 4 glumes and 1 female-fertile floret, rachilla extension absent; palea keels lacking wings. Stamens (2) 3, filaments free. Ovary glabrous, with a short style; stigmas 2. Caryopsis basic. Base chromosome number $x = 10$ (11, 12); $2n = (20) 40$ (44, 48).

Included genus: *Chusquea* Kunth (160) (including *Neurolepis* Meisn., *Rettbergia* Raddi, *Swallenochloa* McClure).

Guaduinae Soderstr. & R. P. Ellis in Soderstr. et al., Grass Syst. Evol.: 238. 1987. Type: *Guadua* Kunth.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short to elongated; internodes of the aerial culms hollow to solid, all subequal; nodes of the aerial culms without a patella. Aerial branching well developed and derived from a single bud per node (1-4 subequal buds per node

in *Apoclada*); thorns absent or present (*Guadua*). Culm leaves well developed; margins of the sheath and blade continuous or nearly so, uncommonly distinct; sheaths often bearing fimbriae or fimbriate auricles at the sheath summit; oral setae usually present (absent in *Guadua*); blades erect or reflexed. Foliage leaf sheaths often with fimbriae or fimbriate auricles at the summit; oral setae present; blades with a complex, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis usually with abundant stomates and well developed papillae, rarely these lacking or infrequent and poorly developed; abaxial epidermis usually lacking a green stripe along the narrow-side margin, with stomates present and abundant (absent in *Apoclada*) and papillae absent to well developed; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences bracteate or not, indeterminate (pseudospikelets) or determinate (spikelets), paniculate; prophylls present or absent. Spikelets (or spikelets proper of the pseudospikelets) consisting of (0-) 1 to 4 (-7) glumes, 1 to many female-fertile florets, and a rachilla extension bearing a rudimentary floret; palea keels wingless to prominently winged. Stamens 3 or 6, filaments free. Ovary glabrous or hairy, with a short style; stigmas 2 or 3. Caryopsis basic, uncommonly baccate (*Olmecca* and *Guadua sarcocarpa*). Base chromosome number $x=12$; $2n=46$ or 48.

Included genera: *Apoclada* McClure (1), *Eremocaulon* Soderstr. & Londoño (4) (including *Criciuma* Soderstr. & Londoño), *Guadua* Kunth (27), *Olmecca* Soderstr. (5), *Otatea* (McClure & E. W. Sm.) C. E. Calderón & Soderstr. (8)

Paleotropical Woody Bamboo Subtribes

Bambusinae J. S. Presl in K. B. Presl, Rel. Haenk. 1: 256. 1830. Type: *Bambusa* Schreb.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short to slightly elongated; internodes of the aerial culms hollow or solid, all subequal; nodes of the aerial culms with or without a patella. Aerial branching well developed and derived from a single bud per node (multiple buds in *Holttumochloa*); thorns usually absent, sometimes present (*Bambusa*). Culm leaves well developed; margins of the sheath and blade continuous or distinct; sheaths bearing fimbriae or fimbriate auricles at the summit or neither; oral setae present or absent; blades erect or reflexed. Foliage leaf sheaths often with fimbriae or fimbriate auricles at the summit; oral setae present or absent; blades with a complex or simple, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis with or without stomates, with or without papillae; abaxial epidermis usually lacking a green stripe along the narrow-side margin, usually with abundant stomates and well developed papillae; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences bracteate or not, indeterminate (pseudospikelets) or less commonly determinate (spikelets), paniculate; prophylls present or absent. Spikelets or spikelets proper of the pseudospikelets consisting of (0-) 1 to several glumes, 1-10 or more female-fertile florets and sometimes a rachilla extension bearing 1-3 rudimentary florets; palea keels wingless to prominently winged. Stamens 6, filaments free or fused. Ovary glabrous or hairy, usually with a short style; stigmas 1, 2 or 3. Caryopsis basic or baccate (*Cyrtochloa*, *Dinochloa*, *Melocalamus*, *Sphaerobambos*). Base chromosome number $x=10$ or 12; $2n=48, 70, 72$.

Included genera: *Bambusa* Schreber (100) (including *Dendrocalamopsis* Q. H. Dai & X. L. Tao, *Isurochloa* Buse, *Leleba* Rumphius ex Nakai, *Lingnania* McClure, *Neosinocalamus* P.C. Keng, *Tetragonocalamus* Nakai), *Bonia* Balansa (5) (including *Monocladus* Chia, H. L. Fung & Y. L. Yang), *Cyrtochloa* S. Dransf. (5), *Dendrocalamus* Nees (41) (including *Klemachloa* R. N. Parker, *Sinocalamus* McClure), *Dinochloa* Buse (31), *Fimbribambusa* Widjaja (2), *Gigantochloa* Kurz ex

Munro (30), *Greslania* Balansa (4), *Holttumochloa* K. M. Wong (3), *Kinabaluchloa* K. M. Wong (2), *Maclurochloa* K. M. Wong (1), *Melocalamus* Benth. (5), *Mullerochloa* K. M. Wong (1), *Neololeba* Widjaja (5), *Neomicrocalamus* P. C. Keng (5) (including *Microcalamus* Gamble), *Oreobambos* K. Schumann (1), *Oxytenanthera* Munro (1), *Parabambusa* Widjaja (1), *Phuphanochloa* Sungkaew & Teerawat. (1), *Pinga* Widjaja (1), *Pseudobambusa* Nguyen (1), *Pseudoxytenanthera* Soderstr. & Ellis (12), *Soejatmia* K. M. Wong (1), *Sphaerobambos* S. Dransf. (3), *Temochloa* S. Dransf. (1), *Temburongia* S. Dransf. & K. M. Wong (1), *Thyrsostachys* Gamble (2), *Vietnamosasa* Nguyen (3).

Hickeliinae A. Camus, Compt. Rend. Acad. Sci. 179: 480. 1924. Type: *Hickelia* A. Camus.

Nastinae Soderstr. & R. P. Ellis in Soderstr. et al., Grass Syst. Evol.: 238. 1987. Type: *Nastus* A. L. Juss.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short to elongated; internodes of the aerial culms usually hollow or rarely solid, all subequal along the aerial culms. Aerial branching well developed and derived from a single bud per node (multiple buds in *Nastus productus*), central branch dominant; thorns absent. Culm leaves well developed; margins of sheath and blade usually discontinuous; sheaths bearing fimbriae or fimbriate auricles or neither; oral setae absent; blades erect or reflexed. Foliage leaf sheaths with fimbriae or fimbriate auricles present or absent; oral setae absent; blades with a complex, adaxially projecting midrib; intercostal sclerenchyma and fiber-like epidermal cells sometimes present; adaxial epidermis lacking stomates and papillae or these infrequent and poorly developed; abaxial epidermis usually lacking a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synfloresences determinate (spikelets), bracteate or ebracteate, paniculate, racemose or capitate; prophylls usually absent. Spikelets consisting of 4-6 glumes and 1 female-fertile floret; rachilla extension present or absent, if present well developed or much reduced bearing a rudimentary or reduced floret; palea usually 2-keeled (without keels when rachilla extension absent), keels wingless. Stamens 6, filaments usually free. Ovary glabrous or hairy, with long or short style; stigmas 3. Caryopsis basic, sessile or stalked (*Cathariostachys*). Base chromosome number and ploidy level unknown.

Included genera: *Cathariostachys* S. Dransf. (2), *Decaryochloa* A. Camus (1), *Hickelia* A. Camus (4) (including *Pseudocoix* A. Camus), *Hitchcockella* A. Camus (1), *Nastus* Juss. (20) (including *Chloothamnus* Büse, *Oreiochloa* Gamble), *Perrierbambus* A. Camus (2), *Sirochloa* S. Dransf. (1), *Valiha* S. Dransf. (2).

Melocanninae Benth., J. Linn. Soc. London 19: 31. 1881. Type: *Melocanna* Trin.

Schizostachyidinae Soderstr. & R. P. Ellis in Soderstr. et al., Grass Syst. Evol.: 238. 1987. Type: *Schizostachyum* Nees.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short or elongated; internodes of the aerial culms moderately long or very long, hollow, with thin walls; nodes of the aerial culms lacking a patella. Aerial branching well developed and derived from a single bud per node; thorns absent. Culm leaves well developed; margins of the sheath and blade distinct; sheaths bearing fimbriae or fimbriate auricles at the summit or neither; oral setae usually absent; blades often reflexed. Foliage leaf sheaths bearing fimbriae or small fimbriate auricles or neither; oral setae present or absent; blades with a complex, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis lacking stomates or these infrequent and poorly developed, papillae often present; abaxial epidermis with (usually) or without a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus with

papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences indeterminate (pseudospikelets), spicate or capitate, prophylls present. Spikelets proper consisting of (0) 2 (or 4) glumes, one female-fertile floret (3 in *Schizostachyum grande*), with or without rachilla extension, if present bearing a rudimentary floret; palea keels wingless or winged. Stamens 6 (15-120 in *Ochlandra*), filaments free or fused. Ovary glabrous, with a long, slender, hollow style; stigmas (2-) 3. Caryopsis basic or baccate (*Melocanna*, *Ochlandra*, *Stapletonia*) or nucoid (*Pseudostachyum*). Base chromosome number $x = 12$; $2n = 72$.

Included genera: *Cephalostachyum* Munro (14), *Davidsea* Soderstr. & Ellis (1), *Dendrochloa* C. E. Parkinson (1), *Melocanna* Trin. (2), *Neohouzeaua* A. Camus (7), *Ochlandra* Thwaites (9), *Pseudostachyum* Munro (1), *Schizostachyum* Nees (50) (including *Leptocanna* L. C. Chia & H. L. Fung), *Stapletonia* Singh, Dash & Kumari (1), *Teinostachyum* Munro (2).

Racemobambosinae Stapleton, Edinburgh J. Bot. 51: 323-324. 1994. Type: *Racemobambos* Holttum. Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short or elongated; internodes of the aerial culms hollow, all subequal; nodes of the aerial culms without a patella. Aerial branching well developed and derived from a single bud per node; thorns absent. Culm leaves well developed; margins of the sheath and blade more or less continuous or distinct; sheaths usually bearing small fimbriate auricles at the summit or rarely efimbriate and exauriculate; oral setae absent; blades erect or reflexed. Foliage leaf sheaths usually bearing small fimbriate auricles at the summit or rarely efimbriate and eauriculate; oral setae absent; blades with an abaxially projecting midrib; blade anatomy and micromorphology unknown. Synflorescences bracteate, determinate (spikelets), racemose; prophylls absent. Spikelets consisting of 2-3 glumes, 3-8 female-fertile florets and a rachilla extension bearing 1 rudimentary floret; palea keels wingless. Stamens 6, filaments free. Ovary usually hairy toward the apex, usually with a short style; stigmas 3. Caryopsis basic. Base chromosome number unknown.

Included genus: *Racemobambos* Holttum (16).

Olyreae Kunth ex Spenn., Fl. Friburg. 1: 172. 1825. Type: *Olyra* L.

Rhizomes (leptomorph) weakly or sometimes strongly developed (*Olyra*, *Pariana*). Culms herbaceous to subwoody, vegetative branching restricted and only one phase of culm development observed. Culm leaves usually absent, sometimes differentiated in taxa with larger culms. Foliage leaves with the outer ligule absent; sheaths sometimes bearing fimbriae (*Eremitis*, *Pariana*) and/or blister-like swellings at or near the summit (*Pariana*), more often fimbriae, swellings, and auriculate appendages absent; blades pseudopetiolate, not articulated, persistent or sometimes deciduous, exhibiting nocturnal folding (nyctinasty) in some genera (e.g., *Eremitis*, *Lithachne*, *Raddia*, *Raddiella*); epidermal silica cells usually with cross-shaped silica bodies in the costal zone and crenate (olyroid) silica bodies in the intercostal zone (these absent in *Buergersiochloa*). Flowering usually annual or seasonal for extended periods, very rarely gregarious and monocarpic. Synflorescences ebracteate or rarely enclosed by a spathaceous leaf sheath (*Eremitis*), apparently determinate. Spikelets unisexual, dimorphic and 1-flowered with no rachilla extension, the plants monoecious; pistillodes or staminodes sometimes present in male or female spikelets respectively. Female spikelets with 2 glumes; lemma chartaceous to more commonly coriaceous, several-nerved, usually non-aristate except in *Agnesia*, *Buergersiochloa* and *Ekmanochloa*; palea with few to several nerves. Male spikelets usually smaller than the females, glumes usually absent or rarely 2 and well developed;

lemmas membranous, 3-nerved. Caryopsis basic; hilum usually linear, sometimes punctate. Base chromosome number $x = 7, 9, 10, 11,$ and (12).

Buergersiochloinae (S. T. Blake) L. G. Clark & Judz., *Aliso* 23: 311. 2007.

Foliage leaf sheaths bearing fimbriae at the apex; blades lacking cross-shaped and crenate (olyroid) silica bodies in both epidermises. Synflorescences paniculate. Female lemmas awned. Stamens 2-3.

Included genus: *Buergersiochloa* Pilg. (1).

Parianinae Hack. in Engler & Prantl, *Naturl. Pflanzenfam.* 2, 2: 88. 1887. Type: *Pariana* Aubl.

Foliage leaf sheaths bearing fimbriae at the apex; blades with cross-shaped and crenate (olyroid) silica bodies in the epidermises. Synflorescences spicate. Female lemmas unawned. Stamens 2 or 6 (to 36-40).

Included genera: *Eremitis* Döll (1), *Pariana* Aubl. (35) (Generic and species delimitations in this subtribe are uncertain, so these numbers represent estimates; de Oliveira and Moreira, pers. comm.)

Olyrinae Kromb., *Fl. Luxembourg* 496. 1875. Type: *Olyra* L.

Foliage leaf sheaths lacking fimbriae at the apex; blade with cross-shaped and crenate (olyroid) silica bodies in the epidermises. Synflorescences paniculate or racemose. Female lemmas usually unawned (awned only in *Agnesia*, *Ekmanochloa*). Stamens 2-3.

Included genera: *Agnesia* Zuloaga & Judz. (1), *Arberella* Soderstr. & C. E. Calderón (7), *Cryptochloa* Swallen (8), *Diandrolyra* Stapf (3), *Ekmanochloa* Swallen (2), *Froesiochloa* G. A. Black (1), *Lithachne* P. Beauv. (4), *Maclurolyra* C. E. Calderón & Soderstr. (1), *Mniochloa* Chase (1), *Olyra* L. (24), *Parodiolyra* Soderstr. & Zuloaga (5), *Piresia* Swallen (5), *Piresiella* Judz., Zuloaga & Morrone (1), *Raddia* Bertol. (9), *Raddiella* Swallen (8), *Rehia* Fijten (1), *Reitzia* Swallen (1), *Sucrea* Soderstr. (3).

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References

- Ades, G. 1999. Important discovery of lesser bamboo bat roosting site in Hong Kong. *Porcupine!*, 19, 22.
- Agro Vitro. 2012. <http://www.bambooinvitro.com/about-bamboo-in-vitro-costa-rica-tissue-technology-production>
- Areta, J.I.; Bodrati, A. 2008. Comportamiento, identificación y relación con la floración de cañas del espiguero negro (*Tiaris fuliginosa*) en Misiones, Argentina. *Hornero*, 23(2), 77-86.
- Areta, J.I.; Bodrati, A.; Cockle, K. 2009. Specialization on *Guadua* bamboo seeds by three bird species in the Atlantic forest of Argentina. *Biotropica*, 10.1111/j.1744-7429.2008.00458.x.
- Bahadur, K.N. 1979. Taxonomy of bamboos. *Indian Journal of Forestry*, 2(3), 222-241.
- Bamboo Biodiversity: <http://www.eeob.iastate.edu/research/bamboo/>
- Bamboo Phylogeny Group. 2006. The Bamboo Phylogeny Project. *BAMBOO*, The Magazine of the American Bamboo Society, December 2006, 27(6), 11-14.
- Barkley, N.A.; Newman, M.L.; Wang, M.L.; Hotchkiss, M.W.; Pederson, G.A. 2005. Assessment of the genetic diversity and phylogenetic relationships of a temperate bamboo collection by using transferred EST-SSR markers. *Genome*, 48, 731-737.
- Bouchenak-Khelladi, Y.; Salamin, N.; Savolainen, V.; Forest, F.; van der Bank, M.; Chase, M.W.; Hodkinson, T.R. 2008. Large multi-gene phylogenetic trees of the grasses (Poaceae): Progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution*, 47, 488-505.
- Brailovsky, H. 1988. Hemiptera—Heteroptera de Mexico. XXXIX. Descripción de una tribu nueva, un género nuevo y una especie nueva de coreidos recolectados en bamboo (*Bambusa* sp.) (Coreidae-Coreinae). *Anales del Instituto de Biología UNAM* 58, ser. Zool. 1, 155-164.
- Bystriakova, N.; Kapos, V.; Lysenko, I. 2004. Bamboo biodiversity: Africa, Madagascar and the Americas. UNEP-WCMC/INBAR, Biodiversity Series 19. Swaingrove Imaging, United Kingdom. <http://www.ourplanet.com/wcmc/19.html>
- Bystriakova, N.; Kapos, V.; Stapleton, C.; Lysenko, I. 2003. Bamboo biodiversity: information for planning conservation and management in the Asia-Pacific region. UNEP-WCMC/INBAR, Biodiversity Series 14. Swaingrove Imaging, United Kingdom. <http://www.ourplanet.com/wcmc/14.html>
- Caccia, F.D.; Chaneton, E.J.; Kitzberger, T. 2009. Direct and indirect effects of understorey bamboo shape tree regeneration niches in a mixed temperate forest. *Oecologia*, 161, 771-780.
- Clark, L.G. 1997. Bamboos: the centerpiece of the grass family. In Chapman, G.P., ed., *The bamboos*. Pp. 237-248. Academic Press, London, England.
- Clark, L.G.; Dransfield, S.; Triplett, J.; Sánchez-Ken, J.G. 2007. Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). *Aliso*, 23, 315-332.
- Clayton, W. D.; Renvoize, S.A. 1986. *Genera Graminum, grasses of the world*. Her Majesty's Stationery Office, London, England. Pp. 389.
- Conover, A. 1994. A new world comes to life, discovered in a stalk of bamboo. *Smithsonian Magazine*, October, 120-129.
- Das, M.; Bhattacharya, S.; Basak, J.; Pal, A. 2007. Phylogenetic relationships among the bamboo species as revealed by morphological characters and polymorphism analyses. *Biologia Plantarum*, 51, 667-672.
- Davidse, G. 1987. Fruit dispersal in the Poaceae. In Soderstrom, T.R., Hilu, K.W., Campbell, C.S., Barkworth, M.E., eds., *Grass systematics and evolution*. Pp. 143-155. Smithsonian Institution Press, Washington, D. C.
- Dierenfeld, E.S. 1997. Chemical composition of bamboo in relation to giant panda nutrition. In Chapman, G.P., ed., *The bamboos*. Pp. 205-211. Academic Press, London, England.
- Duvall, M.R.; Davis, J.I.; Clark, L.G.; Noll, J.D.; Goldman, D.H.; Sánchez-Ken, J.G. 2007. Phylogeny of the grasses (Poaceae) revisited. *Aliso*, 23, 237-247.
- Emmons, L. 1997. *Neotropical rainforest mammals: A field guide*. 2nd ed. University of Chicago Press, Chicago, IL, U.S.A. Pp. 307.
- Fisher, A.; Triplett, J.K.; Ho, C.-S.; Schiller, A.; Oltrogge, K.; Schroder, E.; Kelchner, S.; Clark, L.G.

2009. Paraphyly in the Chusqueinae (Poaceae: Bambusoideae: Bambuseae). *Systematic Botany*, 34, 673-683.
- Franklin, D.C.; Bowman, D.M.J.S. 2004. A multi-scale biogeographical analysis of *Bambusa arnhemica*, a bamboo from monsoonal northern Australia. *Journal of Biogeography*, 31, 1335-1353.
- Friar, E.; Kochert, G. 1994. A study of genetic variation and evolution of *Phyllostachys* (Bambusoideae: Poaceae) using nuclear restriction fragment length polymorphisms. *Theoretical and Applied Genetics*, 89, 265-270.
- Gielis, J.; Everaert, I.; De Loose, M. 1997. Genetic variability and relationships in *Phyllostachys* using random amplified polymorphic DNA. In Chapman, G.P. ed., *The Bamboos*. Pp. 107-124. Academic Press, London, England.
- Goh, W.-L.; Chandran, S.; Kamiya, K.; Wong K.M. 2011. A natural hybrid between *Dendrocalamus pendulus* and *Gigantochloa scortechinii* (Poaceae: Bambusoideae: Bambuseae) in Peninsular Malaysia. *Gardens' Bulletin Singapore*, 62(2), 223-238.
- Goh, W.-L.; Chandran, S.; Lin, R.-S.; Xia, N.-H.; Wong K.M. 2010. Phylogenetic relationships among Southeast Asian climbing bamboos (Poaceae: Bambusoideae) and the *Bambusa* complex. *Biochemical Systematics and Ecology*, 38(4), 764-773.
- Grass Phylogeny Working Group II. 2012. New grass phylogeny resolves deep evolutionary relationships and discovers C₄ origins. *New Phytologist*, 193(2), 304-312.
- Grass Phylogeny Working Group. 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). *Annals of the Missouri Botanical Garden*, 88, 373-457.
- Guala, G.F.; Bogler, D.; Sadle, J.; Francisco-Ortega, J. 2000. Molecular evidence for polyphyly in the genus *Apoclada* (Poaceae: Bambusoideae). *Bamboo Science and Culture*, 14, 15-20.
- Guo, Z.-H.; Chen, Y.-Y.; Li, D.-Z. 2002. Phylogenetic studies on the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae) based on ITS sequence data. *Molecular Phylogenetics and Evolution*, 22, 20-30.
- Guo, Z.-H.; Chen, Y.-Y.; Li, D.-Z.; Yang, J.-B. 2001. Genetic variation and evolution of the alpine bamboos (Poaceae: Bambusoideae) using DNA sequence data. *Journal of Plant Research*, 114, 315-322.
- Guo, Z.-H.; Li, D.-Z. 2004. Phylogenetics of the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae): inference from the sequences of GBSSI gene and ITS spacer. *Molecular Phylogenetics and Evolution*, 30, 1-12.
- Hidalgo, N.P.; Martínez-Torres, D.; Collantes-Alegre, P.M.; Villalobos Muller, W.; Nieto Nafria, J.M. 2012. A new species of *Rhopalosiphum* (Hemiptera, Aphididae) on *Chusquea tomentosa* (Poaceae, Bambusoideae) from Costa Rica. *ZooKeys*, 166, 59-73.
- Higgins, K.L.; Coley, P.; Kursar, T.; Arnold, A.E. 2010. Culturing and direct PCR suggest prevalent host-generalism among diverse fungal endophytes of tropical forest grasses. *Mycologia*, 103, 247-260.
- Hisamoto, Y.; Kashiwagi, H.; Kobayashi, M. 2008. Use of flowering gene *FLOWERING LOCUS T* (*FT*) homologs in the phylogenetic analysis of bambusoid and early diverging grasses. *Journal of Plant Research*, 121, 451-461.
- Hodkinson, T.R.; Ni Chonghaile, G.; Sungkaew, S.; Chase, M.W.; Salamin, N.; Stapleton, C.M.A. 2010. Phylogenetic analyses of plastid and nuclear DNA sequences indicate a rapid, late Miocene radiation of the temperate bamboo tribe (Poaceae: Bambusoideae). *Plant Ecology & Diversity*, 3(2), 109-120.
- Hodkinson, T.R.; Renvoize, S.A.; Ni Chonghaile, G.; Stapleton, C.M.A.; Chase, M.W. 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). *Journal of Plant Research*, 113, 259-269.
- Ishii, H.T.; Kobayashi, T.; Uemura, S.; Takahashi, K.; Hanba, Y.T.; Sumida, A.; Hara, T. 2008. Removal of understory dwarf bamboo (*Sasa kurilensis*) induces changes in water-relations characteristics of overstory *Betula ermanii* trees. *Journal of Forest Research*, 13, 101-109.
- Jaksic, F.M.; Lima, M. 2003. Myths and facts on ratadas: Bamboo blooms, rainfall peaks and rodent outbreaks in South America. *Austral Ecology*, 28, 237-251.

- Judziewicz, E.J.; Clark, L.G. 2007. Classification and biogeography of New World Grasses: Anomochlooideae, Pharoideae, Ehrhartoideae, and Bambusoideae. *Aliso*, 23, 303-314.
- Judziewicz, E.J.; Clark, L.G.; Londoño, X.; Stern, M.J. 1999. American bamboos. Smithsonian Institution Press, Washington, D. C., U.S.A. Pp. 392.
- Judziewicz, E.J.; Sepsenwol, S. 2007. The world's smallest bamboo: *Raddiella vanessiae* (Poaceae: Bambusoideae: Olyreae), a new species from French Guiana. *Journal of the Botanical Research Institute of Texas*, 1, 1-7.
- Kelchner, S.A.; Clark, L.G. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution*, 8, 385-397.
- Kobayashi, M. 1997. Phylogeny of world bamboos analysed by restriction fragment length polymorphisms of chloroplast DNA. In Chapman, G.P. ed., *The bamboos*. Pp. 227-234. Academic Press, London, England.
- Kovac, D. 1993. Abundant species in bamboo stems. Model for complex ecosystems. Reports of the DFG 3/93 German Research, 21-24.
- Larpkern, P.; Moe, S.R.; Totland, O. 2010. Bamboo dominance reduces tree regeneration in a disturbed tropical forest. *Oecologia*, 165, 161-168.
- Lentino, M.; Restall, R. 2003. A new species of *Amaurospiza* blue seedeater from Venezuela. *The Auk*, 120(3), 600-606.
- Lewis, A.; Pande, K.K.; Tewari, S.K.; Gahalain, S.S.; Manikandan, R.; Sah, P. 2010. A comprehensive analysis of genetic divergence in Indian Bamboo. *New York Science Journal*, 3, 90-93.
- Li, D.-Z. 1997. The Flora of China Bambusoideae project—problems and current understanding of bamboo taxonomy in China. In Chapman, G.P. ed., *The bamboos*. Pp. 61-82. Academic Press, London, England.
- Li, D.-Z.; Xue, J.-R. 1997. The biodiversity and conservation of bamboos in Yunnan, China. In Chapman, G.P. ed., *The bamboos*. Pp. 83-94. Academic Press, London, England.
- Li, X.-L.; Lin, R.-S.; Fung, H.-L.; Qi, Z.-X.; Song, W.-Q.; Chen, R.-Y. 2001. Chromosome numbers of some bamboos native in or introduced to China. *Acta Phytotaxonomica Sinica*, 39(5), 433-442.
- Loh, J.P.; Kiew, R.; Set, O.; Gan, L.H.; Gan, Y.-Y. 2000. A study of genetic variation and relationships within the bamboo subtribe Bambusinae using amplified fragment length polymorphism. *Annals of Botany*, 85, 607-612.
- Louton, J.; Gelhaus, J.; Bouchard, R. 1996. The aquatic macrofauna of water-filled bamboo (Poaceae: Bambusoideae: *Guadua*) internodes in a Peruvian tropical lowland forest. *Biotropica*, 28, 228-242.
- Marchesini, V.A.; Sala, O.E.; Austin, A.T. 2009. Ecological consequences of a massive flowering event of bamboo (*Chusquea culeou*) in a temperate forest of Patagonia, Argentina. *Journal of Vegetation Science*, 20, 424-432.
- Martins, S.V.; Júnior, R.C.; Rodrigues, R.R.; Gandolfi, S. 2004. Colonization of gaps produced by death of bamboo clumps in a semideciduous mesophytic forest in southeastern Brazil. *Plant Ecology*, 172, 121-131.
- McClure, F.M. 1966. *The bamboos: A fresh perspective*. Harvard University Press, Cambridge, MA, U.S.A. Pp. 347.
- Medway, Lord. 1964. The Marmoset Rat. *Malayan Nature Journal* 18, 104-110.
- Medway, Lord. 1969. *The wild mammals of Malaya and offshore islands including Singapore*. Oxford University Press, Kuala Lumpur.
- Montti, L.; Campanello, P.I.; Gatti, M.G.; Blundo, C.; Austin A.T.; Sala, O.E.; Goldstein, G. 2011. Understory bamboo flowering provides a very narrow light window of opportunity for canopy-tree recruitment in a neotropical forest of Misiones, Argentina. *Forest Ecology and Management*, 262, 1360-1369.
- Mutschler, T.; Tan, C.L. 2003. *Hapalemur*, bamboo or gentle lemurs. In S. M. Goodman, S.M.; Benstead, J.P. eds., *The natural history of Madagascar*. Pp. 1324-1329. The University of Chicago Press, Chicago, IL, U.S.A.

- Nayak, S.; Rout, G.R.; Das, M. 2003. Evaluation of the genetic variability in bamboo using RAPD markers. *Plant Soil Environment*, 49, 24-28.
- Ní Chonghaile, G. 2002. Systematics of the woody bamboos (Tribe Bambuseae). Unpublished Ph.D. thesis, University of Dublin, Trinity College, Ireland.
- Numata, M. 1979. The structure and succession of bamboo vegetation. In M. Numata ed., *Ecology of grasslands and bamboolands in the world*. Pp. 237-257. VEG Gustav Fischer Verlag, Jena, Germany.
- Ohrnberger, D., 1999. *The bamboos of the world*. Elsevier, Amsterdam, the Netherlands. Pp. 585.
- Oliveira, R.P.; Clark, L.G. 2009. A tiny new species of *Diandrolyra* (Poaceae, Bambusoideae, Olyreae), with notes on the systematics of the genus. *Novon*, 19, 209-214.
- Oliveira-Filho, A.T.; Vilela, E.A.; Gavilanes, M.L.; Carvalho, D.A. 1994. Effect of flooding regime and understorey bamboos on the physiognomy and tree species composition of a tropical semideciduous forest in Southeastern Brazil. *Vegetatio*, 113, 99-124.
- Oprins Plant. 2012. <http://flandersbio.be/life-sciences-database/oprins-plant>
- Pattanaik, S.; Hall, J.B. 2011. Molecular evidence for polyphyly in the woody bamboo genus *Dendrocalamus* (subtribe Bambusinae). *Plant Systematics and Evolution*, 291, 59-67.
- Peng, S.; Yang, H.-Q.; Li, D.-Z. 2008. Highly heterogenous generic delimitation within the temperate bamboo clade (Poaceae: Bambusoideae): evidence from GBSSI and ITS sequences. *Taxon*, 57, 799-810.
- Poulsen, A.D.; Balslev, H. 1991. Abundance and cover of ground herbs in an Amazonian rain forest. *Journal of Vegetation Science*, 2, 315-322.
- Ramanayake, S.M.S.D.; Meemaduma, V.N.; Weerawardene, T.E. 2007. Genetic diversity and relationships between nine species of bamboo in Sri Lanka, using Random Amplified Polymorphic DNA. *Plant Systematics and Evolution*, 269, 55-61.
- Ravaloharimanitra, M.; Ratolojanahary, T.; Rafalimandimby, J.; Rajaonson, A.; Rakotonirina, L.; Rasolofoharivelo, T.; Ndriamiary, J.N.; Andriambololona, J.; Nasoavina, C.; Fanomezantsoa, P.; Rakotoarisoa, J.C.; Youssouf; Ratsimbazafy, J.; Dolch, R.; King, T. 2011. Gathering local knowledge in Madagascar results in a major increase in the known range and number of sites for critically endangered greater bamboo lemurs (*Prolemursimus*). *International Journal of Primatology*, 32, 776-792.
- Ridley, H.N. 1908. Bats in a bamboo. *Journal of the Straits Branch, Royal Asiatic Society*, 50, 103-104.
- Ruiz-Sanchez, E.; Sosa, V. 2010. Delimiting species boundaries within the Neotropical bamboo *Otatea* (Poaceae: Bambusoideae) using molecular, morphological and ecological data. *Molecular Phylogenetics and Evolution*, 54, 344-356.
- Ruiz-Sanchez, E.; Sosa, V.; Mejía-Saules, M.T. 2008. Phylogenetics of *Otatea* inferred from morphology and chloroplast DNA sequence data, and recircumscription of Guaduinae (Poaceae: Bambusoideae). *Systematic Botany*, 33, 277-283.
- Sage, R.D.; Pearson, O.P.; Sanguinetti, J.; Pearson, A.K. 2007. Ratada 2001: A rodent outbreak following the flowering of bamboo (*Chusquea culeou*) in southwestern Argentina. In Kelt, D.A.; Lessa, E.P.; Salazar-Bravo, J.; Patton, J.L., eds., *The Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson*. University of California Publications in Zoology, 134, 177-224.
- Schaller, G.B. 1994. *The last panda*. The University of Chicago Press, Chicago, IL, U.S.A. Pp. 299.
- Sharma, R.K.; Gupta, P.; Sharma, V.; Sood, A.; Mohapatra, T.; Ahuja, P.S. 2008. Evolution of rice and sugarcane SSR markers for phylogenetic and genetic diversity analyses in bamboo. *Genome*, 51, 91-103.
- Singleton, G.R.; Belmain, S.R.; Brown, P.R.; Hardy, B. 2010. Rodent outbreaks: Ecology and impacts. International Rice Research Institute, Manila, Philippines. Pp. 289.
- Soderstrom, T.R. 1981. Some evolutionary trends in the Bambusoideae. *Annals of the Missouri Botanical Garden*, 68, 15-47.
- Soderstrom, T.R.; Calderón, C.E. 1971. Insect pollination in tropical rain forest grasses. *Biotropica*, 3, 1-6.

- Soderstrom, T.R.; Calderón, C.E. 1979. Ecology and phytosociology of bamboo vegetation. In Numata, M., ed., Ecology of grasslands and bamboolands in the world. Pp. 223-236. VEG Gustav Fischer Verlag, Jena, Germany.
- Soderstrom, T.R.; Ellis, R.P. 1987. The position of bamboo genera and allies in a system of grass classification. In Soderstrom, T.R.; Hilu, K.; Campbell, C.; Barkworth, M., eds., Grass systematics and evolution. Pp. 225-238. Smithsonian Institution Press, Washington, D.C., U.S.A.
- Soderstrom, T.R.; Judziewicz, E.J.; Clark, L.G. 1988. Distribution patterns of neotropical bamboos. In Vanzolini, P.E.; Heyer, R.E., eds., Proceedings of a Workshop on Neotropical Distribution Patterns. Proceedings of a Workshop on Neotropical Distribution, Rio de Janeiro, Brazil, 12-16 January 1987. Academia Brasileira de Ciencias, Rio de Janeiro. Pp. 121-157.
- Sun, Y.; Xia, N.; Lin, R. 2005. Phylogenetic analysis of *Bambusa* (Poaceae: Bambusoideae) based on Internal Transcribed Spacer sequences of nuclear ribosomal DNA. *Biochemical Genetics*, 43, 603-612.
- Sun, Y.; Xia, N.; Stapleton, C.M.A. 2006. Relationships between *Bambusa* species (Poaceae, Bambusoideae) revealed by random amplified polymorphic DNA. *Biochemical Systematics and Ecology*, 34, 417-423.
- Sungkaew, S.; Stapleton, C.M.A.; Salamin, N.; Hodkinson, T.R. 2009. Non-monophyly of the woody bamboos (Bambuseae; Poaceae): a multi-gene region phylogenetic analysis of Bambusoideae s.s. *Journal of Plant Research*, 122, 95-108.
- Takahashi, K.; Watano, Y.; Shimizu, T. 1994. Allozyme evidence for intersectional and intergeneric hybridization in the genus *Sasa* and its related genera (Poaceae; Bambusoideae). *Journal of Phytogeography and Taxonomy*, 42, 49-60.
- Takahashi, M.; Furusawa, H.; Limtong, H.; Sunanthapongsuk, P.; Marod, V.; Panuthai, S. 2007. Soil nutrient status after bamboo flowering and death in a seasonal tropical forest in western Thailand. *Ecological Research*, 22, 160-164.
- Triplett, J.K.; Clark, L.G. 2010. Phylogeny of the temperate woody bamboos (Poaceae: Bambusoideae) with an emphasis on *Arundinaria* and allies. *Systematic Botany*, 35, 102-120.
- Triplett, J.K.; Oltrogge, K.; Clark, L.G. 2010. Phylogenetic relationships within *Arundinaria* (Poaceae: Bambusoideae) in North America. *American Journal of Botany*, 97, 471-492.
- Tyrrell, C.D.; Santos-Gonçalves, A.P.; Londoño, X.; Clark, L.G. In review. Molecular phylogeny of the arthrotyliidioid bamboos (Poaceae: Bambusoideae: Bambuseae: Arthrotyliidiinae) and new genus *Didymogonyx*. *Molecular Phylogenetics and Evolution*.
- Watanabe, M.; Ito, M.; Kurita, S. 1994. Chloroplast DNA phylogeny of Asian bamboos (Bambusoideae, Poaceae) and its systematic implication. *Journal of Plant Research*, 107, 253-261.
- Widmer, Y. 1997. Life history of some *Chusquea* species in old-growth oak forest in Costa Rica. In Chapman, G.P., ed., The bamboos. Pp. 17-31. Academic Press, London, England.
- Wong, K.M. 1989. Current and Potential Uses of Bamboos in Peninsular Malaysia. *Journal of the American Bamboo Society*, 7(1-2), 1-15.
- Wong, K.M. 1991. The growth architecture and ecology of some tropical bamboos. *Journal of the American Bamboo Society*, 8, 31-46.
- Wong, K.M. 1995. The bamboos of Peninsular Malaysia. Forest Research Institute Malaysia, Kuala Lumpur.
- Wong, K.M. 2004. Bamboo, the amazing grass. A guide to the diversity and study of bamboos in Southeast Asia. International Plant Genetic Resources Institute & University of Malaya, Kuala Lumpur. Pp. 80.
- Wong, K.M.; Low, Y.W. 2011. Hybrid zone characteristics of the intergeneric hybrid bamboo × *Gigantocalamus malpenensis* (Poaceae: Bambusoideae) in Peninsular Malaysia. *Gardens' Bulletin Singapore*, 63(1&2), 375-384.
- Wu, Z.-Q.; Ge, S. 2012. The phylogeny of the BEP clade in grasses revisited: evidence from the whole-genome sequences of chloroplasts. *Molecular Phylogenetics and Evolution*, 62, 573-578.
- Yang, H.-Q.; Peng, S.; Li, D.-Z. 2007. Generic delimitations of *Schizostachyum* and its allies (Gramineae: Bambusoideae) inferred from GBSSI and *trnL-F* sequence phylogenies. *Taxon*, 56, 45-54.

- Yang, H.-Q.; Yang, J.-B.; Peng, Z.-H.; Gao, J.; Yang, Y.-M.; Peng, S.; Li, D.-Z. 2008. A molecular phylogenetic and fruit evolutionary analysis of the major groups of the paleotropical woody bamboos (Gramineae: Bambusoideae) based on nuclear ITS, GBSSI gene and plastid *trnL-F* DNA sequences. *Molecular Phylogenetics and Evolution*, 48, 809-824.
- Yang, J.-B.; Yang, H.-Q.; Li, D.-Z.; Wong, K.M.; Yang, Y.-M. 2010. Phylogeny of *Bambusa* and its allies (Poaceae: Bambusoideae) inferred from nuclear GBSSI gene and plastid *psbA-trnH*, *rpl32-trnL* and *rps16* intron DNA sequences. *TAXON* 59, 1102-1110.
- Yang, Y.; Wang, K.; Pei, S.; Hao, J. 2004. Bamboo diversity and traditional uses in Yunnan, China. *Mountain Research and Development*, 24(2), 157-165.
- Zeng, C.-Z.; Zhang, Y.-X.; Triplett, J.K.; Yang, J.-B.; Li, D.-Z. 2010. Large multi-locus plastid phylogeny of the tribe Arundinarieae (Poaceae: Bambusoideae) reveals ten major lineages and low rate of molecular divergence. *Molecular Phylogenetics and Evolution*, 56, 821-839.
- Zhang, W.P.; Clark, L.G. 2000. Phylogeny and classification of the Bambusoideae (Poaceae). In Jacobs, S.W.L.; Everett, J. eds. *Grasses: Systematics and Evolution*. Pp. 35-42. CSIRO, Melbourne, Australia.
- Zhou, G.; Meng, C.; Jiang, P.; Xu, Q. 2011. Review of carbon fixation in bamboo forests in China. *Botanical Review*, DOI 10.1007/s12229-011-9082-z.
- Zhou, M.-B.; Zhong, H.; Zhang, Q.-H.; Tang, K.-X.; Tang, D.-Q. 2010. Diversity and evolution of *Ty1-copia* retroelements in representative tribes of Bambusoideae subfamily. *Genetica*, 138, 861-868.
- Zhuge, Q.; Ding, Y.; Xu, C.; Zou, H.; Huang, M.; Wang, M. 2005. A preliminary analysis of phylogenetic relationships of *Arundinaria* and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (*trnL-F* intergenic spacer). *Journal of Forestry Research*, 16(1), 5-8.
- Zuloaga, F.O.; Judziewicz, E.J. 1991. A revision of *Raddiella* (Poaceae: Olyreae). *Annals of the Missouri Botanical Garden*, 78, 928-941.
- Zuloaga, F.O.; Morrone, O.; Judziewicz, E.J. 1993. Endemic herbaceous bamboo genera of Cuba. *Annals of the Missouri Botanical Garden*, 80, 846-861.

Footnote

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KEYNOTE LECTURE

A personal reflection on 60 years of bamboo passion and work

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Abstract

This paper offers a glimpse on my 60 years involvement in bamboo research during various professional stages:

1951-52 Institute for Plant Growth, Düsseldorf; 1952-1953 Wood Preservation Industry, Mannheim; 1953-59 Institute for Forest Botany, University Freiburg; 1959-1963 Institute for Forest Botany, University München; since 1963 Institute for Wood Biology, University Hamburg.

Starting from incidental studies on the structure of bamboo culms over systematic work on improving bamboo preservation, on structural details and other properties of the culm resulted in international bamboo missions in a number of countries around the world. Some projects are briefly described.

The references listed can provide further information.

Keywords

Bamboo, Anatomy, Biology, Protection, National and International Projects.

1. The begin

My first contact with bamboo dates back to 1951, when I was involved in a trial use of bamboo culms imported especially from Indonesia to be used as pit props in the coal mines of the Ruhr Valley. Six years after the end of the 2nd World War, there was a severe timber shortage in Germany, because of the compulsory export of wood to France and the UK as part of “reparation payments”. Therefore, the imported bamboo was to be tested as an alternative to timber. However, when used in mine shafts as support structures, the culms crushed under pressure between the nodes - without emitting sounds to warn the miners to escape.

Having such exotic material at hand, led to its integration into ongoing studies on the fine structure of wood. I had the unique chance to use an electron microscope in Düsseldorf at the Institute for Electron Microscopy under Prof. von Borries, one of the co-inventors of this fascinating research tool. At that time, the electron microscope opened up a miraculous micro-cosmos. Thus, some bamboo samples were investigated, but the photos filed away (Fig.1) (reference 1). Few years later they were looked at again, when a prominent Indian scientist on wood preservation, Dr. A. Purushotham, came to Germany during an international fact finding mission. I told him about my industrial work on the treatment of spruce by creosote, whereupon he smiled, commenting that his special concern was bamboo as well. So the slumbering electron micrographs from 1951/52 came back to light. He was thrilled, stating that such knowledge could help to solve problems on bamboo preservation, and he asked the Food and Agriculture Organisation (FAO) of the UN for a consultancy on bamboo preservation.

So it happened that in December 1957 I arrived in India together with my wife Katrin as house guest of the family Puroshotham (Fig. 2). To the astonishment of the Indian experts, I was just 31 years old and neither had seen any bamboo culms in their natural environment, nor had I read any relevant literature, which in any case would hardly have been available in Europe by that time. Furthermore, communication was initially difficult since my English was poor, my Latin and Greek from school not very helpful, but I was assisted by my wife’s language skill (Fig. 3). I worked at the famous Forest Research Institute in Dehra Dun on preservation techniques for bamboo until May 1958 (Fig. 4). The main task was to find an improvement of the sap-replacement method for bamboo, which promised a good protection, but did not work in a satisfactory manner with bamboo (Fig. 5). But after a few months the treatment was improved (2).

The Indian bamboo project was the starting point of my scientific bamboo career. It has led to ten further assignments in India concerning various bamboo and timber projects, also on the preservation of cooling towers. The last Indian project was an UNDP mission 2003, 2004 at the “Cane and Bamboo Development Centre” (CBTC) at Guwahati, North Eastern Region, India, with Kamesh Salam as project leader (Figs. 6, 7). Its task was to improve the regional bamboo-housing situation and to develop an industrial utilization of the regional vast bamboo with further work by Jörg Stamm (3, 4, 5).

The experiences gained in India 1955-1958 led in the same year to a further FAO mission to Indonesia on wood preservation (Fig. 8) (6), immediately followed by work at the Forest Products Research Division of the CSIRO, Melbourne, Australia, with Dr. Dadswell and Dr. Wardrop, for anatomical studies, also on bamboo from India. This resulted in the first bamboo paper of over 100 to follow (Fig. 9), (7). On the return via the USA some of the results, also on bamboo were lectured at the new Centre

of Wood Research at the University Syracuse, New York State, before reaching home just for Christmas.

The next assignment on bamboo was 1968 in East Pakistan, now Bangladesh (8).

The following reflections on bamboo activities have to be concentrated on some general areas, like culm structure, biology and preservation so that not all projects and countries involved in my bamboo relations can be mentioned.

2. Culm structure

The mission in India and having realized the great importance of bamboo for the welfare of the people urged me to continue work on bamboo, beside my ongoing research on wood structure, pathology and preservation. In the following years main emphasis was placed on laboratory work of the various structural details of the bamboo culm. The first studies, still in Freiburg 1959, concerned the general structure of culm tissue of two species brought from India (9). The later permanent position at Hamburg University from 1963 provided the opportunity to extend the bamboo research in constructive cooperation with colleagues, students and guests. At first, comprehensive studies were done on the variability of the fibres and on the arrangement of vascular bundles with their significance for classification in cooperation with Dietger Grosser (Figs.10, 11). The results on the vascular bundle types are widely used for characterising bamboo tissue; an extended version of the arrangement and classification was presented at the Chiang Mai Bamboo Congress in 2000, still to be finalized by Dr. Grosser (10, 11, 12, 13, 14, 15).

A new dimension of knowledge was obtained by the electron microscope, together with Narayan Parameswaran, until his tragic death in 1985 (Fig. 12). It concerned the manifold fine structural details of the culm tissue (16, 17, 18, 19, 20, 21, 22, 23). Following studies with Yulong Ding, Nanjing Forestry University (Fig. 13) clarified the nodal structure of the bamboo culm and has led to an instructive three-dimensional perspective. Further investigations on bamboo rhizomes showed the presence of air canals in the cortex, an important criterion for their growth in flooded areas (Fig. 14), (24,25, 26).

Structural changes occur during the lifetime of a bamboo culm due to ageing, and also by wounding. These fields were elaborated with Gudrun Weiner (Fig.15), beside her comprehensive rattan contributions. During the first years, the cell walls of fibres and parenchyma thicken, important for their adequate use (Fig. 16), (27). Symptoms of wounding and senescence consist mainly by occlusion of vessels and sieve tubes by plugs, slime, and additional wall layers (Fig.17), (28, 29, 30, 31, 32, 33).

In addition, various aspects of culm characteristics of Malayan bamboos were studied in cooperation with Abd. Latif Mohmod from FRIM, Malaysia (Fig. 18), (34, 35, 35a). The manifold relations of the bamboo structure on its properties and utilization were presented at several conferences and contributions (36, 38, 39, 40, 41 42, 43, 42, 43).

The state of knowledge on the culm structure about 15 years ago was summarized in the INBAR Technical Report No. 18 (Fig. 19), (44).

3. Culm biology

One of the striking phenomena of bamboo biology is the rapid expansion of the growing culm, often leading to the assumption of an extraordinary potential for carbon sequestration. Some bigger bamboo, such as *Guadua*, may produce about 500 cm³ wall substance per day (Fig. 20), (45).

However, the impressive growth of a young culm does not originate from its own photosynthesis with carbon capture, but is based on the carbohydrates produced by the previous culms to be stored in the culms and the rhizome system. At the beginning of the growing season, this energy is mobilized and transported to the growing culm top. The soluble sugar content of up to 50% results in a high osmotic pressure for the telescope-like expansion of the culm tip (Fig. 21) (46).

In Tanzania this osmotic pressure is utilized to collect the flowing sap from cut culms for a rather unusual purpose. Due to its high sugar content, the sap tastes at first pleasant, but soon after turns alcoholic by fermentation, thus providing a most welcome way for getting drunk (Fig. 22). Repeating the cut, one culm can produce up to 10 litres of sap. Such use of bamboo provides many locals with occasional “happiness” (47) and a danger for the traffic. Fortunately, this is only known for *Oxythenanthera braunii* in Iringa State, Tanzania. At the last XXIII IUFRO World Congress August 2010 in Korea investigations about harvesting and utilization of bamboo sap were reported, but with no details about a general use (48).

The impressive growth of bamboo culms is often forcefully advocated for a special ability of bamboo to store CO₂. However, the individual culm with its amount of CO₂ has only a limited lifetime of 7-10 years (Fig.23). At its death, the incorporated carbohydrates will biologically decompose and the CO₂ will be released into the atmosphere again (49, 50, 51).

A serious issue for the CO₂ is also the flowering of bamboos, the gregarious flowering of most species occurs generally on a worldwide scale which is followed by their death (Figs. 24, 25,26),(52). The consequence is a massive release of the stored carbon by the decomposing culms. Also to mention here are the large fires in the wild bamboo forests, leading to an explosive increase of CO₂.

Consequently, for a longer storage of the CO₂ in bamboo culms, capture of the CO₂ in bamboo products is one possibility, albeit limited. Used for constructions, furniture, handicrafts or pulp, such products, however, will often not last very long. Bamboo has a low natural durability against fungi and insects (Figs.27, 28). Recent work in our institute contributed to the protection of bamboo material (53, 54, 55).

4. Preservation

The bamboo preservation in India, 1957/58 on the initiative of Dr. Ashtakala Purushotham started my “bamboo life”.

Bamboo culms are easily attacked by insects and affected by white-, brown- and soft rot; they will be decomposed after some storage time (Fig. 29). Earlier, a treatment by pressure was often undertaken, quite successfully, but was not considering the resulting environmental damage (Figs. 30, 31). It is the merit of Dr. Purushotham to introduce the “Sap-Replacement Method”, applied in Europe as Boucherie Method for the treatment of fresh conifer stems. A proper treatment is most effective, environmentally safe and the equipment can be transported even to a bamboo forest for immediate application (Fig.32). This was the splendid idea, but insufficient results with bamboo brought me to India to address the problems involved. If the treatment is well done, the inner most endangered culm

tissue is protected, whereas the skin remains clean, important for further handling. By applying pressure into the bottom a steady stream replaces the water in the vessels and the preservative is flowing out from the top. (Fig.33). It is sufficient to say that one important part is a valve at the pressure cap to release the air before the preservative is pressed in (Fig.34). To assure a good permanent flow of the preservative through the culm, its high moisture content is crucial. This was investigated in India with P.N.Grover (56) and in Malaysia with Abd. Latif Mohmod (57). Nevertheless, difficulties might always occur, which brought me to Thailand in 2005 to solve those problems (Fig. 35).

As far as preservation techniques for bamboo are concerned, certainly the Environmental Bamboo Foundation, EBF, Bali, Indonesia, has to be mentioned here (Fig. 36). By the “Bamboo Queen” Linda Garland, it was founded 1991 (Fig.37) as the “Environmental Bamboo Foundation” (EBF) in Ubud, Bali, with a wide spectrum for bamboo promotion. For bamboo preservation a new and most efficient method had been developed, the Vertical Soak Diffusion System (VSD). For its application the diaphragms of a fresh culm are punched, except the lowest one. The culms were placed vertically in a covered burn and the internodes to be filled with the preservative up to top (Fig. 38). The preservative then diffuses into the culm tissue. The first, inner layer may have some structural difference, which influence the velocity of diffusion (Fig.39), (58). In 2005, I have also seen the system well working at the SALA – TANUM Company, Thailand (Fig.40). Noteworthy to mention is the high-quality of the operation manual with 26 pages (Fig.41).

A similar system of treatment has been applied in Vietnam since long, called the “Gravity Method. Here only the lacuna of the upper culm internode serves as a reservoir. To ease penetration its inner wall is scraped and at the bottom a round incision made for the diffusion of the preservative. As for the VDS, the internode has to be refilled daily (59).

Details of bamboo preservation were demonstrated at the glamorous 5th International Bamboo Congress 1995 in Ubud, which will be happily remembered by all participants (Figs. 43, 44, 45, 46).

The “Bamboo Preservation Compendium” with Satish Kumar presents a comprehensive overview (INBAR Technical Report No 22, 2003) (Fig.42), (60).

5. International Co-operation

My early work in India and the following laboratory studies in Hamburg have led to numerous scientific contacts. These derived also from consultancies in about 20 countries in Asia, Africa, South-America and Europe for organizations such as FAO, UNIDO, INBAR, UNHABITAT, ITTO, CIFOR, EU and GTZ. Likewise, it resulted in many guests coming for training and research to our institute in Hamburg from the countries where I had worked in (Fig. 47). From these numerous larger and smaller activities only a few can be mentioned here.

5.1 IUFRO and INBAR

The various FAO assignments gave the possibility to inform about the urgent problems on “Bamboo Preservation” at 5th FAO-Conference on Wood Technology in 1963, Madison/USA (61).

To be mentioned first are the bamboo relations with IUFRO and INBAR because of my involvement in their related activities. When elected as President of IUFRO at the Oslo Congress in 1976, - IUFRO by the way is the oldest International Scientific Organization, founded in 1892 in my home town Eberswalde near Berlin - at this Congress my enduring passion for bamboo led to the establishment of

the first international working group on “Protection and Utilization of Bamboo and Related Species”. Dr. Omar Ali, FRI, Chittagong, chaired the first session with already numerous papers on bamboo. At the following XVII IUFRO Congress 1981 in Kyoto (Fig. 48) bamboo had its special visibility, documented as a comprehensive report by Prof. Taykayoshi Higuchi with the key lecture by Dr. Koichiro Ueda on “Bamboo industry in Japan” and 34 additional contributions (Figs. 49,50), (62). The significance of bamboo in Japan was also underlined by a Memorial Planting of *Phyllostachys pubescens* var. *heterocycla* by the Prince and the Princess of Japan. Those first few culms have since developed into an impressive stand (Figs. 51, 52).

At the next XVIII IUFRO Congress in Ljubljana, Yugoslavia. Prof. Wenyu Hsiung and I presented the bamboo situation. And at this IX International Bamboo Congress our dynamic chairman Dr. Jinhe Fu, INBAR, will lead the session.

Among other bamboo events in Japan, the 3rd International Bamboo Congress in Minamata will certainly stay in our memory, with Dr. Masatoshi Watanabe playing an important role. Its bamboo bridge was often used (Figs. 53).

Now a few words about my bamboo path from IUFRO to INBAR: In my first presidential year, 1977, I attended a FAO/IUFRO consultation in Canberra on “Fast Growing Trees”. On the return flight I was seated next to Dr. Gilles Lessard, Associate Director-Forestry of the International Development Research Centre, Canada (IDRC). Of course, I told enthusiastically about my bamboo work and he became quite interested. As a result, IDRC organized a first meeting of the IUFRO Work Group in May 1980 in Singapore, attended by bamboo specialists from 20 countries. Most of them met for the first time, resulting in many new contacts. Following this conference, IDRC initiated several projects and conferences on bamboo and rattan in Asia (Fig. 54) and created in 1985 an organizational entity in their Forestry Program on Bamboo and Rattan. After intensive discussions the name INBAR (International Network for Bamboo and Rattan) was voted for as a milestone for the international development of bamboo and rattan, culminating 1997 in the creation of INBAR by treaty as an intergovernmental, international organization with its headquarter in Beijing. Dr. Cherla Sastry as the IDRC Regional Representative (Fig. 55) was the driving force of this impressive development.

5.2 China-Taiwan

My initial contacts with China were not bamboo specific, but politically related. As President of IUFRO, I was responsible for the organization of its 17th Congress 1981 in Kyoto. It was the first congress in Asia with the intention to strengthen the cooperation among and with the countries of the region. Both China and Taiwan were quite interested in joining IUFRO at this occasion. However, their respective country’s names “People’s Republic of China” and “Republic of China” were not politically acceptable. So, in 1980, I initiated negotiations in Beijing and Taipei for an acceptable solution (Figs. 56, 57). It resulted in an agreement to use only the names “China-Beijing” and “China-Taipei”, and to not show their respective flags. This nomenclature worked also at other occasions. Scientists from both countries, who had been friends or classmates with no communication for a long time, were thus given the opportunity to meet again. For me it was a most emotional moment, since part of my family lived in East Germany and at that time, 1981, no contacts were allowed or foreseen so I could deeply feel the motions. I consider these contacts as a main achievement in my life.

Later on, a number of bamboo symposia and projects were carried out in China, with Prof. Wenyu Hsiung, also as a Concurrent Professor at Nanjing Forestry University (Figs.58, 59) and many visits to follow. At last, in 2005 a consultancy for the ITTO project “Sustainable Management and Utilization

of Sympodial Bamboo” with Prof. Fu Maoyi, Institute of Subtropical Forestry, Fuyang, strengthened the mutual contacts.

An equally strong relationship had developed with colleagues in Taiwan. I remember well the travels with Prof. Shuen Chao Wu to the bamboo forest at Taichung with a house made completely out of bamboo, as also visiting bamboo processing factories and presenting various bamboo lectures (Figs. 60, 61).

5.3 Tanzania

Tanzania had been mentioned before with the region Iringa where the bamboo *Oxytenanthera braunii* can make people happy by consuming its fermented sap (Fig.62). The reason for my visit, however, was an inspection of a bamboo water project, where water was to be transported with a pipeline of 150 km to about 28 villages with 10.000 people (Fig. 63), (63, 64). This was partly done in supported culm halves above ground. Another possibility was tried with full culms, nodal walls interrupted, chemically treated for protection against termites and fungi and buried in soil. At that time, 1986, the buried culms, however were severely degraded (Fig. 64). Furthermore, the treated culms released toxic components into the water to be used by the consumers. Several trials were made, including covering the culm with plastic (Fig. 65).

5.4 La Reunion

In 1993, Yves Crouzet from the Bamboueraie, Prafrance, was requested by the President of the Region La Reunion, near Mauritius, to explore the possibility of building a similar impressive Bambouserie (Fig. 66). Among the many bamboo species, *Nastus borbonicus* was especially attractive with its decorative appearance (Fig. 67). But this endeavour should be planned without any funding by taxpayers. However, due to the non-competitive wages, which were the same as in France, the results were not encouraging. As example, a simple bamboo wall made locally was more expensive than a plastic, durable wall imported from Asia (Fig. 68). So it was suggested to create a cottage industry for handicraft items from bamboo produced at facilities for disabled people and to be sold as souvenirs to tourists (65).

5.5 Ethiopia

Ethiopia also has to be briefly mentioned with about 1 million hectares of bamboo. Only two main species grow in this densely populated country of Africa. The monopodial “high land” bamboo *Arundaria alpina*, in altitudes from 2.200 to 3.000 meters (Figs.69, 70) and the sympodial “lowland” bamboo *Oxytenanthera braunii* from 700-1.800ms (Figs.71, 72). At our project in 1996 the latter was flowering and subsequently dying. The processing by this time was rather poor, with simple tools for furniture to be made in the villages and sold along the street (66, 67, 68, 69, 70, 71).

Noteworthy in the lowland is the general scarcity of firing material. Because there were hardly any trees left and the bamboo loved by the cattle (Fig.73), children had to carry big bundles of bamboo, sufficient only for two days family cooking. The fast burning of bamboo with no glow is well known, and it was tragic to see the misery (Fig.74). In our report 1996 the strengthening of bamboo-charcoal was strongly recommended, but the Central Government saw other priorities. The present activities by INBAR, ITTO and other organizations for building up charcoal production are seen with great relief (Figs.75, 76, 77, 78), (72). Likewise, this concerns now the many activities for the industrial production of panels and related products as by INBAR and for laboratory work on in vitro

propagation of *O. braunii* and *A. alpina* plants by a German company “piccoplant” for culturing in Ethiopia, just to mention a few.

5.6 Colombia and Costa Rica

Colombia and Costa Rica are known as countries, where bamboo has been used for ages. Of interest are the fine city houses in Manizales, Colombia, which were demonstrated already in 1981 at the Premier Simposio Latino americano Sobre Bambu, chaired by Oscar Hidalgo (Fig. 79, 80). To increase the use of bamboo, the International Organisation for Human Settlements started the project FUNBAMBU in 1987 in Costa Rica. Dr. Jules Janssen acted as supervisor with me as consultant with visits in 1988, 1989, 1990 and 1992 (Fig.81, 82). The intention was to build more than 2.000 houses in rural areas to counteract the fast disappearing timber resources. Failures in construction had to be overcome, but quite a number of comfortable houses were the result. Great efforts were made (Figs. 83, 84, 85, 86, 87) but the project came to an end in 1999, because the new Government had other priorities.

Nevertheless, what will stay in memory are several impressive Bamboo Conferences in Costa Rica and in Colombia to underline the importance of bamboo in this region. Special memories are attached to our 5th International Bamboo Congress with FUNBAMBU 1998 in San Jose, Costa Rica, as well as the Congreso Mundial de Bambù Guadua August 1992 in Pereira, Colombia, and finally the GUADUA 2004 Simposio Internacional in Pereira (Fig. 88), (73).

5.7 European Commission, Brussels

Holding the 9th World Bamboo Congress in Belgium, the European Commission in Brussels has certainly to be mentioned. An EU-project “BAMBOO FOR EUROPE” was undertaken from 1996-1999 and brought together research institutions from Belgium (University of Gent & Centre Genie Rural), France (Centre Technique du Bois et de l’Ameublement - CTBA), Germany (University of Hamburg, Bundesforschungsanstalt für Forst- und Holzwirtschaft and Forschungsanstalt für Landwirtschaft FAL, Braunschweig), Portugal (Centro da Biomassa para a Energia, Direccao Regional de Agricultura de Tras-Os-Montes) and the UK (University of Wales, Bangor) under the coordination of Cobelgal and Oprins Plant.

Another EU project “Sustainable Management and Quality Improvement of Bamboos” from 1997-2002 under the coordination of Dr. Richard Murphy brought together bamboo scientists from Belgium (Oprins Plant), Indonesia (Forest and Natural Conservation Research and Development Centre in Bogor), Germany (University Hamburg, Bundesforschungsanstalt für Forst -und Holzwirtschaft), Malaysia (University Sains Malaysia USM and Forest Research Institute of Malaysia – FRIM) and the Philippines (University of the Philippines at Los Banos). At annual meetings in the respective countries the results were discussed, joint results published (74, 75) and field visits made (Fig. 89).

After finalization these research projects, forces were combined in the EU Project “Bamboo Thematic Network” BTN (2002-2004) with additional participation of CABI and INBAR. A Final Report by Johan Gielis and Victor Brias summarizes the many valuable results and cooperations (Fig. 90), (76).

At the end of these reflections an impressive bamboo construction in Vergiate, Italy, should be mentioned. It was conceived by the Italian association EMISSIONIZERA and built by its founder Valeria Chioetto and Neri Braulin in 2002-2003 with Guadua culms imported from Colombia (Fig.

91). The goal was a municipal recreation area of 480 m² (32x16m). Details and lessons learnt have been summarized as guidance for similar projects by Paul Vantomme (77).

6. Concluding remarks

After 60 years passion for many aspects of bamboo my work is now slowly coming to an end. Nevertheless, I am still mentoring a few on-going doctoral theses, such as on the susceptibility of bamboo by fungi by Mr. Dong Sheng Wei and on the treatment of Vietnamese bamboo by Ms. Thi Kim Hong Tang. Both will present their papers at the congress (78, 79).

Finally, I would like to express my special thanks to Ms. Thi Kim Hong Tang in helping me to prepare this paper.

References

1. Liese, W. 2007. Electron Microscopy of Wood: the Pioneering Years. The Plant Cell Wall. In Recent Advances and New Perspectives. Proceedings of the 2nd New- Zealand-German Workshop on Plant Cell Walls (Eds. U. Schmitt, A.P. Singh, Ph. J. Harris), Hamburg, 4-6 October, 2006. Mitt. der Bundesforschungsanstalt für Forst- und Holzwirtschaft, No. 223. pp. 3-12.
2. Liese, W. 1959. Bamboo preservation and soft rot. FAO Report to the Government of India. Rome, No. 1106, pp. 1-37.
3. Liese, W. 2003. Report to UNIDO on the Cane & Rattan Technology Upgrading and Networking Project in Guwahati, North India. 17 pp.
4. Liese, W. 2004. Opportunities and Constraints for Processing and Utilisation of Bamboo and Rattan in North-East-India. In Cane and Bamboo Technology Centre's Technical Papers II, Guwahati, India, pp. 211-217.
5. Liese, W. 2010. Protection of Bamboo Structure. Newsletter of Cane & Bamboo Technology Centre, Guwahati, India. 2(7), 16-19.
6. Liese, W. 1959. Wood Preservation. FAO Report to the Government of Indonesia. Rome, No. 1080, 46 pp.
7. Liese, W.; Dadswell, H. E. 1959. Über den Einfluß der Himmelsrichtung auf die Länge von Holzfasern und Tracheiden. Holz und Roh- Werkstoff, 17(11), 421-427.
8. Liese, W. 1968. Bamboo Preservation in East Pakistan. GTZ, 32 pp.
9. Liese, W.; Mende, C. 1969. Histometrische Untersuchungen über den Aufbau der Sproßachse zweier Bambusarten. Holzforschung-Holzverwertung, 21(5), 113-117.
10. Liese, W.; Grosser, D. 1971. Über das Vorkommen von anomalem Gewebe in der Sproßachse von Monokotyledonen. Holz Roh-und Werkstoff, 29(9), 339-344.
11. Grosser, D.; Liese, W. 1971. On the anatomy of Asian bamboos, with special reference to their vascular bundles. Wood Science and Technology, 5(4), 290-312.
12. Liese, W.; Grosser, D. 1972. Untersuchungen zur Variabilität der Faserlänge bei Bambus. Holzforschung, 26(6), 202-211.
13. Grosser, D.; Liese, W. 1973. Present status and problems of bamboo classification. Journal Arnold Arboretum, 54(2), 293-308.
14. Grosser, D.; Liese, W. 1974. Verteilung der Leitbündel und Zellarten in Sproßachsen verschiedener Bambusarten. Holz Roh- Werkstoff, 32(12), 473-482.
15. Liese, W.; Grosser, D. 2000. An expanded typology for the vascular bundles of bamboo culms. Proceedings of International Symposium on Bamboo 2000, Chiang Mai, Thailand, 2-4 August 2000. Royal Project Foundation. Kasetsart University, Royal For. Dep., ICDF, ROC, eds. L. Puangchit, B. Thaiutsa, S. Thamnichia. pp. 121-134.
16. Parameswaran, N.; Liese, W. 1975. On the polylamellate structure of parenchyma wall in *Phyllostachys edulis* Riv. IAWA Bull., 4, 57-58.
17. Parameswaran, N.; Liese, W. 1976. On the fine structure of bamboo fibres. Wood Science and Technology, 10(4), 231-246.

18. Parameswaran, N.; Liese, W. 1977. Structure of septate fibers in bamboo. *Holzforschung*, 31(2), 55-57.
19. Parameswaran, N.; Liese, W. 1977. Occurrence of warts in bamboo species. *Wood Science and Technology*, 11(4), 313-318.
20. Parameswaran, N.; Liese, W. 1978. A note on the fine structure of protoxylem elements in bamboo. *IAWA Bull.*, 2(3), 29-32.
21. Parameswaran, N.; Liese, W. 1980. Ultrastructural aspects of bamboo cells. *Cellulose Chemistry and Technology*, 14(5), 587-609.
22. Parameswaran, N.; Liese, W. 1981. Occurrence and structure of polylamellate walls in some lignified cells. In *Cell Walls '81. Proceedings of the Second Wall Meeting*, Göttingen, 8-11 April 1981 (ed. by D.G. Robinson and H. Quader). Stuttgart Wiss. Verlagsges. pp. 171-188.
23. Parameswaran, N.; Liese, W. 1981. The fine structure of bamboo. In *Bamboo Production and Utilization. Proceedings of the Congress Group 5.3A "Production and Utilization of Bamboo and Related Species"*, XVII IUFRO World Congress, Kyoto, Japan, 6-17 September 1981, (ed. by T. Higuchi). pp. 178-183.
24. Ding, Y.; Grosser, D.; Liese, W.; Hsiung, W. 1992. Anatomical studies on the rhizome of monopodial bamboos. In *Bamboo and its Use. Proc. Int. Symp. on Industrial Use of Bamboo*, Beijing, China 7-11. December. 1992. pp.143-150.
25. Liese, W.; Ding, Y. 1994. Structure and functions of the nodes in bamboo. *Proc. Intern. Bamboo Workshop*, Chiang Mai, Thailand, November 1991. FORSPA Publ. 6, IDRC/FAO/UNDP, Bangkok. pp. 213-217.
26. Ding, Y.; Liese, W. 1995. On the nodal structure of bamboo. *Journal of Bamboo Research*, 14(1),24-32.
27. Liese, W.; Weiner, G. 1996. Ageing of bamboo culms. *Wood Science and Technology*, 30(1), 77-89.
28. Ding, Y.; Weiner, G.; Liese, W. 1997. Wound reactions in the rhizome of *Phyllostachys edulis*. *Acta Botanica sinica*, Beijing, 39(1), 55-58.
29. Weiner, G.; Liese, W. 1997. Wound reactions in bamboo culms and rhizome. *J. Tropical Forest Science*, 9(3), 379-397.
30. Liese, W.; Weiner, G. 1997. Modifications of bamboo culm structures due to ageing and wounding. In *The Bamboos*, ed. G. Chapman, Linnean Soc. London, Academic Press, 313-322.
31. Liese, W.; Weiner, G. 1995. Wundreaktionen bei Bambus. *Bambusbrief*, (4),13-14.
32. Ding, Y.; Weiner, G.; Liese, W. 1997. Wound reactions in the rhizome of *Phyllostachys edulis*. *Acta Botanica sinica*, Beijing, 39(1), 55-58.
33. Dujesiefken, D.; Liese, W. 2008. Die Wundreaktionen von Bambus und Palmen. In: *Das CODIT-Prinzip. Von den Bäumen lernen für eine fachgerechte Baumpflege*. Haymarket Media Verlag, Braunschweig, 137-144.
34. Latif, A. M.; Liese, W. 2001. Anatomical features of *Bambusa vulgaris* and *Gigantochloa scortechinii* from four harvesting sites in Peninsular Malaysia. *J. Tropical Forest Products*, 7(1),10-28.
35. Latif, A. M.; Liese, W. 2002. Culm characteristics of two bamboos in relation to age, height and site. *Bamboo for Sustainable Development. Proceedings of the Vth and the VIth International Bamboo Workshop*, San José, Costa Rica, 2-6 November 1998. Eds. A. Kumar, I.V. Ramanuja Rao and Cherla Sastry, INBAR Proc. No.7. pp. 223-233.
- 35.a Liese, W., Latif Mohmod, Abd. 2000. The starch content of two Malaysian bamboos in relation to age, culm site and harvesting month. *Proceedings 21st IUFRO World Congress*, Kuala Lumpur, 7-12 August 2000. Poster, Abstract. Vol. 3. pp. 261.
36. Liese, W. 1986. Characterization and Utilization of Bamboo. *Proceedings 18th IUFRO World Congress*, Ljubljana, 7-21 September 1986. Div. 5 Forest Products. pp. 475-485.
37. Liese, W. 1987. Anatomy and properties of bamboo. *Proceedings Int. Bamboo Workshop Hangzhou*, 6-14 October 1985. In *Recent Research on Bamboo*, Ed. Chin. Acad. of Forestry, Peking, and IDRC, Canada. pp. 196-208.
38. Liese, W. 1992. Production and utilization of bamboo and related species in the year 2000. *Proceedings of the IUFRO All Division V Conference on Forest Products*, Nancy, 23-28 August 1992. Vol. 2. pp. 743-751.

39. Liese, W. 1992. The structure of bamboo in relation to its properties and utilization. In *Bamboo and its Use. Proc. Int. Symp. on Industrial Use of Bamboo*, Beijing, China, 7-1 December 1992. Beijing. pp. 95-100.
40. Liese, W. 1995. Anatomy and Utilization of Bamboo. *European Bamboo Society Journal*, Meise, Belgien, (1), 5-12.
41. Latif, A. M.; Liese, W. 1995. Utilization of Bamboo. *Planting and Utilization of Bamboo on Peninsula Malaysia*, Eds. Abd. Razak, O.; Abd. Latif M.; Liese W.; Norini H. FRIM Research Pamphlet No. 118. Forest Research Institute Malaysia, Kuala Lumpur. pp. 50-102.
42. Shanmughavel, P.; Peddappiah, R. S.; Liese, W. 2003. Recent Advances in Bamboo Research. *Scientific Publ.*, Jodhpur, India, 228 pp.
43. Liese, W. 2004. Structures of a bamboo culm affecting its utilization. Proc. INBAR International Workshop "Bamboo Industrial Development and Utilization", Xianning, China, 12 November 2003. INBAR, Beijing. pp. 1-8.
44. Liese, W. 1998. The Anatomy of Bamboo Culms. INBAR Technical Report No. 18. International Network for Bamboo and Rattan, Beijing. 204 pp.
45. Liese, W. 2005. GUADUA in Kolumbien. *Bambus Journal*, 16(1), 4-6.
46. Magel, E.; Kruse, S.; Lütje, G.; Liese, W. 2005. Soluble carbohydrates and acid invertases involved in the rapid growth of developing culms in *Sasa palmata* (Bean) Camus. *Bamboo Science and Culture*, (19), 23-29.
47. Liese, W. 1999. *Oxytenanthera braunii*, der Wein- Bambus. *Bambus Brief*, (10), 17-19.
48. Kwon, S. D.; Park, S. B.; Moon, H. S. 2010. Harvesting and utilization of bamboo sap in Korea. XXIII IUFRO World Congress, Seoul, Republic of Korea, 23-28 August 2010. Abstracts. pp.222.
49. Liese, W.; Düking, R. 2009. Bambus als CO₂- Speicher? *Naturwissenschaftliche Rundschau*, 62(7), 341-348.
50. Liese, W. 2009. Bamboo as carbon-sink fact or fiction. *Journal of Bamboo and Rattan*, 8(3&4), 103-114.
51. Düking, R.; Gielis, J.; Liese, W. 2011. Carbon Flux and Carbon Stock in a Bamboo Stand and their Relevane for Mitigating Climate Change. *Bamboo Science and Culture*, 24(1), 1-6.
52. Liese, W. 2005. Das Blühen von *Melocanna baccifera* in Nordost-Indien und seine Folgen. *Bambus Journal*, 16(1), 12-13.
53. Tang, T. K. H.; Schmidt, O.; Liese, W. 2009. Environment-friendly Short-term Protection of Bamboo against Molding. *Timber Development Association of India Vol.55*, 8-17.
54. Schmidt, O.; Wei, D. Sh.; Liese, W.; Wollenberg, E. 2011. Fungal degradation of bamboo samples. *Holzforschung*, 65, 883-888.
55. Tang, T. K. H.; Schmidt, O.; Liese, W. 2012. Field test for protection of bamboo by environment-friendly chemicals against short-term moulding. *Tropical Forest Science* 24 (2), accepted.
56. Liese, W.; Grover, P. N. 1961. Untersuchungen über den Wassergehalt von indischen Bambushalmen. *Ber. Dt. Bot. Ges.* 74, 105-117.
57. Latif, A. M.; Liese, W. 2002. The moisture content of two Malaysian bamboos in relation to age, culm height, site and harvesting month. *Bamboo for Sustainable Development, Proceedings of the Vth Internal Bamboo Congress and the VIth Intenational Bamboo Workshop*, San Jose, Costa Rica, 1998. Eds. A. Kumar, I.V. Ramanuja Rao and Ch. Sastry. INBAR Pro. No. 7, pp. 257-268.
58. Liese, W.; Schmitt, U. 2006. Development and structure of the terminal layer in bamboo culms. *Wood Science and Technology*, 40 (1), 4-15.
59. Tang, T. K H. Bamboo preservation in Vietnam (2009). Documents of the 40 Conference of the International Research Group on Wood Protection (IRG), 24-28 May 2009, Beijing, China. IRG/WP 09-40457. 11pp.
60. Liese, W.; Kumar, S. 2003. *Bamboo Preservation Compendium*. CIBART, ABS, INBAR Technical Report. No. 22, New Delhi, 231 pp.
61. Liese, W. 1963. *Bamboo Preservation in Asian Countries*. Background Paper Fifth Conference on Wood Technology, Madison, USA. FAO/WTC//63/UP.11 pp.
62. Higushi, T. 1981. *Bamboo Production and Utilization*. Proceedings of Congress Group 5.3, XVII

- IUFRO World Congress, Kyoto, Japan, 213 pp.
63. Lipangile, T. N. 1979. The wood and bamboo project for water supplies and irrigation in Tanzania. Project Seminar in Iringa, September 1979.
 64. Van Heuvel, K. 1981. Wood and Bamboo for rural water supply- a Tanzanian initiative for self-reliance. Delft University press. 76 pp.
 65. Crouzet, Y.; Liese, W.; Thebaut, P. 1993. Etude de faisabilite socio-economique des filiéres bambou á La Réunion. 111 pp.
 66. Liese, W. 1996. Report on given aspects for the bamboo management in Ethiopia. GTZ/LUSO CONSULT. 35 pp.
 67. Liese, W. 1996. Second report on the bamboo management and utilization in Ethiopia. GTZ/LUSO CONSULT. 19 pp.
 68. Liese, W. 1996. Bambus in Äthiopien. Bambus Brief (4), 26-29.
 69. Liese, W. 2005. The Bamboos of Ethiopia. Bamboo Bulletin, Bamboo Soc. of Australia 7(3), 18-19.
 70. Liese, W. 1996. Bamboo Study in Ethiopia. Report on given aspects for the bamboo management in Ethiopia. GTZ/ LUSO Consult. 28pp.
 71. GTZ/ LUSO CONSULT. 1997. Study on sustainable bamboo management. Second Interim report, Hamburg. 58pp.
 72. Liese, W.; Silbermann, S. 2010. Bamboo charcoal. BAMBOO, American Bamboo Soc. 31(3), 2-10.
 73. Liese, W. 2004. La preservación de un tallo de Bambú en relación a su estructura. Proceedings of Internacional Symposium Guadua , Pereira, Colombia, 27 Semtember -2 October 2004. Universidad Tecnológica de Pereira, Facultad de Ciencias Ambientales. pp.9 -19.
 74. Fernandez, E.; Palijon, A. M.; Liese, W.; Esguerra, F. L.; Murphy, R. N. J. 2003. Growth Performance of Two Bamboo Species in New Plantations. Journal of Bamboo and Rattan, 2(3), 225-239.
 75. Abasolo, W.P.; Fernandez, E. C.; Liese, W. 2005. Fiber Characteristics of *Gigantochloa levis* and *Dendrocalamus asper* as influenced by organic fertilizers. Journal of Tropical Forest Science, 17(2), 297-305.
 76. Brias, V.; Gielis, J. 2002. Bamboo Thematic Network. Final Report, INCO-EU, 71 pp.
 77. Vantomme, P. ; Braunlin, P. ; Chioetto, V.; Liese, W. (2003). Public constructions made with Bamboo: Lessons learnt from the “Vergiate Bamboo Pavilion” in North-Italy. Journal of Bamboo and Rattan , 2(4), 225-239.
 78. Dong, S. W.; Schmidt, O.; Liese, W. 2012. Susceptibility of Bamboo to Fungi. The 9th World Bamboo Congress, Antwerp, April, accepted.
 79. Tang, T. K. H; Welling, J; Ho, T. D.; Liese, W. 2012. Investigation on kiln drying of the bamboo species *Bambusa stenostachya*, *Dendrocalamus asper* and *Thyrostachys siamensis*. The 9th World Bamboo Congress, Antwerp, April, accepted.

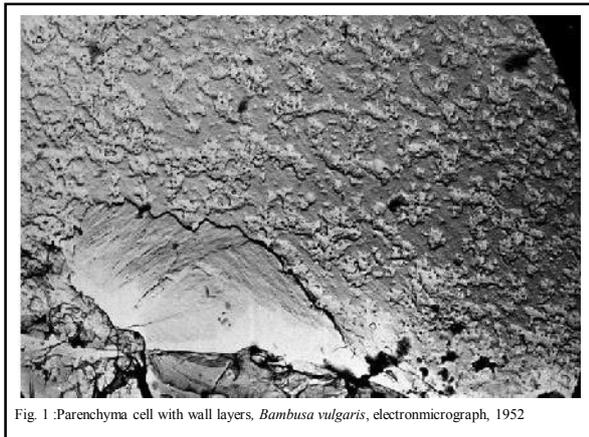


Fig. 1 :Parenchyma cell with wall layers, *Bambusa vulgaris*, electronmicrograph, 1952

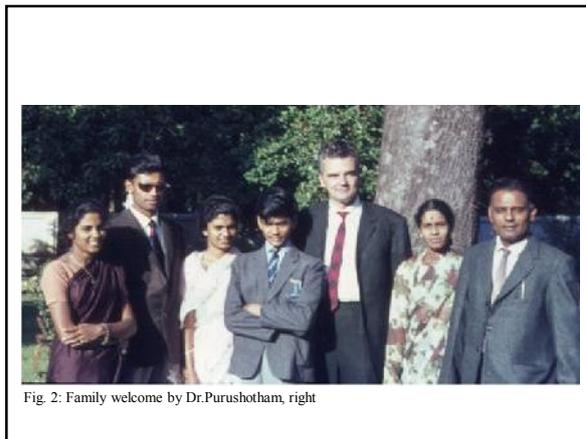


Fig. 2: Family welcome by Dr.Purushotham, right



Fig.3: Christmas Day 1957,Katrin feeding the monkeys in the bamboo forest

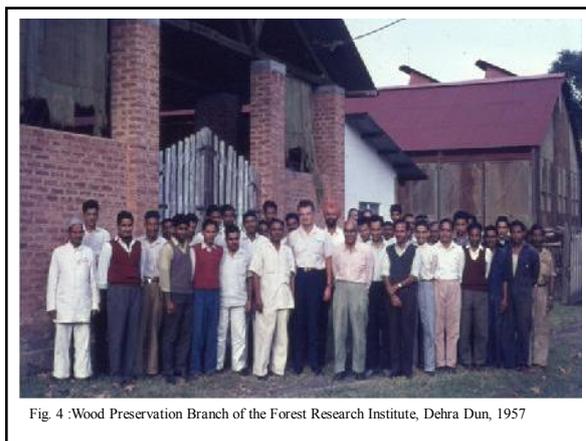


Fig. 4 :Wood Preservation Branch of the Forest Research Institute, Dehra Dun, 1957

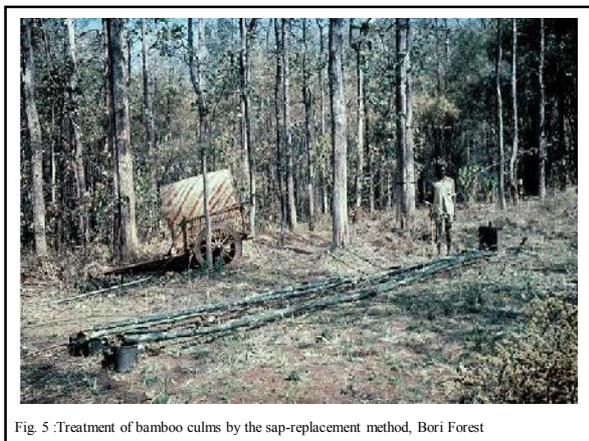


Fig. 5 :Treatment of bamboo culms by the sap-replacement method, Bori Forest



Fig. 6: Kamesh Salam with his wife Hussina, Guwahati, 2003



Fig. 7: Bamboo for houses, UNDP Project 2003

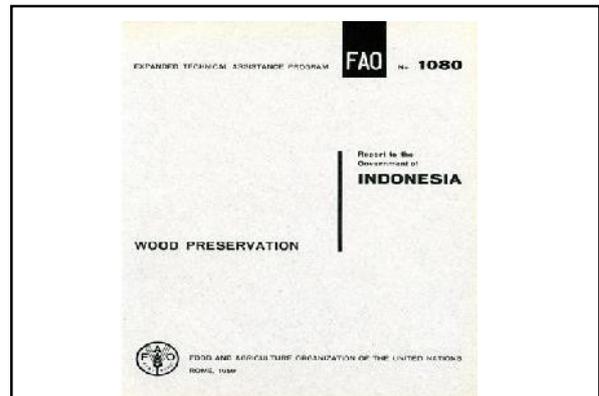


Fig. 8: FAO Project on Wood Preservation in Indonesia, 1958



Fig. 9: First work on Indian bamboo with Dr. Dadswell and Dr. Wardrop, CSIRO, Melbourne, 1958

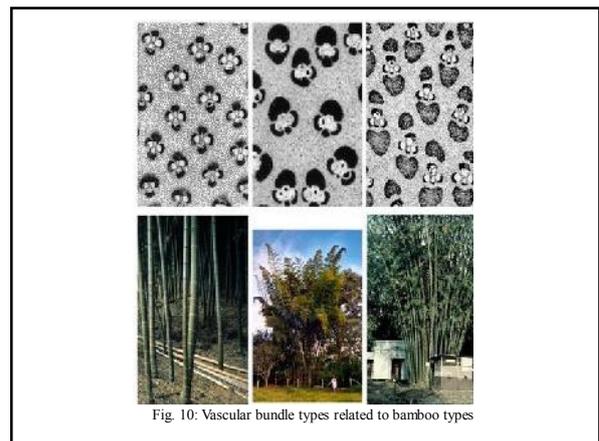


Fig. 10: Vascular bundle types related to bamboo types

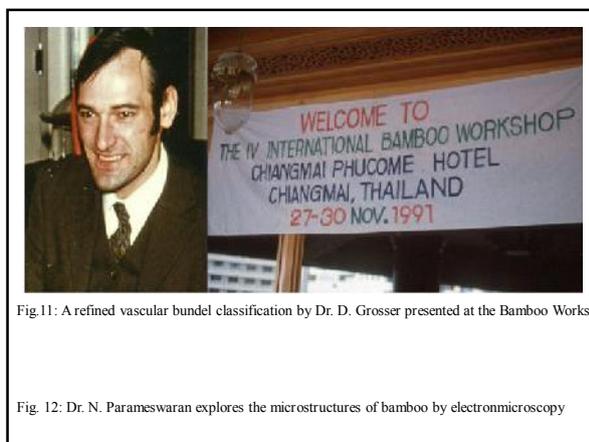


Fig. 11: A refined vascular bundle classification by Dr. D. Grosser presented at the Bamboo Workshop 1991, Chiang Mai



Fig. 12: Dr. N. Parameswaran explores the microstructures of bamboo by electron microscopy

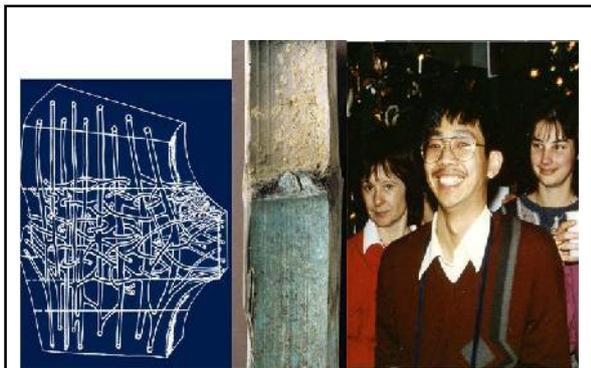


Fig 13: Dr.Yulong Ding clarifies the nodal structure of the bamboo culm

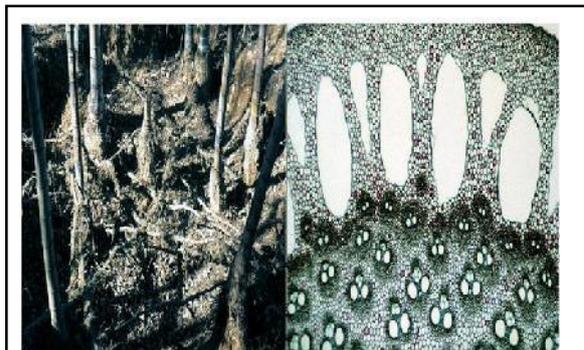


Fig. 14: Structural differences of the bamboo rhizome with air canals, *Phyllostachys heteroclada*



Fig. 15: Cooperation between Yulong Ding and Gudrun Weiner

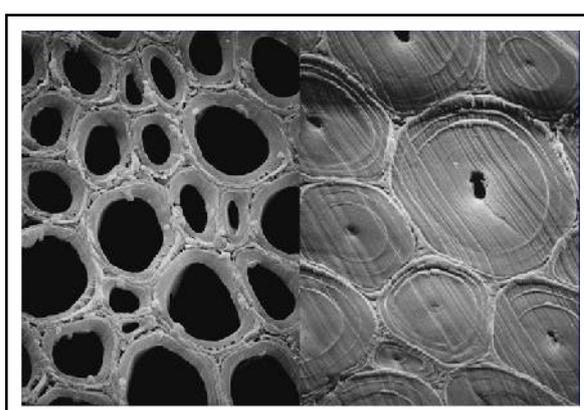


Fig 16: Bamboo fibre cell walls at 1 year and 6 years, *Phyllostachys viridiglaucens*

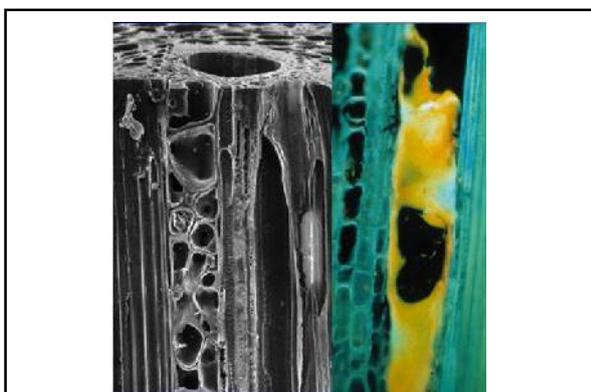


Fig. 17: Vessel closure by tyloses and slime

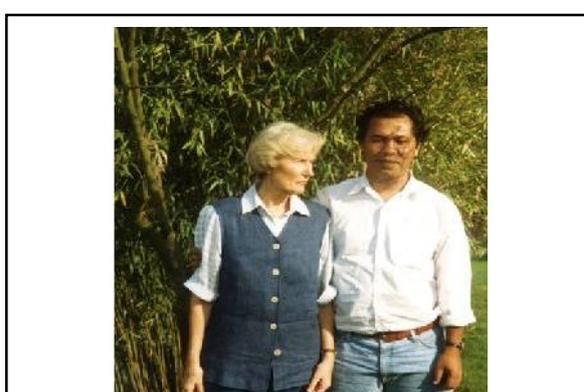


Fig. 18: Discussion between Katrin Liese and Abd. Latif Mohmod, Malaysia in our bamboo garden

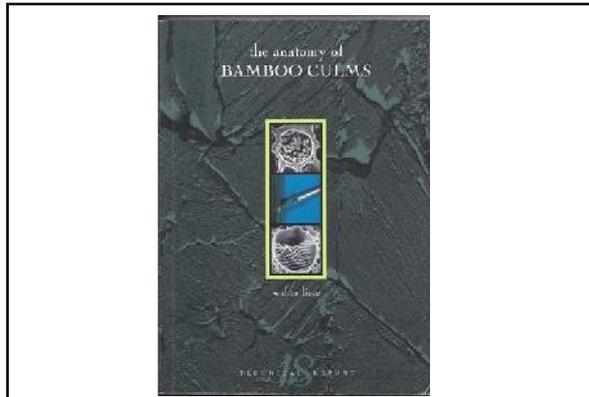


Fig. 19: The anatomy of bamboo culms
INBAR, Rep. 18, 1998
with X. Sarmiento, L. Chelona, Pereira, Colombia



Fig. 20: Expansion of bamboo culms, *Guadua angustifolia*,

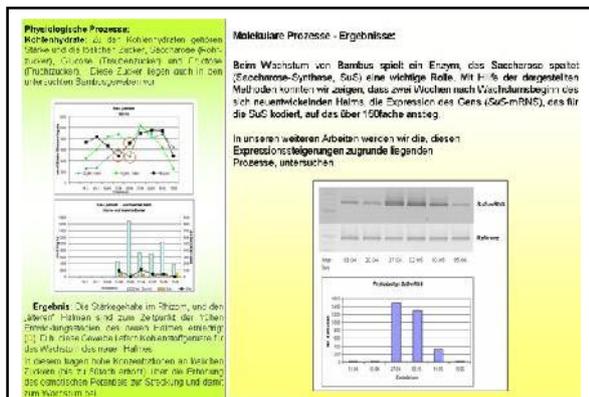


Fig. 21: Changes of carbohydrates during culm expansion
alcoholic, Itanga, Tanzania



Fig. 22 : Collection of bamboo sap from *Oxythenthera braunii*, fermenting from tasty to

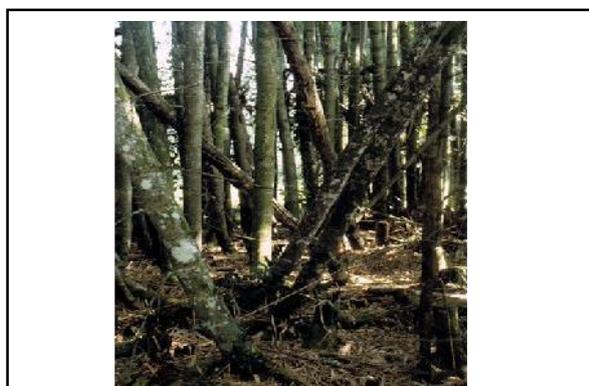


Fig. 23: Natural bamboo forest with young and dying culms,
Guadua angustifolia, Colombia

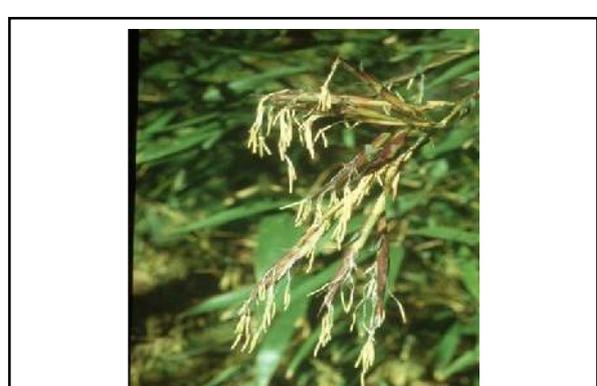


Fig. 24 :Flowering of *Fargesia murielae*



Fig. 25: Flowering of *Melocanna baccifera* : 1969 in Bangladesh; 2004 in Colombia as well as in North-India. The pear-like fruits-with vivipary-appear delicious, specially for rats



Fig. 26: Bamboo flowering with their subsequent dying, *Dendrocalamus strictus*



Fig. 27: Bamboo structures with captured CO₂ will naturally degraded and release the CO₂ again into the atmosphere



Fig. 28: A newly built bamboo hotel is soon infested by fungi for degradation



Fig. 29: Bamboo storage at a pulp mill, degraded after 2 years

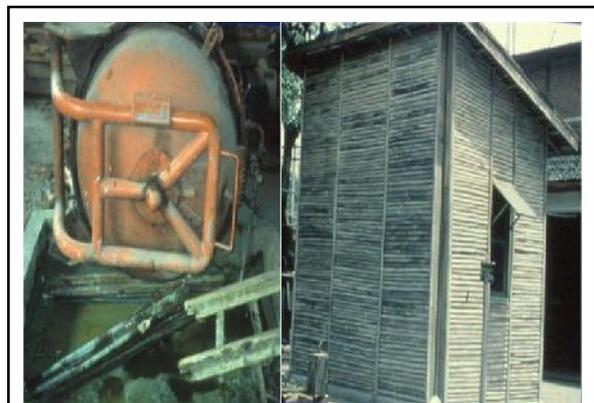


Fig. 30: Earlier pressure treatment protects this construction since over 50 years



Fig. 31: Pressure treatment with creosote caused environmental damage

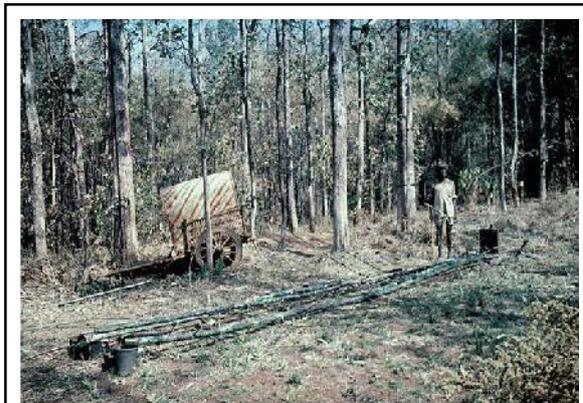


Fig.32: Trials to treat bamboo culms directly in the forest with the sap-replacement system



Fig. 33: Bamboo culms treated by the sap replacement system, EBF, Bali



Fig. 34: A valve at the pressure cap releases air to let preservative pressed in



Fig. 35: Practical demonstration of the sap-replacement system, Thailand

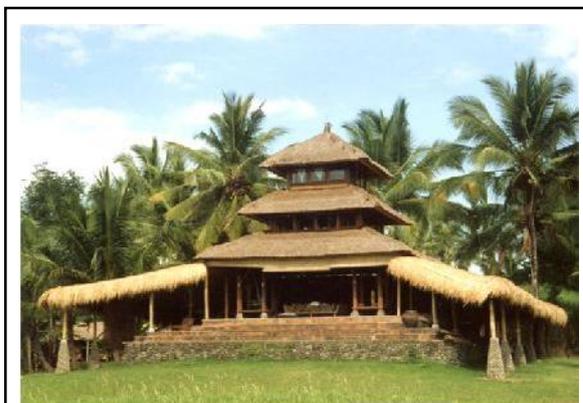


Fig. 36: Centre for the Environmental Bamboo Foundation, EBF, Ubud, Bali



Fig. 37: Linda Garland, Founder of the Environmental Bamboo Foundation (EBF)

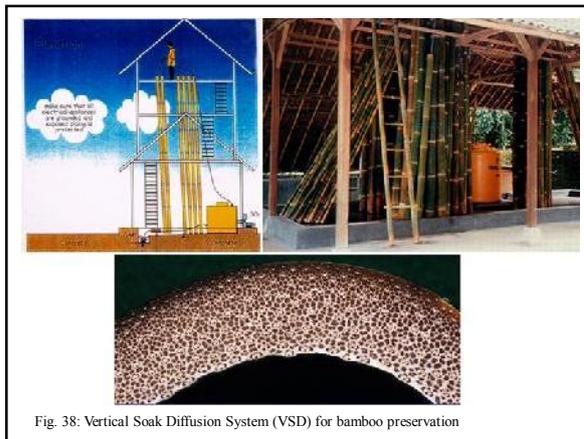


Fig. 38: Vertical Soak Diffusion System (VSD) for bamboo preservation

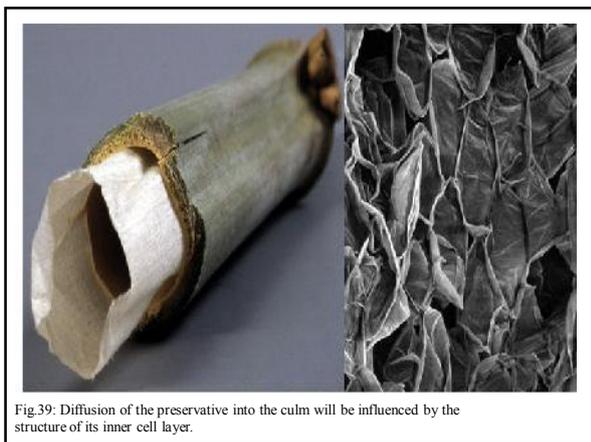


Fig. 39: Diffusion of the preservative into the culm will be influenced by the structure of its inner cell layer.

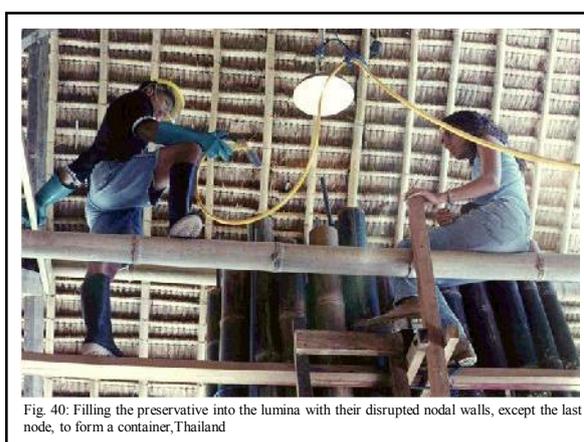


Fig. 40: Filling the preservative into the lumina with their disrupted nodal walls, except the last node, to form a container, Thailand

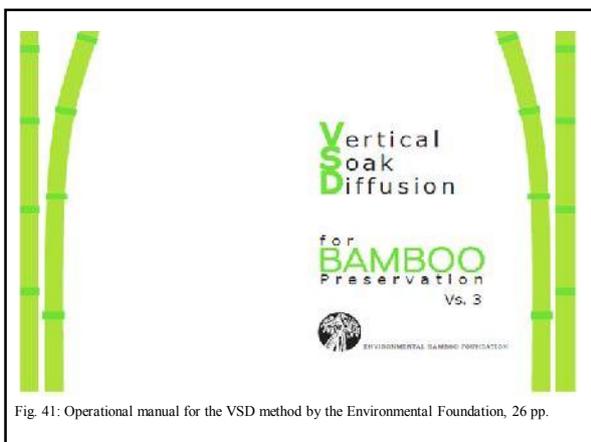


Fig. 41: Operational manual for the VSD method by the Environmental Foundation, 26 pp.

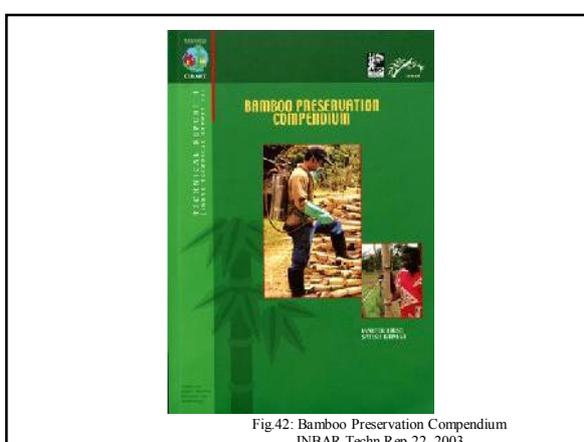


Fig. 42: Bamboo Preservation Compendium INBAR Techn.Rep 22, 2003



Fig. 43: The IV International Bamboo Congress, Festival and Trade Fair, Ubud 1995

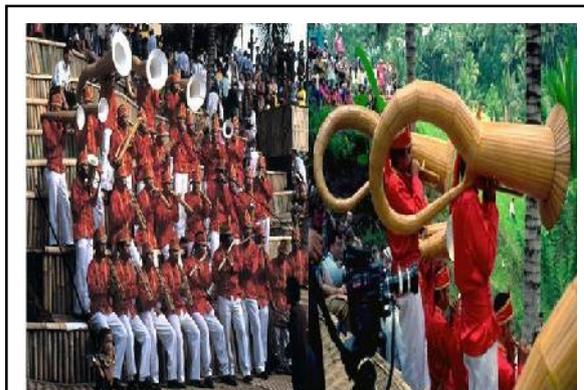


Fig. 44: Bamboo music is always a happening, Bali

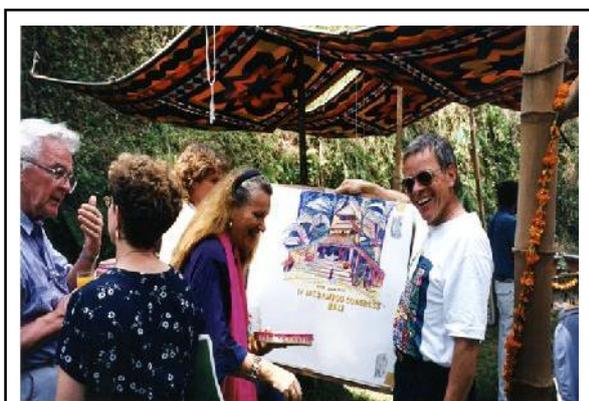


Fig. 45: Lively inter-change at the congress



Fig. 46: Farewell with great thanks to the organisers and contributors, Ubud, 1995



Fig. 47: Laboratory work with Bhot Anuwongse, Thailand and Abdurachim Widjaja, Indonesia

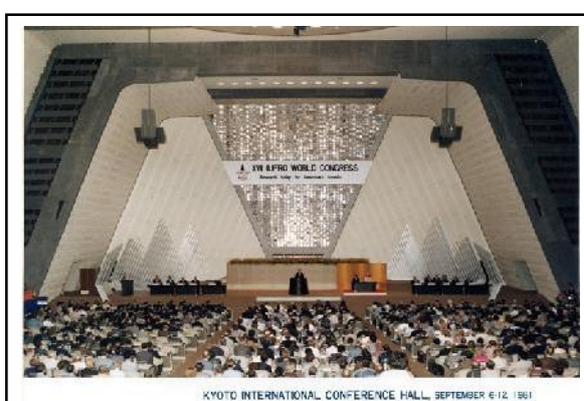


Fig. 48: Opening of the XVII IUFRO WORLD CONGRESS, Kyoto, 1981

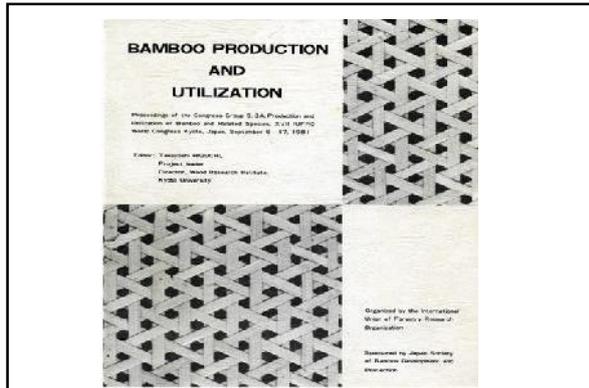


Fig. 49: Proceedings on „Bamboo Production and Utilization“, IUFRO CONGRESS 1981, 213 pp.



Fig.50: At the IUFRO Congress, from right Prof. Koichiro UEDA, Prof. Takayoshi HIGUSHI, Prof. Ken SHIMAJI



Fig.51: Prince and Princess of Japan planting *Phyllostachys pubescens* var. *heterocycla*



Fig. 52: The bamboo grove after 30 years

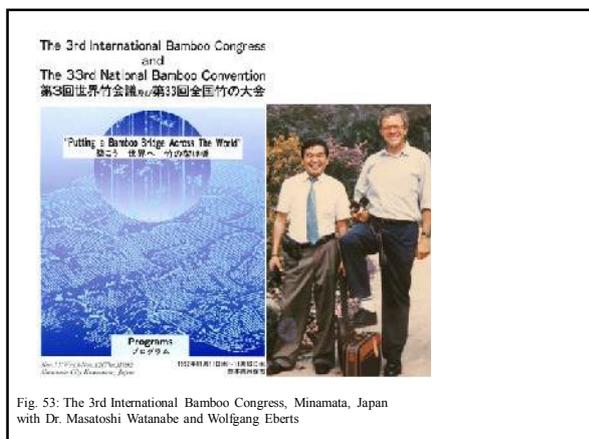


Fig. 53: The 3rd International Bamboo Congress, Minamata, Japan with Dr. Masatoshi Watanabe and Wolfgang Eberts



Fig.54: INBAR International Bamboo Workshops, Xianning Hubei,China, 2003

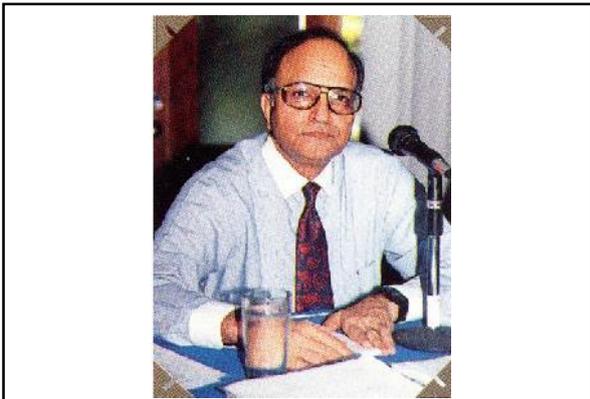


Fig. 55: Dr. Cherla Sastry, IDRC Regional Representative, Beijing



Fig. 56: Discussion with Chinese delegation about IUFRO participation, with Prof. Wenyu Hsiung, left, 1980, Beijing



Fig. 57: Welcome by the Taiwan Forestry Research Institute, Prof. Shuen Chao Wu, at right



Fig.58: Welcome at Nanjing Forestry University by Prof. Wenyu Hsiung left



Fig. 59: Lecturing as Concurrent Professor, Nanjing Forestry University

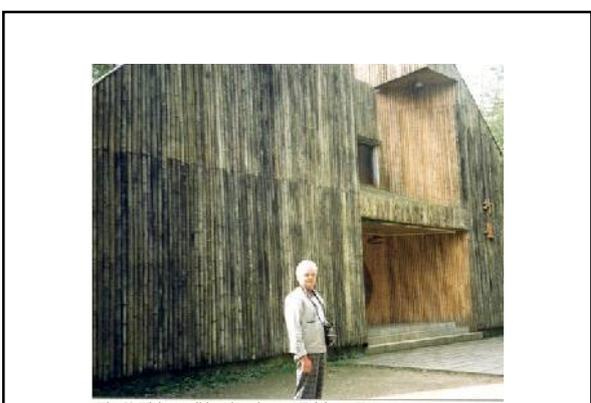


Fig.60: Visit an all bamboo house, Taichung Forest



Fig. 61: Prof. Wu trusting and enjoying a bamboo-laminated chair



Fig.62: Happy villagers after enjoying bamboo wine, Tanzania



Fig.63: Water transport in split culms and burried in soil Mr. T.N. Lipangile, project manager, Tanzania.



Fig.64 :Fungi growing from the water pipes



Fig.65 : Trials to protect the culms with plastic, inside and outside

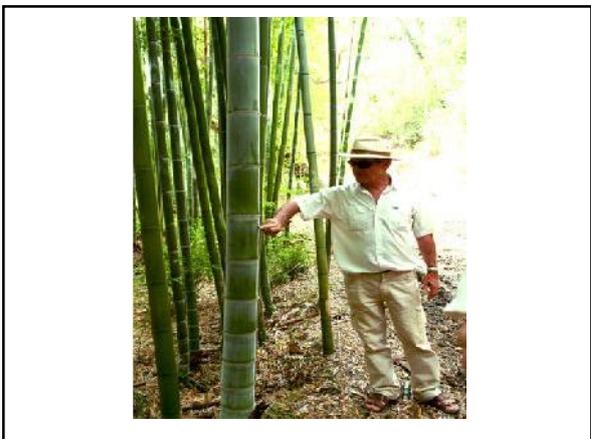
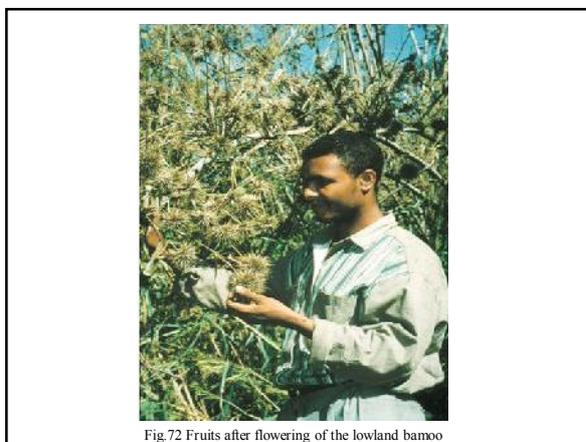
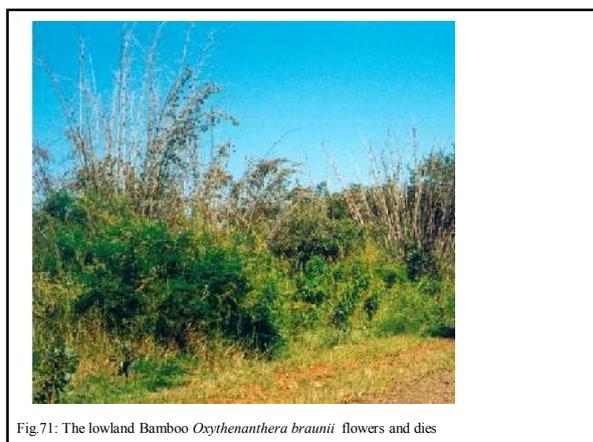
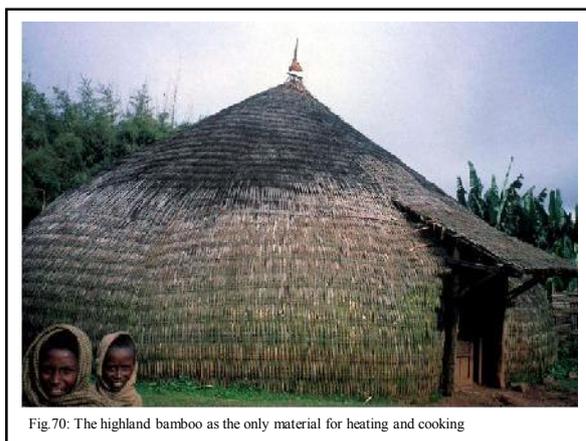


Fig.66: Yves Crouzet at the Bambouseaie, Prafrance



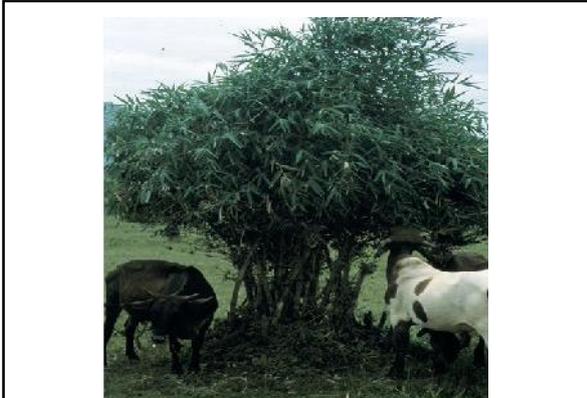


Fig.73: The lowland bamboo is loved by cattle and thus reduced



Fig.74: Children transport heavy bundles of bamboo for the daily meal. Since bamboo does not keep glow, a constant resupply is needed



Fig.75 Workshops by ITTO provide information and training for various needs, Thailand 2002

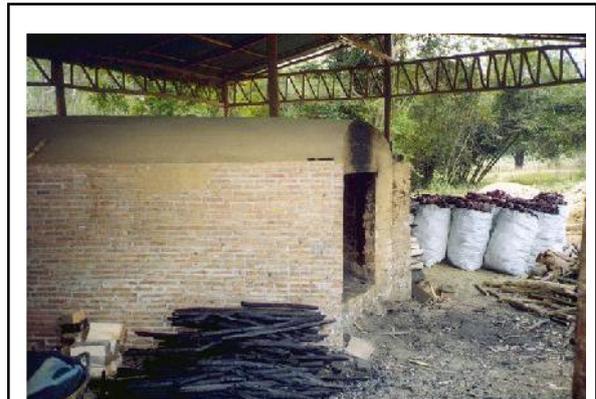


Fig.76: Workshop on production of bamboo coal, Thailand



Fig.77: Bamboo coal from Thailand

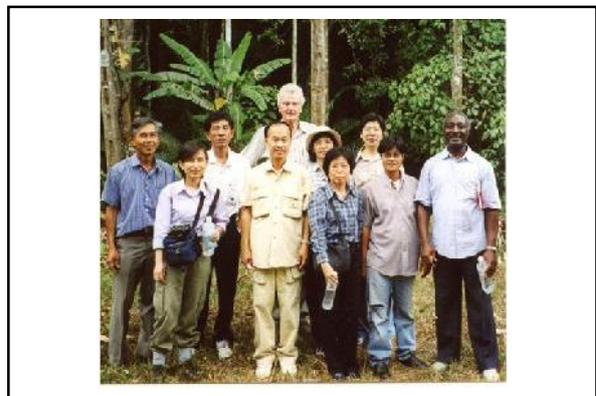


Fig.78: ITTO Workshop for production of bamboo charcoal, with the ITTO DG Emmanuel Ze Meka at right

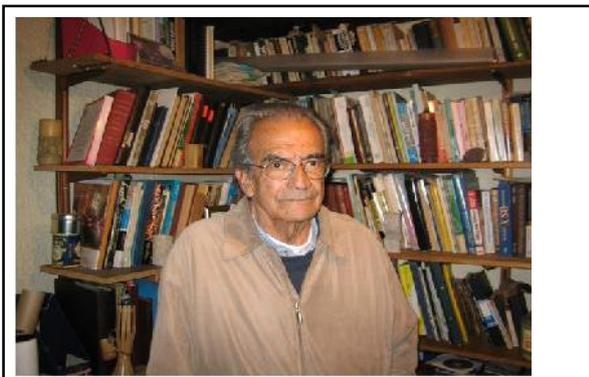


Fig.79: Oscar Hidalgo Lopez

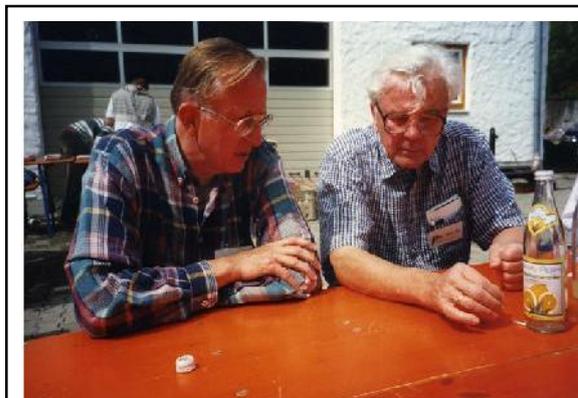


Fig.80: Jules Janssen and Walter Liese discussing bambo issues



Fig.81: Proyecto Nacional BAMBU, Manizales, Colombia



Fig.82: Bamboo house in Costa Rica



Fig.83: Construction failures due to weathering and splitting

Fig.84: Bamboo as foundation embedded for protection by cement





Fig.85: Bamboo embeded by cement causes shrinkage, with water collection causing fungal decay



Fig.86: FUNBAMBU; Production of prefab. panels. Photo J.Janssen



Fig.87: FUNBAMBU; Sap replacement treatment for constructions, Photo J. Janssen



Fig.88: Farewell at the Simposio International GUADUA 2004, Pereira, Colombia

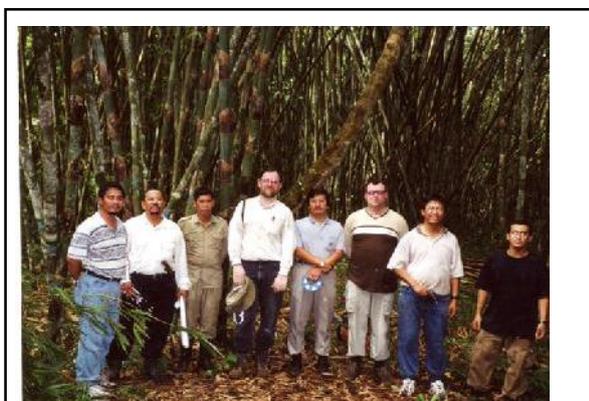


Fig.89: Field excursion, Philippines, 2003

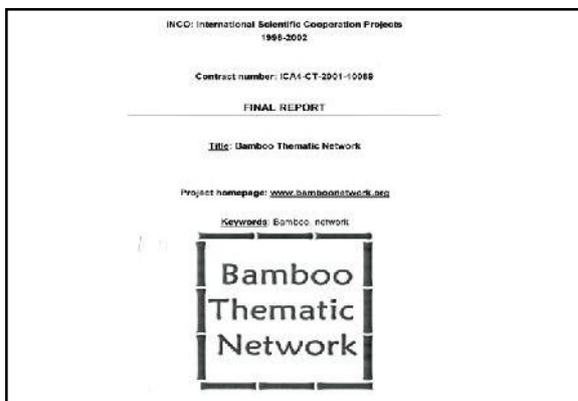


Fig.90: Bamboo Thematic Network. Final Report, 2004



Fig.91: Convocation hall at Vergiate, Italy made with Guadua culms from Colombia

KEYNOTE LECTURE

A Genomic Approach to Identify Genes Expressed during Fiber Development in *Bambusa balcooa*

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Abstract

Bamboo fiber development is a complex but ordered phenomenon. First hand information on bamboo fiber development *in planta* is based mainly on speculations derived from simple anatomical and sophisticated confocal laser scanning microscopic evidences. Nevertheless, knowledge on the molecular mechanism of fiber development is not fully explored. In this study, PCR-based suppressive subtractive hybridization technique was employed between cDNA isolated from leaf tissues and internodal tissues at different stages of development to identify genes involved in fiber development of *Bambusa balcooa*. Differential screening revealed 53% of total ESTs were up-regulated during three phases of fiber development, i.e. initiation, elongation and maturation. Of which, 156 assembled ESTs with known functions were further categorized into different functional categories using Blast2GO software. Fifty-one of these classified ESTs were exclusively fibers specific. Distribution and differential expression patterns of 13 selected *B. balcooa* fiber specific cDNAs at different stages of internode development were studied. The present findings clearly suggest that fiber related genes are involved in several integrated cellular processes involving Ca⁺ signaling pathway, cell wall synthesis, hormone regulation, maintenance of cell turgor pressure and cytoskeleton synthesis pathway signifying complex nature of fiber development yet a controlled metabolic process involving differential expressions of fiber associated gene cascades. A hypothetical model for cellulose and lignin biosynthesis during fiber development in *B. balcooa* has been proposed involving identified genes. The molecular interactions between different predicted proteins were envisaged using Pathway Studio software. The network of various cellular pathways that are either directly or indirectly linked with few nodal proteins were noted of these sucrose synthase serves as a nodal protein which initiates several cellular functions and plays a pivotal role in lignin and cellulose biosynthesis.

Keywords

CLSM, cellulose, ESTs, fiber, lignin,

Introduction

Members of tree bamboo have gained significant attention as non-wood fiber resource during the last two decades. Bamboo fiber made panels and boards are tough, resilient and already proved their potentials as a dependable substitute of hardwood for quality merchandise production. Additionally, fibers of several bamboo species are endowed with high tear index, almost equal strength and durability of papers made from hardwood (Anon, 1997). Bamboo species have the potential to substitute wood in many industrial applications. It quickly restores forest plantations due to the fast growth habit. *Bambusa balcooa* is an abundant tropical species and recognized as priority bamboo species by FAO, amongst eighteen other bamboo species distributed globally (www.unepwcmc.org, www.inbar.int; Das *et al.*, 2005). It is mostly preferred for construction purposes and fiber based mat-board and panel fabrication (Ganapathy, 1997); and also to produce quality paper-pulp (Bhatt *et al.*, 2003) primarily due to its mechanical strength (Kabir *et al.*, 1991).

Prof. Liese (1987) was the pioneer in emphasizing the urgent need of a thorough understanding of the relations between anatomical structures and chemical properties of bamboo fiber for the promotion of bamboo utilization at the best. Unfortunately, despite its enormous potential, little basic investigation has been carried out on precise assessment of bamboo fiber quality (Liese, 1992).

Quality of fiber depends on variable quantities of chemical constituents like hemicelluloses, cellulose, silica, ash, α -cellulose and lignin. Among these, cellulose and lignin are the main determinants of fiber quality. Lignin is one of the major cell wall components rendering the mechanical strength and accounting for approximately 30% of the organic carbon in the biosphere (Boerjan *et al.*, 2003). It is composed of collective phenylpropanoid macromolecules, derived primarily from three cinnamyl alcohols (monolignols) namely, p-coumaryl, coniferyl, and sinapyl alcohols (Sarkanen and Ludwig, 1971). Several attempts have been made to reveal the process of lignin formation and to study the distribution in the cell wall of different tree species (Donaldson *et al.*, 1999; Fromm *et al.*, 2003) involving diverse techniques (Saka and Goring, 1985; Maurer and Fengel, 1991; Donaldson *et al.*, 2001). Although the high lignin content provides stiffness to the bamboo culm yet it has a negative impact on paper production (Bhattacharya *et al.*, 2010). Nevertheless, a comprehensive investigation towards proper evaluation of bamboo-non-wood fiber source is limited (Parameswaran and Liese, 1976; Wang *et al.*, 1980; Xu *et al.*, 2006), especially with respect to lignin contents. It was also felt that there is a dearth of any noninvasive, rapid method to measure cell wall lignin contents accurately (Hatfield and Fukushima, 2005). In the previous study we had employed bright field and confocal laser scanning microscopy (CLSM) technique to reveal the anatomical features of *B. balcooa* to understand the ontogeny of fiber sheaths from parenchymatous pith cells (Bhattacharya *et al.*, 2010). *In situ* estimations of lignin contents in isolated fibers and in sections of lignified fiber-bundles were performed using suitable CLSM based software following orthochromatic labeling with acridine orange. Similarly cellulose contents were estimated labeling with Congo red. Results were verified with the quantitative data obtained from the biochemical assays of lignin and cellulose contents, respectively.

Recently, we have employed PCR-based suppression subtractive hybridization (SSH) between cDNAs isolated from fiber less tissues of leaf and different stages of internodal tissues to identify ESTs/genus involved in fiber development of *B. balcooa* (Rai *et al.*, 2011). An in depth analysis of functions of these ESTs on the background of known functional genes has been presented in this paper; followed by a predicted model involving derived proteins encoded by these genes showing probable pathways of lignin and cellulose formation during the fiber development.

Materials and Methods

Plant materials

Young leaves, 2nd, 5th and 10th internodes (from apex to base) of the secondary branches of mature field grown *Bambusa balcooa* Roxb were collected from the Regional Plant Resource Center, Bhubaneswar, Orissa, India.

Isolation of RNA

Total RNA was isolated from 3.0 gm of leaf apices and 1.0 gm each of 2nd, 5th and 10th internodes of *B. balcooa* grown under natural condition following Rai et al. (2010). The quality and quantity of all RNA samples were checked spectrophotometrically (NanoPhotometer, Sl. No. 1137, Implen, UK).

Subtraction Library Construction

mRNA was purified from the total RNA samples isolated from leaf and internodes of *B. balcooa* using PolyAtract® mRNA Isolation System III (Cat# Z5300, Promega, Madison, WI, USA) following manufacturer's instruction. First strand cDNA was prepared following steps outlined in the Smart cDNA synthesis kit (Super SMART PCR cDNA synthesis kit, Clontech, Palo alto, USA). RsaI-digestion, subtractive hybridization and subsequent PCR amplification were carried out using the Smart PCR-Select cDNA subtraction kit (Cat# 637401, Clontech, Canada, USA) as per manufacturer's instruction. Forward subtraction was performed using cDNA from internodal tissue (2nd, 5th and 10th internodal tissues in 1:1:1 ratio as tester) and that from leaf (as driver). PCR product was purified using Qiaquick PCR purification kit (Cat# 28004, Qiagen, Tokyo), ethanol precipitated, cloned into pGEMT Easy-Vector (Cat# A1360, Promega, Madison, WI, USA) and sequenced (Sanger et al., 1977).

Data Analysis

EST sequences (n=521) were trimmed from the vector and 65 low quality EST sequences were removed with the Phred software using the default parameters. The remaining 456 EST sequences were reprocessed with Univec and VecScreen from NCBI for the removing vector sequence or adaptor contaminant sequences, if any. The program was applied repetitively to the sequences in FASTA format until no further nucleotide could be excluded. Total EST sequences (n=456) were assembled separately into contigs by using Contig Assembly Program 3 (Cap3). Using default values for all the parameters. 156 unigenes were found (contig + singletons) and submitted to NCBI EST Database (Genbank ID: GW687706 to GW687756, GW820160 to GW820235 and HO001786 to HO001814).

Plausible functions for the established contigs and singletons were designated by gene homology based on BLAST with an e-value of 10^{-5} or less. The biological meaning of the unique sequences was investigated according to gene ontology (GO) terms based on BLAST definitions using the program BLAST2GO which is a comprehensive bioinformatics tool for functional annotation and analysis of gene or EST sequences. The distribution of genes in each ontology categories was examined; percentages of unique sequences in each of the assigned GO term namely, biological process, molecular function and species distribution were computed and presented.

Semi quantitative RT-PCR

RT-PCR was performed with the primer-pairs as shown in Table 1 to check the abundance of the transcripts in leaf and in 2nd, 5th and 10th internodal tissues of *B. balcooa* as stated in Rai et al. (2011).

Table 1. Gene specific primer sequences used for transcript expression analyses

PRIMER CODE	FORWARD PRIMER SEQUENCE 5'-3'	REVERSE PRIMER SEQUENCE 5'-3'
Bb.1	TCATCACCCCTCCTGAGTAT	CGTTCTCCCCGTTGATATAG
Bb.2	ATTGTAAATGGCCACGACTT	ACGTTGCTGATCTTCTGTGA
Bb.3	AGCACCCATCTAGCGATAAC	TACTCAGCTGTGGGAGTCAA
Bb.4	TCACCTCCTTCACCTTCTTC	GACCTCGTCAAGAAGCACTC
Bb.5	CCATCAGAGGAGAGCACTTT	ATTCGTTGTCCCTAGTTTCG
Bb.6	TACTCTTGCCTCACAGGATG	CGTGACAAGCAGGATAAGTG
Bb.7	CGCTGCCGTTGATCTATT	GGAGATACGGGAACAGCA
Bb.8	TTCGGGGTGTTTAGCATC	AGGCCAAATGTTGACTGG
Bb.9	ACTTCATGAACCCCAAGGT	CAGTTCGAAGTCTGAACCCTA
Bb.10	ATCTGAGCAGCCTAAGCATT	CAAATGAAATGCACCAAGAA
Bb.11	GCGCAAGCATGAACTAATTT	GAGTTTGAAGCAACCTTCGT
Bb.12	CCATACACCGAGACTGACAA	AAATCCCTCAGATGTGCATT
Bb.13	TCATCACCGACGTGTATCTC	GAGAAGTTCATCACGCACAG
β - Actin	ATGACTCAGATCATGTTTGAG	AGCCTTCGCAATCCACATCTG

Real Time-PCR

Thirteen pairs of gene specific primers were designed to study the differential gene expression levels at different stages of fiber development (Table 1). Real-time PCR reactions were performed with an Opticon-2 qRT-PCR machine (MJ Research, Bio-Rad) using the LightCycler FastStart DNA Master SYBR Premix Ex TaqTM II (Perfect Real Time, Takara Bio Inc.). The PCR reaction mixture contained the following concentrations of reactants: 2 ml of SYBR premix Ex Taq II (1 X concentration), 4 mM MgCl₂, 0.5 mM of each primer, 2 ml of template cDNA template and sterile PCR grade water to a total volume of 20 ml per capillary. Each LightCycler run contained one negative control consisting of distilled water without any template to monitor for possible contamination. The thermal cycling program for the LightCycler has four phases: denaturation, amplification, melting and cooling. In the initial denaturation phase the capillary was heated at 95° C for 10 min, followed by 40 to 45 cycles of amplification phase of 10 s at 95 °C, annealing for 10 sec at 60 °C, and extension for 20 sec at 72 °C. Signal detection was performed at the end of the extension step with a single fluorescence acquisition for each capillary. The melting curve analysis phase began with 95 °C for 10 sec, and then cooled to 73 °C for 30 sec before the temperature was raised to 95 °C at a rate of 0.1 °C/sec. Fluorescence acquisition was performed continuously during this phase. Finally, the cooling phase lasted for 1 min at 40 °C. After completion of amplification reactions, threshold cycle (CT) value for each reaction was obtained and the differences in the transcript level (in fold) between the leaf (control) and 2nd, 5th and 10th internodes were calculated using Pfaffl method (Pfaffl, 2001) keeping the CT value of β -actin as the internal control.

In-situ hybridization

In-situ hybridization was carried out following the protocol of Rai et al (2011). Thin cross sections of 2nd, 5th and 10th internodes of *B. balcooa* were prepared and immediately fixed in FAA (50% ethanol + 10% formalin + 5% acetic acid) at 42°C for overnight. Digoxigenin-labelled RNA probes were synthesized by *in vitro* transcription using 1 μ g of purified and linearized DNA template from putative fiber specific genes V1BB139, V1Bb154 and V1Bb88 digoxigenin-11-UTP and SP6 or T7 RNA polymerases, according to the protocol of the DIG RNA Labelling Kit (Sp6/T7) (Cat# 11 175 025 910, Roche, Mannheim, Germany). The RNA probe was then allowed to hybridize with the pre-

fixed tissue sections at 42°C for overnight. Sections were then washed with DIG Wash and Block Buffer Set (Cat# 11 585 762 001, Roche, Mannheim, Germany) as per manufacturer's instruction. The hybridized mRNA was detected by incubating the sections with anti-digoxigenin antibody conjugated rhodamine (Cat# 11 207 750 910, Roche, Mannheim, Germany) diluted by 1:4 factor for 1 hr at room temperature in dark to avoid photo-bleaching or quenching. After washing, the fluorescence of rhodamine was analysed in a CLSM with a pin hole setting at 1 Array and PMT setting at 1143V by exciting the sections at 488 nm and capturing the emission between 520-530 nm. A few sections were kept as control, which was processed in a similar manner except for probe addition.

Pathway analysis

Pathway Studio 7.1 software (Ariadne Genomics, Rockville, MD, USA) was used to study functional interactions and possible pathways among the proteins encoded by fiber related genes (Nikitin et al., 2003). The accession numbers (NCBI nr) of these proteins were converted to TAIR (The Arabidopsis Information Resource) IDs by performing TAIR BLAST 2.2.8. To establish the relationship between proteins and cellular processes, the TAIR IDs were imported to Pathway Studio 7.1 and an interaction network was constructed. Each identified cellular process was confirmed via the Pub Med/Medline hyperlink embedded in each node.

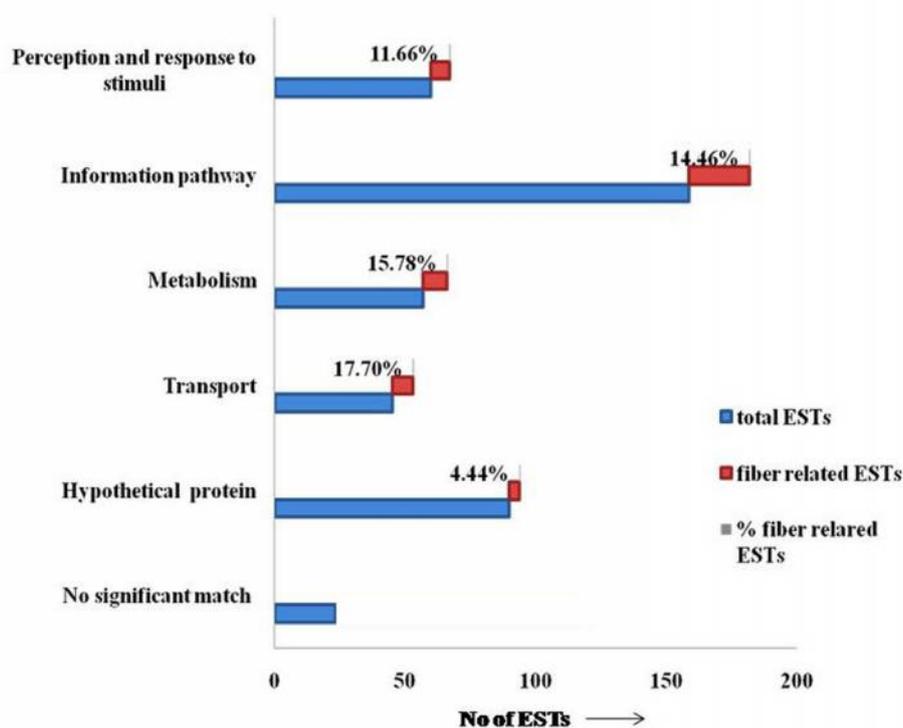


Fig. 1

Fig. 1. Functional categorization of 456 ESTs obtained from forward SSH library into 6 sub-clusters depending on their physiological roles. Out of which 50, 57, 165 and 63 ESTs were categorized under transport, metabolism, information pathway and stimuli responses, respectively while 89 ESTs were under the hypothetical proteins and the remaining were non-significant. The percentage of fiber specific ESTs under different categories is presented on the top of respective bars.

Results and Discussion

Among 456 ESTs isolated by forward SSH, of which ca. 75% were clustered into 41 contigs and remaining ESTs were singletons (n=115, Table 2).

Table 2. Classification of ESTs obtained from forward SSH cDNA library of *Bambusa balcooa* generated from mRNA isolated from leaf (apical) and internodes (2nd, 5th and 10th internodes in 1:1:1 ratio).

Sl. No.	Descriptive Category	Values
1.	No. of high quality ESTs	456
2.	Mean length of ESTs	422
3.	EST size range (bp)	150-1199
4.	No. of singeltons	115
5.	No. of contiguous sequence	41
6.	No. of unigenes	156

Functional annotation of *B. balcooa* unigenes

All the *B. balcooa* unigenes (n=156) were searched by BLASTX for homology analysis against the protein sequences deposited in public databases. These unigenes were further annotated on the basis of existing annotation for the proteome of other species, in which functions were categorized according to the Gene Ontology Consortium. During the annotation, when multiple hits were found, the one with the lowest E-value was selected.

Functional categorization of 456 ESTs obtained from forward SSH library into 6 sub-clusters depending on their physiological roles. Out of which 50, 57, 165 and 63 ESTs were categorized under transport, metabolism, information pathway and stimuli responses, respectively; while 89 ESTs were under the hypothetical proteins and the remaining were non-significant (Fig. 1). Relative abundance of fiber specific ESTs under the above stated functional categories are computed, of which transport, metabolism and information pathway related ESTs are more abundant than other ESTs (Fig. 1).

Thirty-nine percent of the classified genes are categorized in relation to the metabolic process, 30.8% related to the cellular process followed by biological regulation, cellular localization, response to stimulus, signaling and developmental process, which are typical to growing tissues (Fig. 2 A). Results of this detail analysis under each of these functional categories are depicted in Figure 2 B.

Among the 156 unigenes, 51 (approximately 33%) were found to be directly or indirectly related to fiber developmental process in other plants. Interestingly, it was noted that bamboo fiber related genes/ESTs were largely distributed among different monocot species. In order to investigate gene conservation these unigenes were compared with the available gene indices (GI) of other species using BLAST2GO software. Maximum sequence match was obtained with *Oryza sativa* followed by *Sorghum bicolor*, *Brachypodium distachyon* (the model grass), as expected. Matches were also found in other plant species, viz. *O. officinalis*, *O. grandiglumis*, *Hordeum vulgare* and with two other bamboo species (Fig. 3). Since availability of information on bamboo genome is limited, therefore top hit scores are low with other bamboo species, viz. *Phyllostachys edulis* and *Bambusa oldhamii*.

Differential transcript analysis at different stages of fiber development

In our previous study we have shown that 2nd, 5th and 10th internodes is representing fiber initiation, elongation and maturation stages (Rai et al, 2011). Expression of V1Bb45 (eukaryotic initiation factor 4A) gene was only detected during fiber elongation and was maximum during fiber maturation

(Fig. 4 A, B). Transcripts of V1Bb56 (Fiber protein Fb2) was detected only during fiber elongation. V1Bb77 (heat shock protein HSP82) transcripts were found to be much higher in the 5th and 10th internodes signifying the involvement of these genes in bamboo fiber elongation and maturation. Higher expression of V1Bb88 gene encoding MYB-domain containing protein was found at the fiber initiation stage, which was more pronounced at the fiber maturation stage. Possibly this MYB domain interacts with AC elements of enzymes involved in lignin biosynthetic genes to promote lignin biosynthesis (Deluc et al., 2006; Patzlaff et al., 2003).

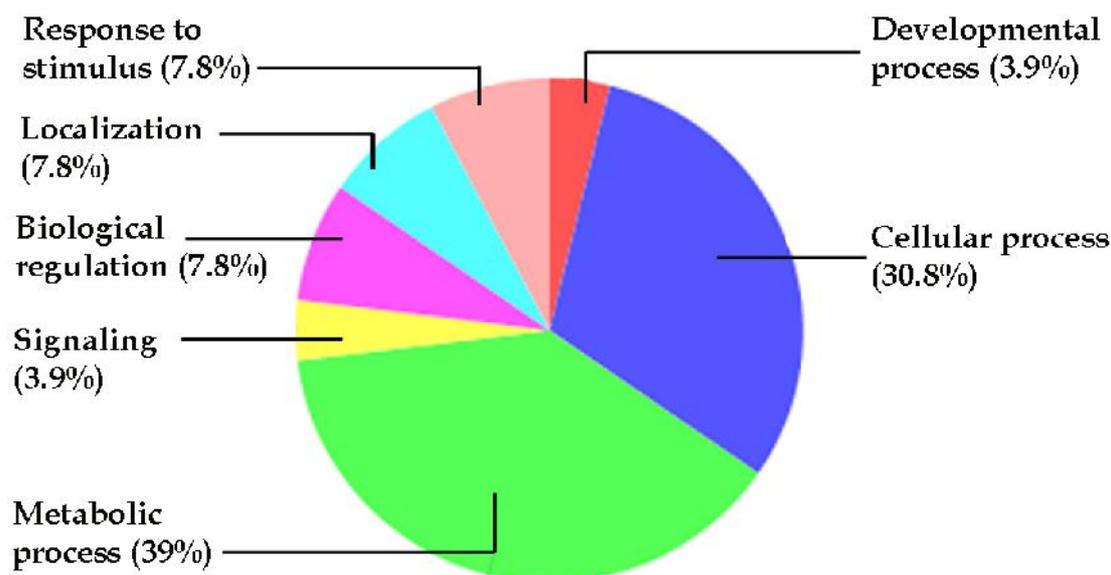


Fig. 2 A

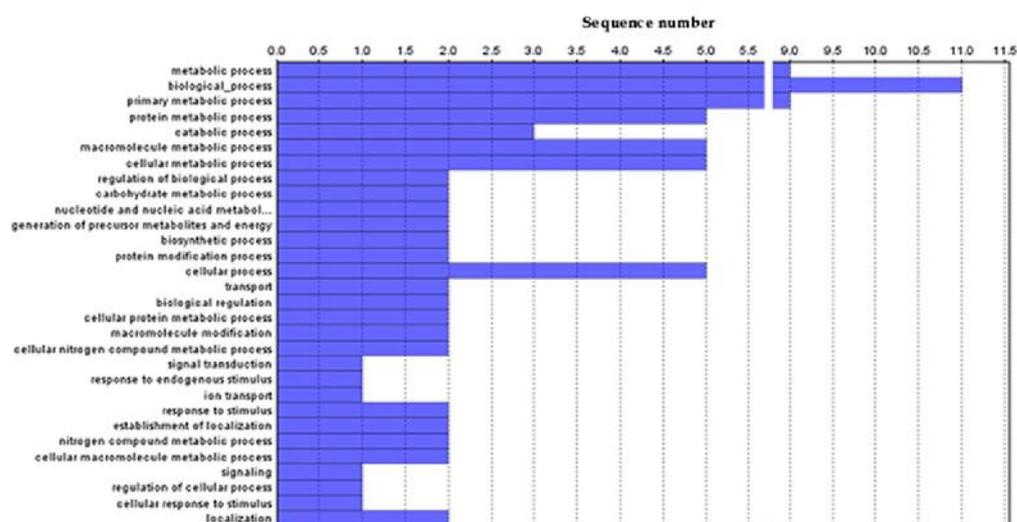


Fig. 2B

Fig. 2A.

Identified ESTs that are expressed during different stages of fiber development in *B. balcooa* were grouped with assigned gene ontology (GO) criteria using Blast2GO software. B. Functional categorization of *B. balcooa* unigenes obtained from forward SSH library involved in fiber development.

Transcripts of V1Bb97 (1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 4) gene were not detected in leaf and in the 2nd internodal tissues, while their expression were detected in the 5th internodal tissues and expression was maximum in the 10th internodes, suggesting that this gene may have an effective role in fiber maturation. The expression study suggests that V1Bb137 (ZIP), V1Bb147 (protein kinase-like protein) and V1Bb139 (acetyltransferase 1-like) genes have a positive role in fiber initiation, while the later also involved in fiber maturation. Transcripts of V1Bb154 (sucrose synthase) was present throughout fiber development, but maximum in the 5th internodes (Fig. 4 A, B). The putative role of some of these above mentioned genes in fiber initiation and elongation have been reported in cotton (Wu et al., 2006, Lee et al., 2007). From molecular perspective, phenomenon of fiber development is a transcriptionally controlled event and physiological changes associated with the development could be accounted for differential expressions of different fiber specific genes.

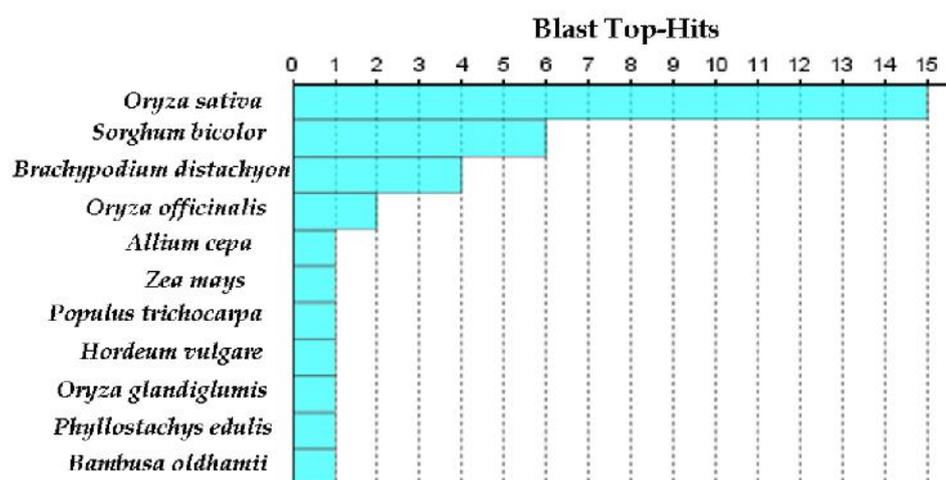


Fig. 3

Fig. 3. Homologs of fiber development related *B. balcooa* 51 unigenes found in other plant species. Graph generated using Blast2GO program based on homology search shows species distribution of unigenes from combined dataset using BLASTX against non-redundant protein database. Top 10 BLAST hits were considered for each transcript.

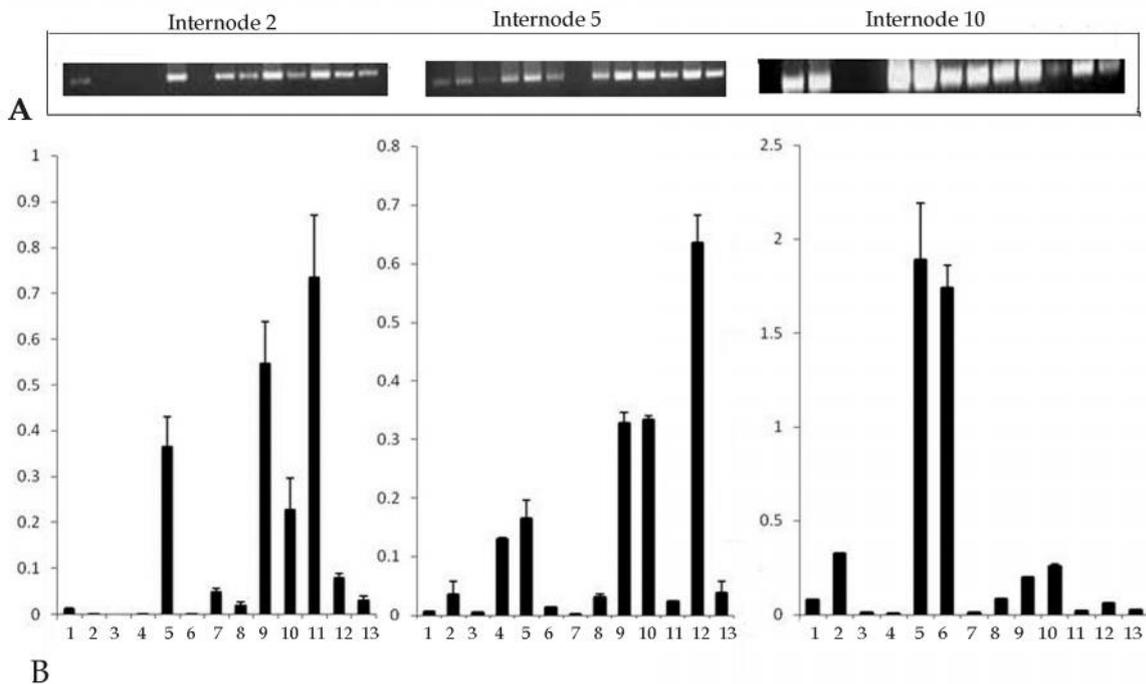


Fig. 4

Fig.

4. Analyses of expression patterns of 13 fiber specific genes of *B. balcooa* in different internodes using semi-quantitative PCR (A) and real time PCR (B).

A. Differential expression patterns of 13 fiber specific transcripts among 2nd, 5th and 10th internodes of *B. balcooa* detected by RT-PCR. Amplified products were run on 1.2% agarose gel. Transcripts in Lane 1= V1Bb26; Lane 2 = V1Bb45; Lane 3 = V1Bb56; Lane 4 =V1Bb77; Lane 5= V1Bb88; Lane 6 = V1Bb97; Lane 7 = V1Bb123; Lane 8 = V1Bb130; Lane 9 = V1Bb137; Lane 10 = V1Bb139; Lane 11 = V1Bb147; Lane 12 = V1Bb 154 and Lane 13 = V1Bb177.

B. Real time PCR was conducted to measure the differential expression of 13 putative fiber specific transcripts (same as shown in the legend of Fig. 2A) among 2nd, 5th and 10th internodes of *B. balcooa*. β -actin transcript was used as an internal control, while RNA from the leaf was used as a base line control to measure the relative up-regulation of fiber specific genes in different internodes of *B. balcooa*. The fold differences in the level of expression of respective cDNA in different internodes were presented as mean of three independent experiments with respective +/- SD.

***In situ* localization of fiber-specific genes**

In situ localization study using fluorescent riboprobes of three randomly selected cDNAs authenticates expression of these genes (VIBb88, VIBb139, VIBb154) in the vascular bundle region (Fig. 5) at all the three stages of fiber development.

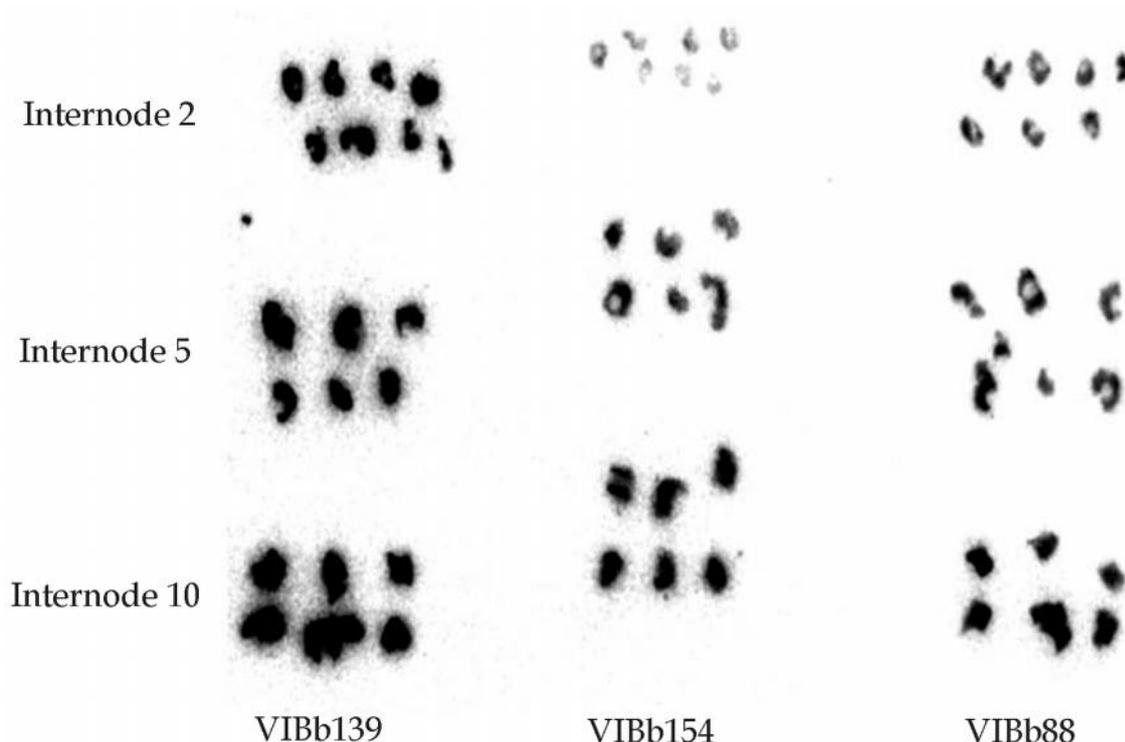


Fig. 5

5. Hybridization of VIBb139 (Acetyl transferase), VIBb154 (sucrose synthase) and VIBb88 (myb domain protein) with thin cross-sections of 2nd, 5th and 10th internodes of bamboo as described in methods.

Fig.

Distribution of fiber related genes at three distinct stages of fiber development

Venn diagram has been generated to illustrate distribution of fiber related genes, of which 14 are consistently present in all the developmental stages, while 2, 3 and 2 genes are exclusively expressed during the fiber initiation, elongation and maturation stages, respectively; while others are overlapping between any two stages (Fig. 6).

It was found that VIBb11 (ataxin-2 C-terminal region family protein), VIBb178 (plasminogen activator inhibitor 1 RNA-binding protein), VIBb83 (metallothionine-like protein type 2), VIBb24 (oryzain gamma chain precursor), VIBb59 (vacuolar H⁺-ATPase catalytic subunit), VIBb76 (calcium binding EF hand protein-like), VIBb88 (myb domain-containing protein), VIBb101 (Rho related protein from plant 2), VIBb116 (BRI 1-associated receptor kinase 1 precursor), VIBb130 (RHM1), VIBb137 (ZIP), VIBb139 (acetyltransferase 1), VIBb147 (protein kinase-like protein) and VIBb154 (sucrose synthase) genes were expressed simultaneously in all internodal tissues, although there were variations in their level of expressions. While some other genes were expressed exclusively in specific internodes, these includes VIBb58 (ABC transporter family protein) and VIBb97 (1, 2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 4) in 10th internode; VIBb21 (calcyclin-binding protein) and VIBb77 (heat shock protein HSP 82) in 5th internode; VIBb23 (ethylene response

element binding protein) and V1Bb26 (GSH-dependent dehydroascorbate reductase 1) in 2nd internode. Still others act as links for the transition from one stage of development to the next and can be grouped as V1Bb64 (eukaryotic initiation factor 5A1) and V1Bb45 (eukaryotic initiation factor 4A) for 5th to 10th internode transition; V1Bb119 (ankyrin-like protein) for 2nd to 5th internode transition.

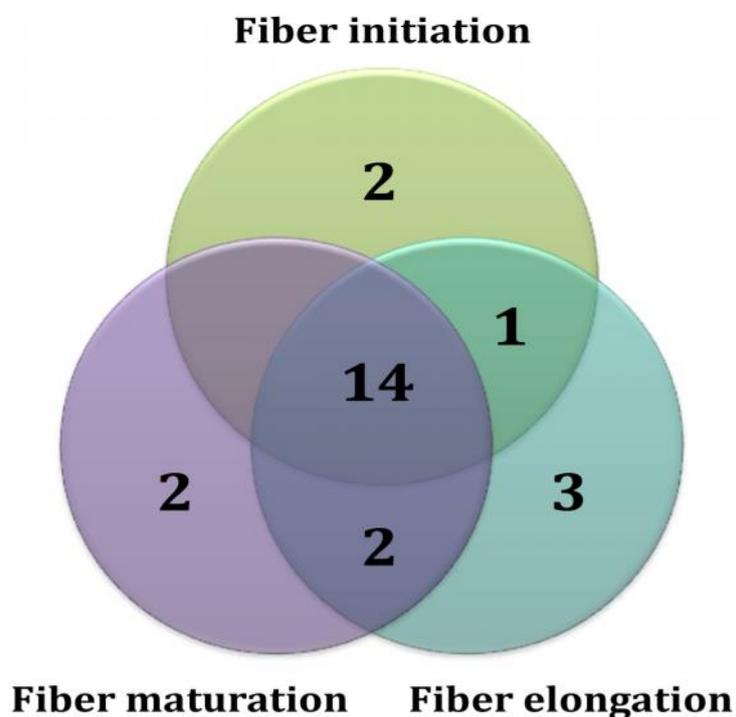


Fig. 6

Fig. 6. Venn diagram showing the overlapping pattern of fiber related genes at different stages of fiber developmental in *B. balcooa*.

Proposed model for cellulose and lignin biosynthesis during fiber development and participation of relevant genes

A model has been proposed emphasizing lignin and cellulose biosynthesis during fiber development, based on the available genomic information (Fig.7). It is suggested that the plasma membrane-associated rosette assembly of cellulose synthase (CelS) is the catalytic unit of cellulose synthesis that triggers the functional cellulose synthesis machinery during fiber development. Cellulose synthesis in plants is carried out by the plasma membrane-associated rosette structure of CelS, which has the UDP-glucose binding activity and possessed β -1,4-glucan (cellulose) synthesis activity. Monomers of CelS when oxidized in presence of Rac-like GTP binding protein (VIBb 101), metallothionine (VIBb74) or thioredoxin (VIBb 28) form dimeric structure which assembled to appear as rosette structure as shown in the model (Fig.7). It was demonstrated by Fujii et al. (2009) that sucrose synthase (SuSy; VIBb154) is an integral component of the cellulose synthesis machinery. In a contemporary investigation Coleman et al (2009) have clearly shown that SuSy is a key regulator of sink strength in poplar trees and demonstrated the close association of SuSy with cellulose synthesis and secondary wall formation. In cotton it was found that suppression of SuSy gene expression inhibited cotton fiber cell initiation and elongation (Ruan et al., 2003). Fujii et al. (2010) designated SuSy as the catalytic sub-unit of CelS which catalyzes degradation of sucrose to UDP-Glucose that serves as the substrate for cellulose biosynthesis. Recently Brill et al. (2011) have identified a novel SuSy that is targeted to the cell wall

during secondary cell wall synthesis in cotton fiber and it was proposed that cell wall-localized SuSy may provide UDP-glucose for cellulose and callose synthesis from extracellular sugars as shown in the model (Fig. 7).

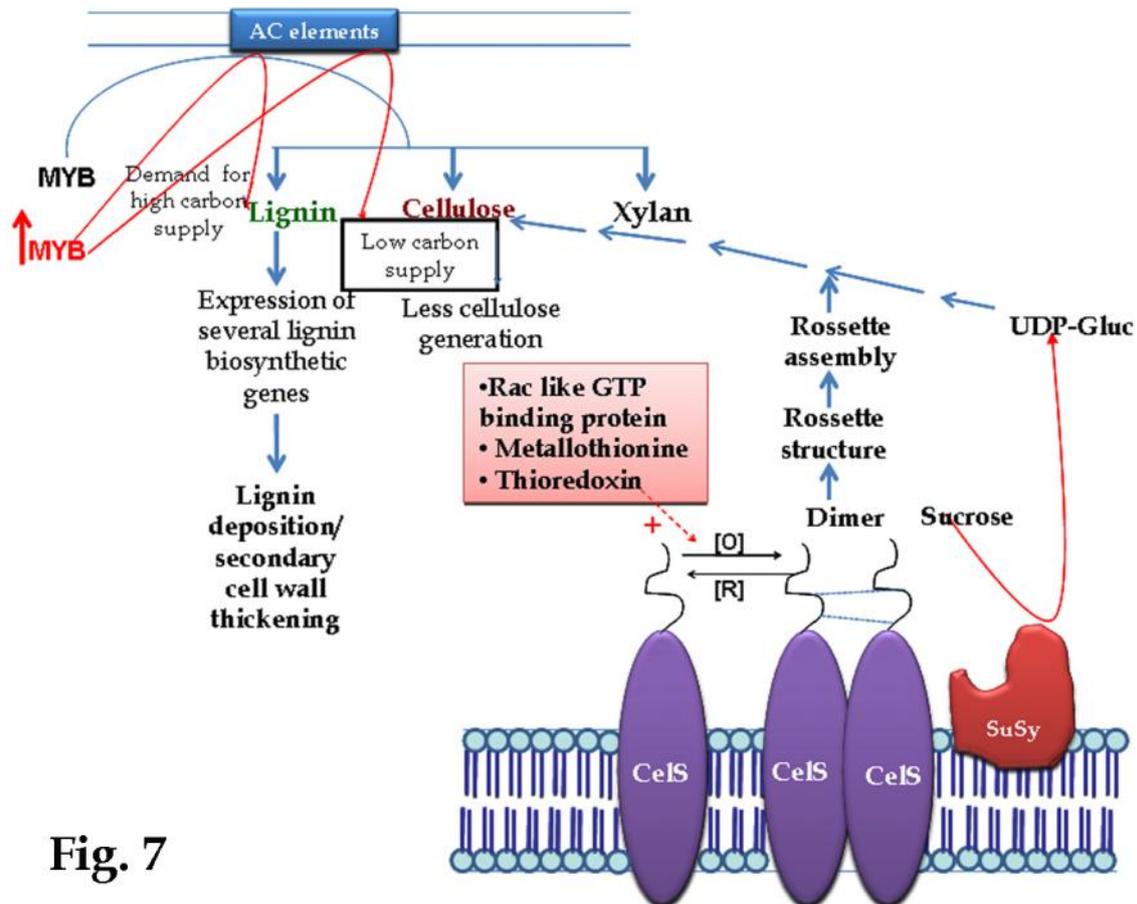


Fig. 7

Fig. 7. Hypothetical model depicting molecular basis of differential interplay of different key genes involved in cellulose and lignin biosynthesis during fiber development in *B. balcooa*.

Thus, SuSy plays a dual role in producing both sucrose and UDP-glucose necessary for cell wall and cellulose biosynthesis. Lignin is the polymerized form of monolignols that are synthesized through the lignin biosynthetic pathway. All the genes in the lignin biosynthetic pathway have to be turned on in an orchestrated manner during lignin biosynthesis. It has been shown that a common cis-element, namely the AC element, is present in the majority of the lignin biosynthetic genes and required for their expression in lignifying cells (Zhong and Ye, 2009). It has been shown that MYB-transcription factors bind to the AC elements and are involved in the coordinated regulation of lignin biosynthesis (Deluc et al., 2006; Patzlaff et al., 2003). It was also found that the transcriptional regulation of lignin biosynthesis is under the control of the same transcriptional network regulating the biosynthesis of other secondary wall components, including cellulose and xylan (Zhong and Ye, 2009). However, cellulose synthesis declines when the carbon supply is low. This model depicts that when the expression of MYB domain protein is high then lignin biosynthesis occurs and cellulose biosynthesis is suppressed as the availability of carbon supply is low. On the other hand cellulose biosynthesis occurs when the expression of MYB is less as CeIS is substrate dependent. In the presence of ample substrate CeIS dimerizes in oxidized condition.

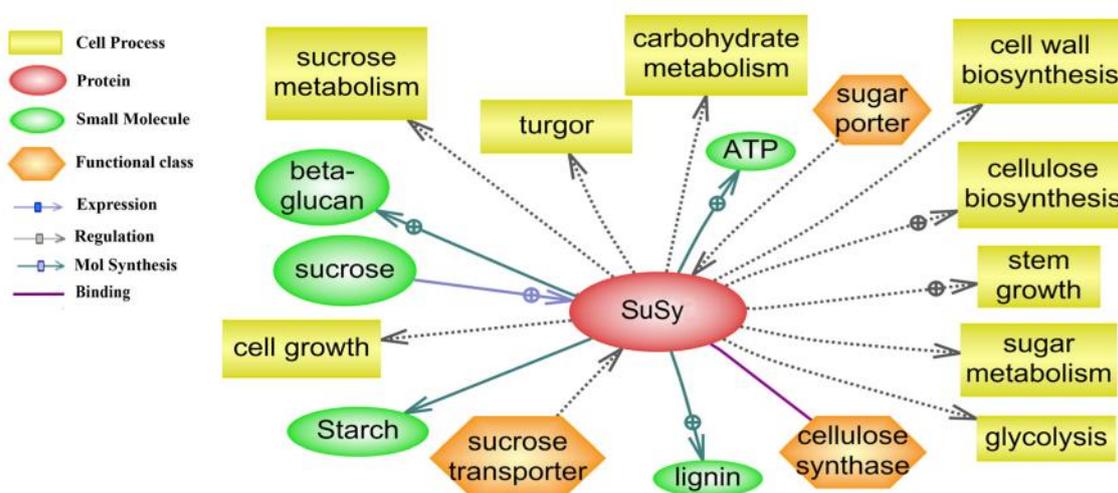


Fig. 8

Fig. 8. Pathway studio analysis revealed involvement of sucrose synthase (SuSy, V1Bb 154) in inducing different important cellular processes that regulate *B. balcooa* fiber development.

The key role of sucrose synthase as a nodal protein which initiates various supportive cellular processes during fiber development has also been predicted using the programme of Pathway Studio software employing fiber related genes of *B. balcooa*. SuSy triggers cell wall biosynthesis, cellulose biosynthesis, sucrose metabolism, cell growth etc. as depicted in the Figure 8. During fiber development committed cells are growing rapidly and growth of such cells is supported by SuSy necessitating rapid nucleic acid synthesis. Enhanced expressions of V1Bb45 (eukaryotic initiation factor 4A), V1Bb158 (ATP/GTP-binding protein) and V1Bb184 (putative poly-A-polymerase) contributes towards nucleic acid biosynthesis either directly or indirectly. Possibly, ESTs like V1Bb26 (chloride intracellular channel 6), V1Bb58 (ABC transporter family protein), V1Bb59 (vacuolar H⁺/-ATPase catalytic subunit) and V1Bb125 (vacuolar ATP synthase catalytic subunit A) are maintaining the turgor pressure and thus implicated in bamboo fiber development. Ruan et al. (2001) also suggested that these genes are involved in maintaining the cell turgor pressure during cotton fiber development.

All these findings firmly indicate that the involvement of several factors belonging to different metabolic and signaling pathways plays a crucial role in bamboo fiber development. Synchronous functioning of all such genes together with genetic control and environmental conditions ultimately determines the overall physiology and the inherent character of bamboo fiber.

Acknowledgements

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References

- Anon. 1997. Provisional outlook for global forest products consumption, production and trade: FAO. Forestry Department, Policy and Planning Division. Rome.
- Bennetzen, J.L.; Coleman, C.; Liu, R.; Ma, J.; Ramakrishna, W. 2004. Consistent over-estimation of gene number in complex plant genomes. *Current Opinion in Plant Biology*, 7, 732–736.
- Bhatt, B.P.; Singh, L.B.; Singh, K.; Sachan M.S. 2003. Some commercial edible bamboo species of North East India: Production, indigenous uses, cost-benefit and management strategies. *Bamboo Science & Culture*, 17, 4-20.
- Bhattacharya, S.; Ghosh, J.S.; Sahoo, D.K.; Dey, N.; Pal, A. 2010. Screening of superior fiber-quality-traits among wild accessions of *Bambusa balcooa*: Efficient and non-invasive evaluation of fiber developmental stages. *Annals of Forest Science*, 67 (6), 611.
- Boerjan, W.; Ralph, J.; Baucher, M. 2003 Lignin biosynthesis. *Annual Review of Plant Biology*, 54, 519-546.
- Brill, E.; van Thournout, M.; White, R.G.; Llewellyn, D.; Campbell, P.M.; Engelen, S.; Ruan, Y.L.; Arioli, T.; Furbank, R.T. 2011. A novel isoform of sucrose synthase is targeted to the cell wall during secondary cell wall synthesis in cotton fiber. *Plant Physiology*, 157(1), 40-54.
- Coleman, H.D.; Yan, J.; Mansfield, S.D. 2009. Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proceedings National Academy of Sciences*, 106 (31), 13118-13123.
- Das, M.; Bhattacharya, S.; Pal, A. 2005. Generation and Characterization of SCARs by Cloning and Sequencing of RAPD Products: A Strategy for Species-specific Marker Development in Bamboo. *Annals of Botany*, 95, 835-841.
- Deluc, L.; Barrieu, F.; Marchive, C.; Lauvergeat, V.; Decendit, A.; Richard, T.; et al. 2006. Characterization of a grapevine R3R3-MYB transcription factor that regulates the phenylpropanoid pathway. *Plant Physiol.* 140:499–511.
- Donaldson, L.A.; Hague, J.; Snell, R. 2001. Lignin distribution in coppice poplar, linseed and wheat straw. *Horzforschung* 2001, 55, 379-385.
- Donaldson, L.A.; Singh, A.P.; Yoshinaga, A.; Takabe, K. 1999 Lignin distribution in mild compression wood of *Pinus radiata*. *Canadian Journal of Botany*, 77, 41–50.
- Fromm, J.; Rockel, B.; Windeisen, E.; Wanner, G. 2003. Lignin distribution in wood cell walls determined by TEM and backscattered SEM techniques. *Journal of Structural Biology*, 143, 77–84.
- Fujii, S.; Hayashi, T.; Mizuno, K. 2009. Sucrose synthase is an integral component of the cellulose synthesis machinery. *Plant and Cell Physiology*, 5 (2); 294-301.
- Ganapathy, P.M. 1997. Sources of non wood fiber for paper, board and panels production: status, trends and prospects for India. Asia-pacific forestry sector outlook study working paper series, Working Paper No: APFSOS/WP/10. Forestry Policy and Planning Division, Rome Regional Office for Asia and the Pacific, Bangkok. 59 pp.
- Kabir, M.F.; Bhattacharya, D.K.; Sattar, M.A. 1991. Physical and mechanical properties of four bamboo species. *Bangladesh Journal of Forest Science*, 20, 31-36.
- Lee, J.J.; Woodward, A.W.; Chen, Z.J. 2007. Gene expression changes and early events in cotton fibre development. *Annals of Botany*, 100, 1391–1401.
- Liese, W. 1987. Anatomy and properties of Bamboo. In: *Recent Research on Bamboos*. Edited by Rao, A.N., Dhanarajan, G., Sastry, C.B. Canada: Chinese Academy of Forestry, China and International Development Research Centre, 196-208.
- Liese, W. 1992. The structure of bamboo in relation to its properties and utilization. In *Proceedings International Symposium on Industrial use of Bamboo (Bamboo and its use)*: Beijing, China, 7-

- 11 December 1992. International Tropical Timber Organization, Chinese Academy of Forestry, China.
- Maurer, A.; Fengel, D. 1991. Electron microscopic representation of structural details in softwood cell walls by very thin ultramicrotome sections. *Holz als Roh- und Werkstoff*, 49, 53–56.
- Nikitin, A.; Egorov, S.; Daraselia, N.; Mazo, I. 2003. Pathway studio—the analysis and navigation of molecular networks. *Bioinformatics*, 19, 2155–2157.
- Parameswaran, N.; Liese, W. 1976. On the fine structure of bamboo fibers. *Wood Science and Technology*, 10, 231–246.
- Patzlaff, A.; McInnis, S.; Courtenay, A.; Surman, C.; Newman, L.J.; Smith, C.; et al. 2003. Characterisation of a pine MYB that regulates lignification. *Plant Journal*; 36:743–754.
- Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, 29(9), e45.
- Rai, V.; Ghosh, J.; Dey, N.; 2010. Isolation of total RNA from hard bamboo tissue rich in polyphenols and polysaccharides for gene expression studies. *Electronic Journal of Biotechnology*, 13,17. DOI 10.2225.
- Rai, V., Ghosh, J.S.; Pal, A.; Dey, N. 2011. Identification of genes involved in bamboo fiber development. *Gene*, 478, 19–27.
- Ruan, Y.L.; Llewellyn, D.J.; Furbank, R.T. 2001. The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and K⁺ transporters and expansin. *Plant Cell*, 13(1), 47–60.
- Ruan, Y.L.; Llewellyn, D.J.; Furbank, R.T. 2003. Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development. *Plant Cell*. 15(4), 952–964.
- Saka, S.; Goring, D.A.I. 1985. Localization of lignins in wood cell walls. In: *Biosynthesis and Biodegradation of Wood Components*. Edited by Higuchi, T., Academic Press, New York, pp. 141–160.
- Sanger, F.; Nicklen, S.; and Coulson, A.R. 1997. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, 74(12), 5463–5467.
- Sarkanen, K.V.; Ludwig, C.H. 1971. Definition and nomenclature. In: *Lignins, occurrence, formation, structure and reactions*. Edited by Sarkanen, K.V.; Ludwig, C.H., Wiley-Interscience, New York, pp.1–18.
- Wang, C.P.; Yu, Z.H., Ye, G.H.; Chu, C.D.; Chao, C.S.; Chen, S.Y.; Yao, C.Y.; Zhao, H.R. 1980. A taxonomical study of *Phyllostachys*. *Acta Phytotaxonomica Sinica*, 18, 168–193.
- Xu, F.; Zhonga, X.C.; Sunb, R.C.; Luc, Q. 2006. Anatomy, ultrastructure and lignin distribution in cell wall of *Caragana korshinskii*. *Industrial Crops and Products*, 24.86–193.
- Zhong, R.; Ye Z-H. 2009. Transcriptional regulation of lignin biosynthesis. *Plant Signal and Behaviour*. 4(11), 1028–1034.

Life Cycle Assessment and Carbon Sequestration; the Environmental Impact of Industrial Bamboo Products

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Abstract

Life Cycle Assessment (LCA) is the commonly accepted methodology to systematically assess the environmental impact of a product or material over the full life cycle, thus from the extraction of resources until the end phase of demolition or recycling (from cradle till grave).

The objective of this study is two-fold. The first objective is to gain a better understanding about the environmental impact of industrial bamboo products and their production processes in terms of their CO₂ equivalent (carbon footprint), toxic emissions, and materials depletion (LCA). The second objective is to clarify how carbon sequestration on a global scale can be defined and calculated for bamboo products, and can be incorporated in the standard LCA calculations.

The study concludes that industrial bamboo products, if based on best-practice technology (in this case production chain of Moso International), even when used in Europe can – depending on the assumptions made - be labelled as being CO₂ neutral.

Keywords

Life Cycle Assessment (LCA); Carbon footprint, Industrial Bamboo products

1. Introduction & Goal

The growing human population in combination with an increase of consumption per capita, is putting more and more pressure on global resources, which results in materials depletion, ecosystem deterioration and human health problems. Because of its rapid growth and applicability, giant bamboo species such as *Phyllostachys pubescens* are perceived as being an environmentally benign alternative that could act as a promising renewable material. In this paper the sustainability of industrial bamboo materials is analysed using Life Cycle Assessment (LCA), analysing a range of environmental effects along the production chain over the full life cycle of a product.

The objective of this study is to gain a better understanding about the environmental impact of industrial bamboo products and their production process in terms of Green House Gas (GHG) balance (carbon footprint), toxic emissions, and materials depletion.

There is a distinction of two issues of carbon sequestration in natural renewable products (like wood, bamboo and agricultural products):

1. the issue of biogenic CO₂ in the life cycle of a product (from cradle-to-grave), which is the domain of standard LCA analyses
2. the issue of the global CO₂ cycles and global storage of CO₂, which is dealt with in LCA by calculation the change of sequestered carbon in relation to transformation of land to bamboo plantations.

Discussions on carbon sequestration are often blurred, since the aforementioned distinction in system levels are often not made clear. This leads to a secondary goal of this paper:

- to clarify the LCA calculation as such, and the way “biogenic CO₂” is dealt within the life cycle
- to clarify how carbon sequestration on a global scale can be defined and calculated for bamboo products, and can be incorporated in the standard LCA calculations

The analyses on biogenic CO₂equivalents in LCA and carbon sequestration on a global scale are according to 2 recent scientific books on this subject (ILCD 2010) (Vogtländer 2010).

2. Scope & Methodology

This study is based on the production process of the company Moso International for all solid bamboo products of this company, i.e. bamboo flooring, panels, veneer and decking. In this paper the LCA results for one typical product is selected for analysis: carbonized 3-layer laminated bamboo board. Details about the other products can be found in (Vogtländer 2011).

The analyses in this paper comply with the ISO specifications (ISO 14040 and 14044) and the manual for LCA (ILCD 2010). Details on the calculations, including detailed production data, have been published in peer reviewed papers (Vogtländer et al. 2010a) and books (Van der Lugt et al. 2009, van der Lugt 2008).

Note: This LCA has been performed for the specific case of the Moso production chain following best practice and can therefore not be perceived as being typical for the production chain of other industrial bamboo material manufacturers.

The system boundary of this LCA is “cradle-to-warehouse-gate” plus “end-of-life” as depicted in Fig. 1. The Use-Phase has been excluded from the analyses, because the emissions in this step are less than 1% (in comparison to the first and the last step)

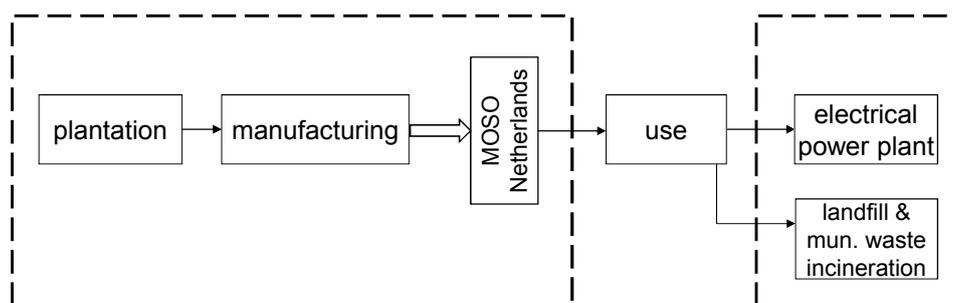


Figure 1: System boundary: cradle-to-gate plus end-of-life.

The LCA methodology is internationally standardized in the ISO 14040 series, and measures the environmental impact in several categories, including resource depletion, air quality (dust, smog), toxicity and Global Warming Potential (GWP). In some life cycle impact assessment methodologies the environmental impact caused by a product can be aggregated and expressed under one number, for example expressed in eco-costs (Vogtländer et al., 2010b). Given the increasing attention with respect to global warming, the GWP of products is often assessed separately in a so-called ‘carbon footprint’. In this assessment all the greenhouse gas emissions during the life cycle of a product are measured and expressed as kg CO₂ equivalent (in short CO₂e).

After the first step in LCA, the Goal and scope definition, the core of the LCA method comprises two basic steps: the Life Cycle Inventory (a list of emissions and used materials) and the Life Cycle Inventory Analyses (a system to express the result of a LCI in one score, the so called “single indicator”) (ISO 14044).

For this study, two single indicators are used:

- the CO₂e (“carbon footprint”) , which can easily be understood and explained, but excludes other polluting emissions (like SO_x, NO_x, carcinogens, fine dust, etc.) that have effects in other impact categories
- the “eco-costs” system which incorporates 3000 polluting substances, as well as materials depletion (Vogtländer et al. 2010b)

3. Scientific Background of LCA and the CO₂ cycle

Additional to the standard LCA (ISO 14040 and 14044), the sequestration (= capture and storage) of CO₂ has been taken into account in this study, in line with (ILCD 2010). Sequestration of CO₂ in wood is an important issue in sustainability.

3.1. Carbon Sequestration at Product Level

There is consensus in science on the way “biogenic CO₂” (related to the carbon which is captured in wood during the growth of a tree) is to be handled in LCA, see Fig.2.

Biogenic CO₂ is first taken out of the air at the bamboo plantation, and then released back to the atmosphere at the End of Life. So biogenic CO₂ is recycled, and its net effect on global warming is zero.

When the bamboo product, however, is burnt at end-of life in an electrical power plant, the total system of figure 2 generates electricity. This electricity can replace electricity from other sources, including fossil fuels. In other words: the use of fossil fuels and the emissions of fossil CO₂ is consequently avoided, which results in a reduction of potential global warming effects. In LCA calculations this can be used as a system credit: the production of electricity from bamboo waste has a negative carbon footprint and negative eco-costs.

The conclusion is that the storage of biogenic CO₂ (carbon sequestration) in bamboo is not counted in LCA (ILCD, 2010), unless the bamboo (or any other bio-product like wood) is burned for electricity or heat.

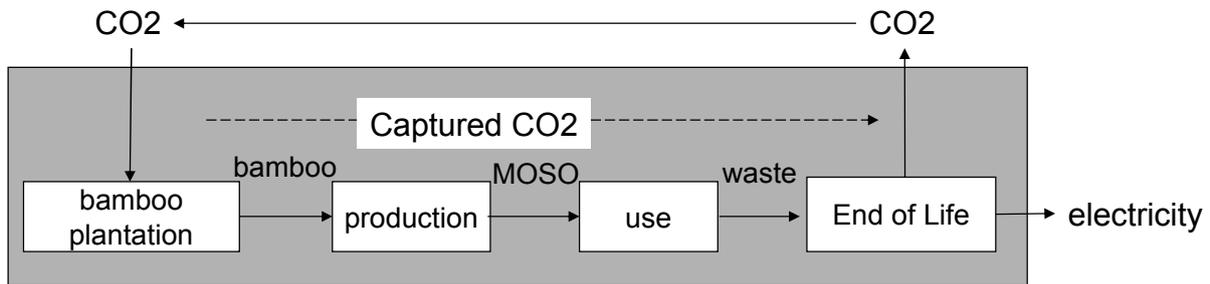


Figure 2: The CO₂e cycle on a product level.

3.2. Carbon Sequestration at Global Level

The effects of carbon sequestration can be understood when we look at a global system level. On a global scale, CO₂ is stored in forests (and other vegetation), in the ocean, and in products (buildings, furniture, etc). A good overview of the global carbon cycle and sequestration of carbon in forests is depicted in Fig. 3 (source NASA Earth Science Enterprise). A short explanation of this Figure is given at the website of the NASA:

http://earthobservatory.nasa.gov/Library/CarbonCycle/carbon_cycle4.html

The issue is that the human role of the CO₂ emissions is three-fold:

5.5 Gt carbon emissions per year caused by burning of fossil fuels

1.6 Gt carbon emissions per year caused by deforestation in tropical and sub-tropical areas

0.5 Gt carbon sequestration per year by re-growth of forests on the Northern Hemisphere.

So it can be concluded that the global carbon cycle can significantly be improved in the short term by:

- less burning of fossil fuels
- stopping deforestation
- forest conservation by better management and wood production in plantations
- afforestation (planting of trees on soils that have not supported forests in the recent past)

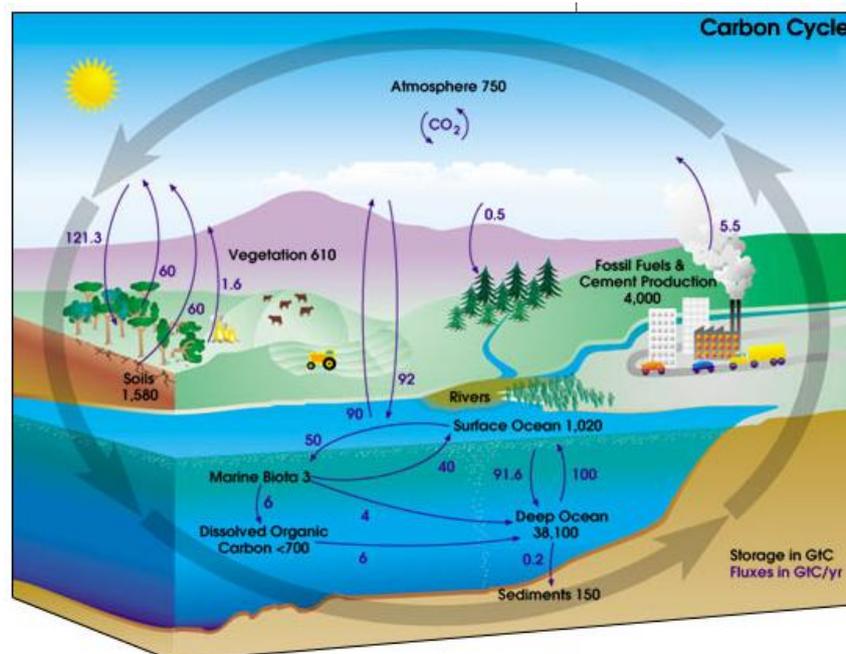


Figure 3: The global carbon cycle (source NASA)

It is far too simple to say that “application of wood in design and construction will lead to carbon sequestration, and therefore it will counteract global warming”. It depends on the type of wood.

There are two issues:

- carbon sequestration of wood in the forests
- carbon sequestration of wood in the houses, offices, etc. during the life time

One should realise that, if there is *no change* in the area of forests and *no change* in the volume of wood in houses, offices, etc., there is *no change* in sequestered carbon. Then, there is no effect on carbon emissions.

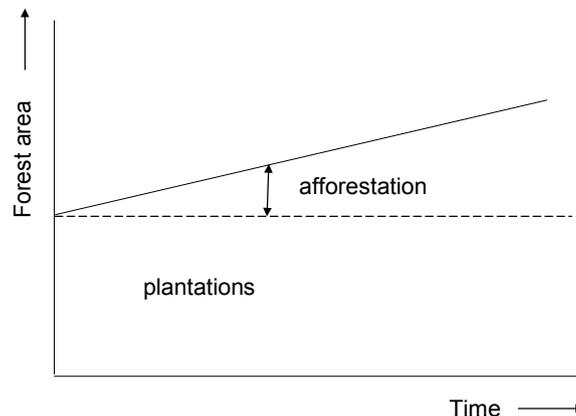


Figure 4: More demand of European wood leads to afforestation (extra forests) in Europe and more carbon sequestration

Only when the global area of forests is increasing, and when the total volume of wood in houses, offices, etc. is increasing, there will be extra carbon sequestration. This is the situation for European wood. See Fig. 4.

So, the issue is related with the *global growth* of production and demand of wood.

The situation is different for tropical hardwood.

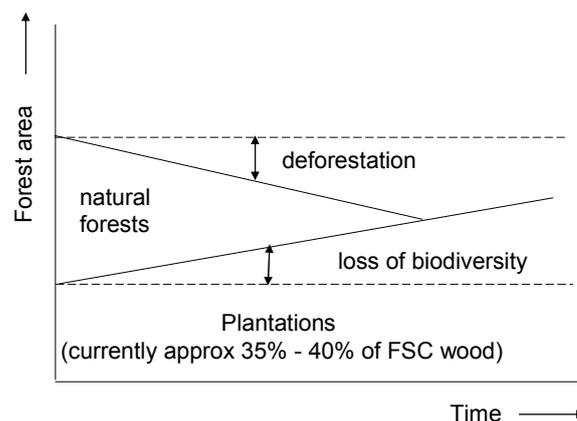


Figure 5: More demand of tropical hardwood leads to deforestation on the short term, and less carbon sequestration

The demand for tropical hardwood is more than the supply from plantations (only 35% - 40% of FSC-wood is from plantations). This leads to deforestation, resulting in carbon emissions caused by less carbon sequestration. See Fig. 5.

The conclusion for the production side of wood is:

- extra demand of European wood leads to an increase in forest area, so more sequestered carbon

- (extra) demand of tropical hardwood leads to a decrease in forest area, so less sequestered carbon
- extra demand of bamboo, however, leads to an increase in forest area, since bamboo is not harvested from natural forests

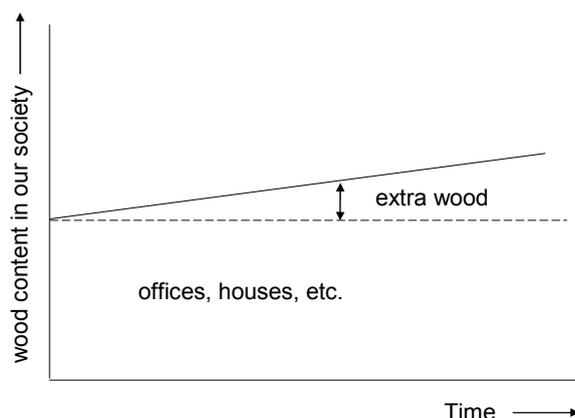


Figure 6: More applications of wood in the building industry leads to more carbon sequestration

The volume of wood in houses, offices, etc. is slowly rising on a global scale (because of increasing population), which is positive in terms of extra carbon sequestration. See Fig. 6. This volume, however, is generally low in comparison with the volume of standing trees in the forests (less than 25% of the wood ends up in housing).

The conclusion for designers, architects and engineers is that carbon sequestration is enhanced whenever more European wood and/or bamboo is applied. The application of tropical hardwood, however, is damaging carbon sequestration.

Note that carbon sequestration is not increasing per house which is built, but per extra house that is built above the number of houses that are required to replace discarded, old, houses

In LCA, the aforementioned global aspect of carbon sequestration is defined in (ILCD,2010) in terms of transformation of land. The issue is then how to allocate the positive or negative effect of carbon sequestration to the wood or bamboo. In this paper, we propose an allocation to the global bamboo production, which will be explained in Section 6.

4. Cradle-to-gate Calculations

The production system of bamboo “from cradle-to-warehouse-gate” is depicted in Fig. 7.

The calculations have been made on the actual product chain of Moso International based on consumption in the Netherlands:

- Type of bamboo: *Phyllostachys pubescens* (density 700 kg/m³, length up to 15 m, diameter on the ground 10-12 cm, wall thickness 9mm).
- Plantation and first processing: the Anji region, the province of Zhejiang, China
- Final processing (Laminated bamboo board, compressed bamboo, veneer): Huangzhou, the province of Zhejiang
- The product is shipped via Shanghai and Rotterdam to the warehouse of Moso International in The Netherlands (Zwaag)

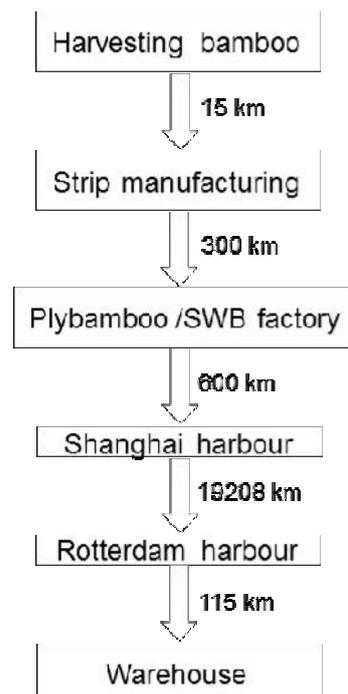


Figure 7: The production system of Moso International (cradle-to-warehouse-gate).

The required heat for the manufacturing process is generated locally by combustion of sawdust and bamboo waste. Electricity is assumed to be from the local grid (modern coal fired power plant, 0.805 kg CO₂e/kWh). Note: a cogeneration plant for electricity and heat is an opportunity for the future, to reduce the carbon footprint of the electricity consumption.

The calculations for the LCAs have been made with the computer program Simapro, applying LCI databases of Ecoinvent v2 (2008) and Idemat 2008 (a database of the Delft University of Technology, partly based on Ecoinvent Unit data).

For this study, the LCA was based on a 3 layer carbonized laminated bamboo board. Laminated bamboo board, a hard aesthetical material which is often used in flooring or table tops, is manufactured in various varieties: 1, 3, or 5 layers, bleached or carbonized, side pressed or plain pressed. Table 1 and 2 provide specific production data for the production of 3 layer carbonized laminated bamboo board (the detailed Functional Units used are described in the legends to Tables 1 & 2). A comprehensive description of the production processes and Tables for the other varieties can be found in van der Lugt et al. (2009) and van der Lugt (2008).

Table 1: Input data and results in CO₂e (carbon footprint) for the environmental impact assessment (cradle to gate) of carbonized 3-layer laminated bamboo board (consisting of two layers of 5 mm plain pressed at the outsides, and one layer of 10 mm side pressed in the core). The Functional Unit used as the base element for this assessment is one board of 2440 x 1220 x 20 mm (2.98 m²), with a weight of 41.7 kilograms (based on a density of 700 kg/m³).

Process step	amount	unit	Carbon fp kgCO ₂ e/unit	Carbon fp kgCO ₂ e/FU	Carbon fp kgCO ₂ e/kg	Carbon fp %
1. Cultivation and harvesting from plantation						
Gasoline consumption	0.224	litre / FU	3.895/ litre	0.873	0.0209	1.5%
2. Transport from plantation to strip manufacturing facility; a 5 ton truck (transport of 23.1 FUs)						
	30	km / truck	0.63/ km	0.818	0.0196	1.4%
3. Strip making	1.38	kWh/ FU	0.805/kWh	1.111	0.0266	1.9%
4. Transport from strip manufacturing facility to factory; a 10 ton truck (transport of 77.6 FUs).						
	600	km / truck	0.825/km	6.379	0.1530	10.8%
5. Rough planing	8.62	kWh/ FU	0.805/kWh	6.939	0.1664	11.8%
6. Strip selection						
7. Carbonization	4.73	kWh/FU	0.805/kWh	3.808	0.0913	6.5%
8. Drying carbonized strips	9.66	kWh/FU	0.805/kWh	7.776	0.1865	13.2%
9. Fine planing	5.8	kWh/FU	0.805/kWh	4.669	0.1120	7.9%
10. Strip selection						
11. Glue application (1-layer boards)	0.894	kg / FU	2.24 /kg	2.003	0.0480	3.4%
12. Pressing strips to 1-layer board	1.89	kWh/FU	0.805/kWh	1.521	0.0365	2.6%
13. Sanding 1-layer board	1.62	kWh/FU	0.805/kWh	1.304	0.0313	2.2%
14. Glue application (3-layer board)	0.983	kg / FU	2.24 /kg	2.202	0.0528	3.7%
15. Pressing three layers to one board	1.65	kWh/FU	0.805/kWh	1.328	0.0319	2.3%
16. Sawing	0.29	kWh/FU	0.805/kWh	0.233	0.0056	0.4%
17. Sanding 3-layer board	0.86	kWh/FU	0.805/kWh	0.692	0.0166	1.2%
18. Dust absorption (during all steps)	8.67	kWh/FU	0.805/kWh	6.979	0.1674	11.8%
19. Transport from factory to harbour	12.51	ton.km/FU	0.086/ton.km	1.076	0.0258	1.8%
20. Transport from harbour to harbour	800.9736	ton.km/FU	0.011/ton.km	8.811	0.2113	14.9%
21. Transport from harbour to warehouse	4.7955	ton.km/FU	0.086/ton.km	0.412	0.0099	0.7%
TOTAL carbon footprint				58.93	1.413	100.0%

Table 2: Input data and results in eco-costs for the environmental impact assessment (cradle to gate) of carbonized 3-layer laminated bamboo board (consisting of two layers of 5 mm plain pressed at the outsides, and one layer of 10 mm side pressed in the core). The Functional Unit used as the base element for this assessment is one board of 2440 x 1220 x 20 mm (2.98 m²), with a weight of 41.7 kilograms (based on a density of 700 kg/m³).

Process step	amount	unit	ecocosts €/unit	ecocosts €/FU	ecocosts €/kg	ecocosts %
1. Cultivation and harvesting from plantation						
Gasoline consumption	0.224	litre / FU	1.04/ litre	0.233	0.0056	1.7%
2. Transport from plantation to strip manufacturing facility; eco-costs of a 5 ton truck (transport of 23.1 FUs)	30	km / truck	0.243/ km	0.316	0.0076	2.3%
3. Strip making	1.38	kWh/ FU	0.109/kWh	0.150	0.0036	1.1%
4. Transport from strip manufacturing facility to factory; eco-costs of a 10 ton truck (transport of 77.6 FUs).	600	km / truck	0.32/km	2.474	0.0593	18.0%
5. Rough planing	8.62	kWh/ FU	0.109/kWh	0.940	0.0225	6.8%
6. Strip selection						
7. Carbonization	4.73	kWh/FU	0.109/kWh	0.516	0.0124	3.7%
8. Drying carbonized strips	9.66	kWh/FU	0.109/kWh	1.053	0.0253	7.7%
9. Fine planing	5.8	kWh/FU	0.109/kWh	0.632	0.0152	4.6%
10. Strip selection						
11. Glue application (1-layer boards)	0.894	kg / FU	0.57/kg	0.510	0.0122	3.7%
12. Pressing strips to 1-layer board	1.89	kWh/FU	0.109/kWh	0.206	0.0049	1.5%
13. Sanding 1-layer board	1.62	kWh/FU	0.109/kWh	0.177	0.0042	1.3%
14. Glue application (3-layer board)	0.983	kg / FU	0.57/kg	0.560	0.0134	4.1%
15. Pressing three layers to one board	1.65	kWh/FU	0.109/kWh	0.180	0.0043	1.3%
16. Sawing	0.29	kWh/FU	0.109/kWh	0.032	0.0008	0.2%
17. Sanding 3-layer board	0.86	kWh/FU	0.109/kWh	0.094	0.0022	0.7%
18. Dust absorption (during all steps)	8.67	kWh/FU	0.109/kWh	0.945	0.0227	6.9%
19. Transport from factory to harbour	12.51	ton.km/ FU	0.033/ton.km	0.413	0.0099	3.0%
20. Transport from harbour to harbour	800.9736	ton.km/ FU	0.0052/ton.km	4.165	0.0999	30.3%
21. Transport from harbour to warehouse	4.7955	ton.km/ FU	0.033/ton.km	0.158	0.0038	1.2%
TOTAL eco-costs (€)				13.75	0.330	100.0%

5. End-of-life Calculations

The end-of-life of bamboo is a combination of:

1. Combustion in an electrical power plant
2. Combustion in a municipal waste incineration plant (no energy recovery)
3. Landfill

In the Netherlands and other West European Countries, wood and bamboo is separated from other waste and ends up in an electrical power plant. Only a small proportion is combusted in a municipal waste incinerator. In Western Europe, the percentage which ends up in landfill is estimated at less than 10%.

The end-of-life credit for electricity production from bamboo waste is based on 17.7 MJth heat recovery per kg of bamboo waste. The heat recovery / CO₂e ratio is 15 MJth/kg CO₂e, being the average of electricity production in the European UCTE countries (data from Ecoinvent v2.2):

- carbon footprint: 1.18 kgCO₂ per kg of bamboo waste
- eco-costs: 0.21 € per kg of bamboo waste

In this study we assume that 90% of the bamboo products will be combusted with heat recovery (10% is landfill), leading to a credit of:

- carbon footprint: $1.18 \times 0.9 = 1.062$ kgCO₂ per kg of bamboo product
- eco-costs: $0.21 \times 0.9 = 0.189$ euro eco-costs per kg of bamboo product

6. Calculation of Carbon Sequestration

As has been explained in section 3, the extra global carbon sequestration is proportional to the growth of the market for bamboo products. According to van der Lugt and Lobovikov (2008) annual growth of the market for industrial bamboo products in EU and China ranges between 17% to 25%. However, the establishment of new plantations often does not directly follow increase in market demand but is following the market growth with a delay. This phenomenon also becomes clear from the 7th Chinese National Forestry Inventory (2010) where is shown that the area of bamboo resources in China in 2004-2008 has grown from 4,84 million ha to 5,38 million ha in 2008, thus a growth of 11,18% in 5 years which refers to an annual growth of 2,24%. Note that the growth of tree forest area in China lies at a similar level (11,74%) with a growth of 174,91 million ha to 195,45 million ha in the same period (2004-2008).

For this study it is assumed that the annual growth in permanent plantations in China will increase to 5% as a result of the high domestic and international market growth of 17-25%. This can be considered a conservative approach as it may be expected that this number will turn out to be higher considering the high market growth.

It is assumed that the additional permanent plantations are established on grassland and do not come at the expense of natural tree forests. This is a plausible assumption as a large portion of the Moso bamboo resources comes from the industrialised provinces around Shanghai (Zhejiang, Anhui, Jiangxi). Furthermore, this assumption fits well in the current policy for afforestation and natural forest protection of the Chinese Government controlled by the Chinese State Forestry. More information on this issue can be found at <http://english.forestry.gov.cn/web/index.do>, which shows the increasing forest area in China.

It is important to realize that one kg of an industrial bamboo product relates to many kg of bamboo in the plantation:

- 1 kg final industrial bamboo product (A-quality bamboo material) consists of approximately 0.9 kg bamboo strip, 0.08 kg water (at 20 degrees Celsius and a relative humidity of 50%) and 0.02 kg glue;
- 0.9 kg bamboo strip is manufactured from 2.14 kg bamboo at the plantation above the ground (production efficiency 42%, see van der Lugt (2008))
- 2.14 kg bamboo contains carbon, equivalent to $2.14 \text{ kg} \times 1.83 \text{ kg CO}_2 / \text{kg bamboo} = 3.92 \text{ kg CO}_2$
- 3.92 kg CO₂ above the ground relates to $3.92/0.32 = 12.2 \text{ kg CO}_2$ above + below the ground, since bamboo has a vast root system¹

Concluding: 1 kg final bamboo product is related to 12.2 kg CO₂ stored at the plantation.

According to the model of the global carbon sequestration, as described in Section 3.2, the growth of bamboo plantations, and its related transformation of land, is defining the effect on global carbon sequestration. Prior to the bamboo plantation, the land was grassland, having an approximate carbon level in the range of 3 – 6 ton biomass per hectare , i.e. 1.1 – 2.2 kg CO₂ per m² (source IPCC website). Transformation of land from grassland to bamboo plantations results in 10 – 11 kg CO₂ per m² carbon sequestration. We allocate (=distribute) the positive effect of land transformation to the rest of the global bamboo production.

At a market growth of 5%, the sequestered carbon per kg global bamboo production is 5% of 10 - 11 kg CO₂, i.e. 0.5 – 0.55 kg CO₂. That means that 0.5 – 0.55 kg CO₂ can be allocated to 1 kg final bamboo product in the building industry. In eco-costs this is a credit of 0.068 – 0.074 euro.

7. Results and Conclusions

Fig. 8 and 9 below presents the total results for carbonized 3-layer laminated bamboo board, based on its carbon footprint and eco-costs over the full life cycle, including the effects of carbon sequestration from bamboo plantation area growth over a 5 year period.

¹ Besides in the trunks, branches and shrub, there is CO₂ stored below ground in the soil and roots of a plantation. Zhou et al. (2004) found that, for a medium intensity managed Moso bamboo plantation in Lin'an, Zhejiang province, the distribution of biomass above ground versus below ground is 32.2% and 68.8% respectively.

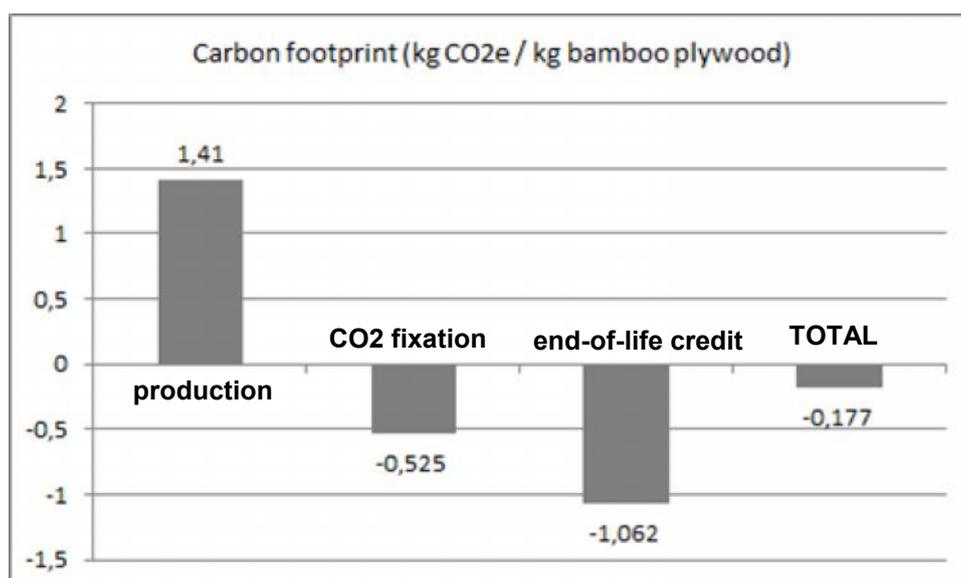


Figure 8: Carbon Footprint over Life Cycle (kgCO₂eq / kg bamboo product) for carbonized 3-layer laminated bamboo board

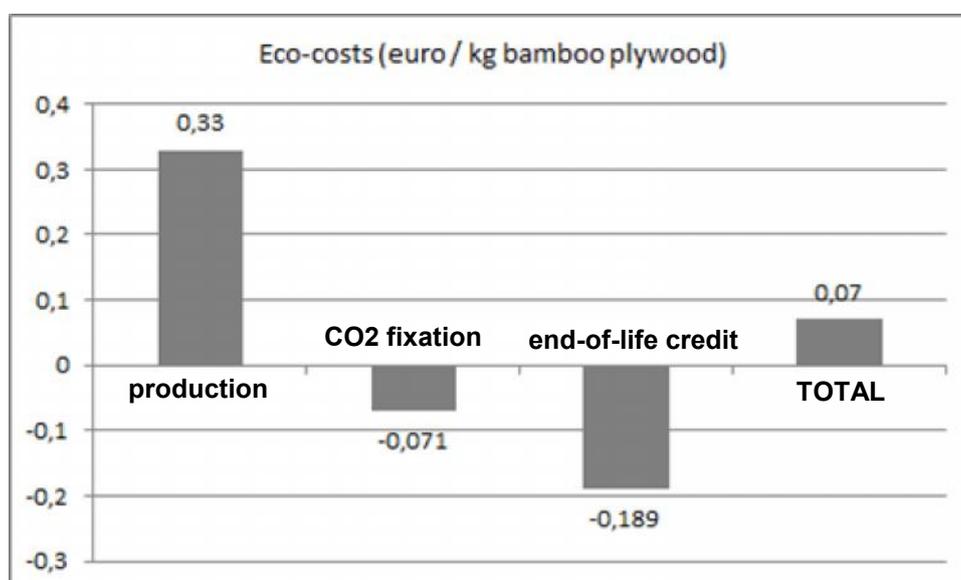


Figure 9: Ecocosts over Life Cycle (€ / kg bamboo product) for carbonized 3-layer laminated bamboo board

From the results it may be concluded that bamboo plywood, if based on best-practice technology (in this case production chain of Moso International), even when used in Europe can actually be labelled “CO₂ neutral or better”. When used in the country of production (China), the results will be even more positive.

Acknowledgements

The authors thank the reviewers for their remarks. Especially the contribution of Richard J. Murphy enhanced the scientific level of this paper.

References

- ILCD, 2010 (European Commission, Joint Research Centre, Institute for Environment and Sustainability); International Reference Life Cycle Data System (ILCD) Handbook: General guide for Life Cycle Assessment (LCA) - Detailed Guidance, First edition, 2010.
- ISO 2006. ISO 14044 Life cycle assessment – Requirements and Guidelines. ISO/FDIS, Geneva, Switzerland
- PhD thesis. Delft University of Technology. ISBN 978-90-5155-047-4, VSSD, Delft, the Netherlands.
- State Forestry Administration of P.R. China 2010. China's Forest Resources Status and Dynamic Change. Forestry Economics. (2):66-72.
- Van der Lugt, P. 2008. Design interventions for stimulating bamboo commercialization.
- Van der Lugt, P., Lobovikov, M. 2008. Markets for bamboo products in the West. Bois et forêts des tropiques, 295(1): pp 81-90. CIRAD, Paris, France.
- Van der Lugt, P., Vogtländer, J.G., Brezet J.C. 2009. Bamboo, a sustainable Solution for Western Europe. Design cases, LCAs and Land-use. ISBN 978-90-6562-196-2, VSSD, Delft, the Netherlands.
- Van der Lugt, P., Vogtländer, J.G., Brezet J.C. 2009. Bamboo, a sustainable Solution for Western Europe. INBAR Technical Report no. 30. International Network for Bamboo and Rattan, Beijing.
- Vogtländer, J.G. 2010. A practical guide to LCA for students, designers and business managers, cradle-to-grave and cradle-to-cradle. ISBN 978-90-6562-253-2, VSSD, Delft, the Netherlands
- Vogtländer, J.G. 2011. Life Cycle Assessment and Carbon Sequestration - Bamboo products of MOSO International. Delft University of Technology
- Vogtländer, J.G. et al. 2010. LCA-based assessment of sustainability: The Eco-costs/Value Ratio (EVR). ISBN 978-90-6562-233-4, ISBN 978-90-6562-234-1 (e-book), VSSD, Delft, the Netherlands
- Vogtländer, J.G., Van der Lugt, P., Brezet, J.C. 2010. The sustainability of bamboo products for local and Western European applications. LCAs and land-use. Journal of Cleaner Production 18 (2010) 1260-1269
- Zhou, G. M., Jiang, P. K. 2004. Density, storage and spatial distribution of carbon in *Phyllostachys pubescens* forest. Scientia Silvae Sinicae, 6: 20-24. (In Chinese with English summary).

BAMBOO PIONEERS

In Honor of Bamboo Pioneers

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In 2009, the World Bamboo Organization initiated the Bamboo Pioneers Award. This award is a small attempt to recognize the great achievements of our contemporaries who have dedicated their lives in the pursuit of bamboo knowledge and progress. We know that living creatures all around the world depend on bamboo for their survival. We also know that for centuries, human cultures have cultivated and utilized bamboo for their daily needs and through innovation improved their livelihoods and economies. We are in awe of the myriad of discoveries and innovations of those early pioneers, and have deep appreciation and respect for the traditional cultural utilization and reverence of bamboo.

Dedication, determination and collaboration are required to advance any scientific endeavor. There exists individuals whose lifelong commitment to bamboo science deserve our attention and honored recognition. Today, as part of the inauguration of the 9th World Bamboo Congress, we honor 5 of these great Bamboo Pioneers:

Oscar Hidalgo-Lopez of Colombia
Wenyu Hsiung of the Peoples Republic of China
Jules Janssen of The Netherlands
Shuen Chao Wu of Taiwan
Masatoshi Watanabe of Japan

Previous Pioneer Recipients:

For the 8th WBC Bangkok (2009):

Ueda Koichiro of Japan
Krit Samapuddhi of Thailand
Floyd Alonzo McClure of the United States
Walter Liese of Germany

For World Bamboo Day, Nagaland (2010):

Richard Belho of Nagaland
Rajeev Goswami of Assam
Vino Kaley of Maharashtra
Cherla Sastry of Canada
Sampurana Singh of Meghalaya

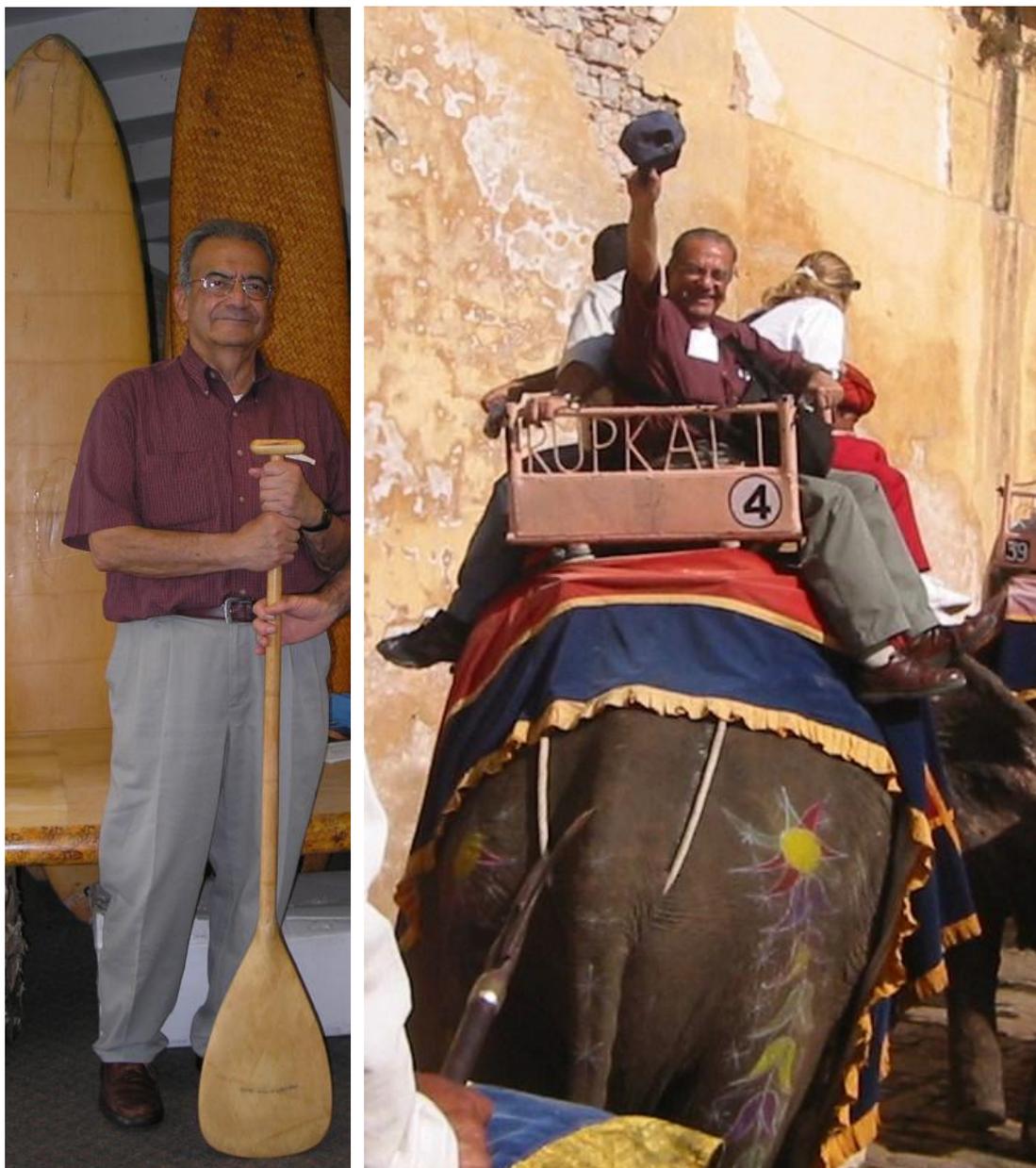
Oscar Hidalgo – Lopez (1930 -)



Oscar Hidalgo was born in a town called Chinchina on the 16th of November in 1930. In this town, it was very common to build houses from local bamboo (the Guadua). His father was an odontologist and his mother was dedicated to child care at home. He had a brother named Fernando who also became an architect, who sadly passed away ten years ago. He has a step-brother named Fabian, who has so kindly brought Oscar to the 9th WBC in order for him to participate here in person and to receive this Bamboo Pioneer Award today.

Oscar studied at the Universidad Nacional de Colombia and graduated as an Architect. Immediately after graduation, Oscar worked locally for several years until leaving for the United States in order to teach at Parsons School of Design in New York City. A pioneer in art and design education since its founding in 1896, Parsons has cultivated outstanding artists, designers, scholars, businesspeople, and community leaders for more than a century. During the years Oscar was at Parsons, the school programs began to encourage students to work on more socially conscious projects, such as public housing, alternatives to substandard urban housing, etc. The philosophy at Parsons during and since Oscar's tenure there emphatically championed art and design as both intellectual practice and social responsibility. This is apparent in the evolution of Oscar's work.

Growing up in Chinchina, surrounded by houses made of bamboo, Oscar watched as bamboo was used for many residential and public buildings. It was cheap and widely available, and was hidden behind plaster exteriors. After his education in architecture, he was further intrigued by the possibilities of bamboo. He embarked on a project to construct a country club kiosk 23 meters in diameter using bamboo. Five days before the opening ceremony, there was a hurricane which extremely distorted the building, moving the kingpost 90 cm off-center. After only two hours of working with a winch, however, the structure was successfully moved back into place without collapsing. Seeing this, he was sold on bamboo.



In 1960, when the Guadua were on the brink of extinction due to the intensive destruction of the natural bamboo plantations which began in the 1950's, it was the Colombian Institute of Natural Resources (INDERENA) that forbade the cutting of bamboo without its permission. Fortunately today the natural Guadua forests are protected, managed and respected; no doubt thanks to people like Oscar.

He later worked for the giant global contractor, Bechtel for 13 years, and as an inspiring teacher and researcher at the Universidad Nacional de Colombia. He founded the Bamboo Research Center (CIBAM). He worked as a consultant in Ecuador and Costa Rica for the United Nations, as well as consultant for the Acuerdo de Cartagena PADT-REFORT in Peru and Bolivia. His research took him to libraries in several universities, including Washington University in Canada, Columbia University in New York, and the University of California in Berkeley.

Architect Oscar Hidalgo set out and dedicated his life to bamboo research, teaching the world about the limitless possibilities of this remarkable plant. "With bamboo we can replace wood or timber in all their applications, but we cannot use wood or timber to make all the things and structures that can only be made with bamboo." (Hidalgo, 2003) Amazed at its structural integrity and aesthetic possibilities,

he traveled extensively throughout the United States, Germany, Japan, Philippines, Taiwan, China, Indonesia, Costa Rica, Brazil, India and elsewhere to study and teach, to experiment and explore. Everywhere he went, he inspired and influenced many students of architecture and design, builders and engineers.

In 2003, Oscar Hidalgo published an incredible book entitled, *Bamboo – The Gift of the Gods*. Essentially it is a testament of his life's discoveries involving his study of bamboo; as a plant, its taxonomy, ecology, silviculture, mechanical and chemical properties, the role of preservation and protection in its durability, its use in traditional uses and handicrafts, manufacture of modern products and materials, bamboo construction technologies, engineering potentials, and modern possibilities. It is a triumph to his dedication and commitment. It is standing proof of his bamboo pioneering spirit.

Wenyu Hsiung (1915 -)



Wenyu Hsiung was born July 9th, 1915 in Chong Qing county of Sichuan Province. He graduated from the Forestry Department of Sichuan University, receiving his Bachelor of Agricultural Science degree in 1940. He remained on the staff as a faculty member after graduation. Four years later, he was promoted to a lecturer. In 1944 he obtained a scholarship and set his heart on pursuing his studies abroad. He first attended classes at the University of Toronto and then transferred to the College of Forestry of Yale University. He received a master's degree from Yale University in 1947. In August of that year, he transferred to the University of Minnesota and received a doctorate degree in 1951, and stayed on to continue his research.

After his return from overseas in August of 1953, he successively held the posts of vice dean, dean and professor of Forestry Department of Nanjing Forestry College (now called the Nanjing Forestry University).

He was a member of the third, fourth and fifth council of the Chinese Society of Forestry; director of Ecological Society of China; member of the third and fourth discipline assessment groups of the academic degree commission of the state council; subeditor of the forestry group of the editorial board of the ministry of forestry; member of the academic committee of environmental ecological center of the Chinese Academy of Sciences; advisor of the integrated survey group of the southern part of the Chinese Academy of Sciences; member and advisor of the scientific and technological committee of the ministry of forestry; followed by president and honorary president of Jiangsu executive council of ecology.

Since 1978, Wenyu Hsiung was appointed as visiting professor in the College of Forestry of the University of Idaho (USA); invited spokesperson of the seventeenth, eighteenth and nineteenth world

conferences of IUFRO; visiting professor of University of Edinburgh, University of Aberdeen, University of Oxford, University of Wales, Bangor, Universität Hamburg and Universität Göttingen; Malaysian forestry advisor of United Nations Development Programme. In 1987, he was selected as leader of P5-04 of IUFRO, mainly in charge of the 19th symposium to be held in August of 1990.

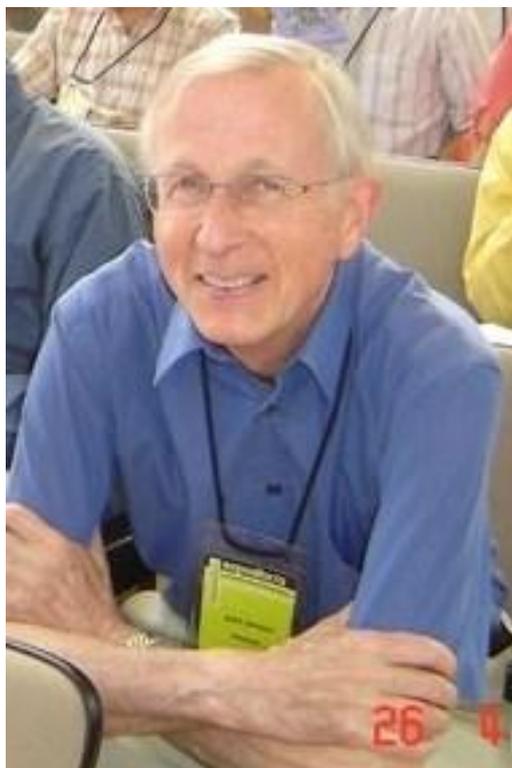
He has been a teacher for more than 50 years, devoting himself to forestry education, training and mentoring of scientific personnel for bamboo research.



Wenyu Hsiung grew up in an environment of bamboo and has deep affection for bamboo. He was very worried about China's lack of forest resources. As China has a large area of bamboo forest which is widely distributed with lots of varieties and rich experience in the cultivation and utilization of bamboo, he believed that we can solve the contradiction between the supply and demand of timber and give full play to bamboo resources by strengthening bamboo production and substituting bamboo for wood. Thus, he decided to be engaged in bamboo research, and selected Moso bamboo as his research object. Moso bamboo is the main species of bamboo in China which is fast growing and of high economic value. He spent all his energy in bamboo research, and led his work team to conduct on-the-spot investigations. In 1958, he published a book called *High-yield Moso Bamboo Stands in Shimen of Zhejiang Province*. He found if we wanted to enlarge the area of bamboo forests and raise the per unit yield, we would be need to make a systematic study. Therefore, he organized the bamboo research group in Nanjing Forestry College in 1962. In addition, he conducted research on the characteristics of population structure of bamboo forest by using principles of ecology and put forward the measures and research direction of management and administration for bamboo forest. In 1964, he put forward the idea of south bamboo north transplanting and has carried out the introduction of moso bamboo ever since. In 1974, he with Prof. Zhou Fangchun, another bamboo expert, compiled a book called *Bamboo Cultivation*, which was welcomed by the reading public and reprinted four times successively. In 1978, his two achievements *High Yield Moso Bamboo* and *South Bamboo North Transplanting* won The National Science Conference Unit Award.

In 1981, Prof. Hsiung was invited to join the 17th world conference held by IUFRO in Japan. He presented the paper called *Study on Intercalary Meristem and internode elongation of Bamboo Plants*, and put forward some new points which roused attendants' interests. In 1986, he was also invited to join the 18th world conference by IUFRO in Yugoslavia. He gave the report on *Current Status and Development of Bamboo Research in China* which was published in *Forestry Research* issued by the United Nations. At that conference, he was selected as leader of P5-04 of IUFRO for a term from 1987 to 1990. He compiled *Bamboo Bulletin* in English, mainly introducing world bamboo production and scientific research trends. In 1987, his name Wenyu Hsiung was listed in the biology volume of *Who's Who in the World* by Longman Group Ltd. In 1988, his name was also listed in the *Celebrity of Far East and Australia and International Intellectuals* by Cambridge International Autobiography. In order to push bamboo research work forward, Prof. Hsiung and Zhou set up Bamboo Institute in Nanjing Forestry University. Wenyu Hsiung has put a great deal of effort and made a great contribution to bamboo development at home and abroad, in December of 1990, he won the outstanding contribution prize by IUFRO and was known as "Mr. Bamboo" by foreign friends.

Jules J.A. Janssen (1935 -)



In 1963, Jules Janssen achieved a Masters of Science degree in Civil Engineering from Delft University, with the main subject of structural design. From 1963-67, he was partner in an architect's office, with major projects involving the structural design for a concert hall containing 3000 seats, and a harbor shed of 7200 square meter (80,000 sq. ft.)

Jules begins his teaching career in September of 1967, at the Eindhoven University, on the faculty of architecture and building, teaching structural design and applied mechanics. A few years later, in 1972, a Dutch volunteer in Indonesia asks him his advice on how to build with bamboo; the only source for his answer is found in a Dutch colonial military handbook from 1890. This raises his interest in bamboo!

In 1974, when the university faculty asks who of the younger staff members would like to begin a PhD research, Jules applies with "bamboo as a building material" as his subject. The faculty considers this as crazy. Jules says, "in fact, they are right. I am headstrong, and starting this research is extremely difficult." Jules struggles with how to determine the mechanical properties of bamboo without knowledge of appropriate testing methods. He discovers there is hardly any literature available, and he has to start with the test methods themselves, which is rather unusual. Later on, in 1997, this research is extremely welcome as a basis for the ISO standard on these methods.

In 1979, the secretary of the Forestry Department of Wageningen University informs him "a German professor" is coming to present a guest lecture on bamboo, and Jules attends that lecture. Afterwards he meets the professor (Walter Liese) and presents his research. Professor Liese is enthusiastic, and invites Jules to the first bamboo workshop in Singapore, May 1980, sponsored by the International

Development Research Centre (IDRC, Canada, the founder of INBAR – the International Network of Bamboo and Rattan).

Three years later, Walter Liese attends the PhD ceremonies of Jules Janssen.



Dr. Janssen's first consultancy involves strategy to diminish the import of timber products by using locally made bamboo products in the country of Burundi. Additionally he was a member of the Intermediate Technology Development Group's (ITDG) building panel, CIB-W18B for several years. He became supervisor of the National Bamboo Project in Costa Rica, serving in that role from 1987-1995, visiting there twice a year for three weeks at a time. Beginning in 1988 while attending the International Bamboo Workshop in Cochin, India, he commits himself to the development of international standards for bamboo and devotes almost sixteen years to this goal.

A consultancy job takes Jules to Bangladesh in 1991 to work on the preservation of bamboo; he is granted the Royal Order of "Officer in the Order of Oranje Nassau" for his work on bamboo by Her Majesty the Queen of the Netherlands in 1994, attends bamboo seminars in Tanzania in 1996, and in 1997, upon invitation of the Hawaii Chapter of the American Bamboo Society, Jules leads lectures on bamboo for three weeks. The lecture notes become the basis for his handbook, "Designing and building with bamboo", which is later published by INBAR in 2000.

Dr. Janssen's efforts on behalf of INBAR achieved significant success in 1997, at which time the Dutch Government, through the department for development cooperation, approved funding of \$1.4 million (USD) for INBAR. This leads to Jules being present at the formal ceremony in Beijing for the establishment of new INBAR headquarters, followed by hectic years of much traveling as he works to build a strong organizational structure within INBAR. Most of his time from 1997-2000, he is occupied with writing draft texts for ISO standards on bamboo.

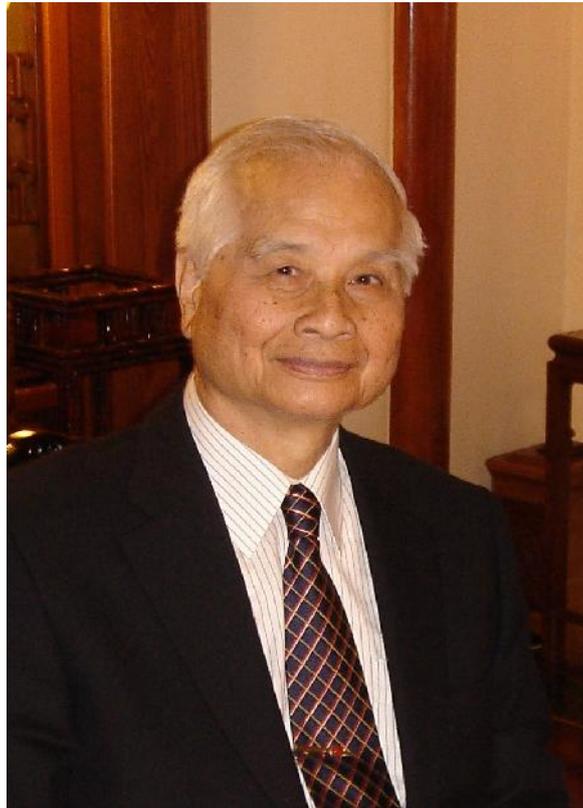
The year 2000 brings formal retirement to Jules, but he continues to work for INBAR. Fortunately, Eindhoven University allows him use of all the facilities, and he continues his efforts in the development of the ISO bamboo standards, and additionally begins the role of editor for INBAR's new Journal of Bamboo and Rattan. He continues mentoring, as he supervised several PhD candidates on bamboo: three in Eindhoven, one in Birmingham, and two in Delft.

Finally, in 2004, the bamboo standards are approved by ISO. This is an incredible achievement in the acceptance of bamboo as a legitimate alternative to traditional timber and opens many doors for the use of bamboo in developed countries.

The next year brings the end of Jules roles as Editor in Chief of the Journal of Bamboo and Rattan, which became the responsibility of the Kerala Forestry Research Institute. He left one job as editor for another, as he becomes the editor for the English language version of the handbook entitled, "Bamboo and Rattan in the World", published by the Chinese Academy of Forestry on the occasion of the tenth anniversary of INBAR. This is a job of 250, 000 words, which is extraordinary considering English is not Jules' first language!

These days Jules Janssen enjoys calmer days at home, writing magazine articles or advising students. Bamboo stays on his mind, and in his heart. We welcome him here today in Antwerp.

Shuen-Chao Wu (1927-)



Professor Shuen-Chao Wu was born on March 2, 1927 at Chai-Yi, a city in southern Taiwan. Wu grew up near the forest train station, and frequently had the chance to see trains carrying huge logs. In his childhood, those scenes gave him a strange feeling and unforgettable deep image in his heart.

Wu's interest in forestry can be traced back to the summer of 1940. At the age of 13, Wu joined the boy scouts and had a chance to climb up to Mountain Morrison (also called Yushan, the highest mountain in northeast Asia), reaching its top to the height of 3950 meters above sea level. Through his many trips to the mountains, Wu had been able to observe many plants, such as bamboos, broadleaf trees and coniferous trees, from tropical, sub-tropical, temperate and sub frigid forests, gradually changing with increasing elevations. Since then, Wu grew up with a deep interest in bamboos, woods and forests.

From 1949 to 1953, Wu attended the National Taiwan University (NTU) and majored in Forestry. After graduation, up to 1956, he worked at National Forests (Taiwan Forest Bureau) as a forest staff member, and later continued his work at the Experimental Forest, National Taiwan University.

Wu studied at the Graduate School of Forestry at the University of Tokyo, Japan from 1956-1962, and obtained a masters and doctorate degrees. In his six-year sojourn in Tokyo, Wu joined research work focusing on forest operation and wood utilization, and worked all over Japan. He presented many research papers in Japan and Taiwan.

On August of 1962, Wu accepted an offer as associate professor, returning to his alma mater, the Department of Forestry, NTU. In 1965, Wu was promoted as professor, and continued his research

works and teaching in Forestry for over 30 years. Under his guidance, 60 diploma students, 47 master students and 8 doctoral students achieved their degrees. After his retirement in 1997, Wu continued to participate in forestry and academic activities by attending several forestry associations and some foundations.



From 1972 to 1978, Wu was appointed as the chairman of the Department and Graduate School of Forestry, NTU. Under his exertion, the masters and doctorate degree programs were promoted. He also developed the scheme of the departmental program by dividing it into four units as “silviculture”, “resource management”, “forest industry” and “forest resources and conservation”. In 1978, Wu was invited as Visiting Professor in the University of Tokyo, where he undertook research works and strengthened the relationship between University of Tokyo and NTU. From 1981 to 1984, Wu took a position as the Dean of General Affairs of NTU, and had many outstanding achievements in the areas of forestry and administration. In 1988, Wu was invited as Visiting Professor in the Tokyo Agricultural University, Tokyo, and was actively involved in research works with scholars all over Japan.

As honorable positions, Wu holds the Professor Emeritus of National Taiwan University, Honorary President of Chinese Forest Products Association-Taiwan, the honorary member of the Chinese Forest Association-Taiwan, and the honorary member of Japanese Forest Engineering Association-Japan.

1. As the Representative of Taiwan (China-Taipei), Wu had participated eight times in the IUFRO World Congress, including the meetings from 16th to 22nd, and the Centennial Anniversary, from 1976 to 2005. His research papers were presented in IUFRO and other international meetings. As one of the previous IUFRO Executive Board Members, Wu attended several Board Meetings of IUFRO, traveling to many countries, such as Manaus-Brazil, Garpenberg-Sweden, Pointe-Noire-Congo, Portland-USA, Los Banas-Philippines, Prague-Czechoslovakia, Quebec-Canada, Beijing-China, and San José-Costa Rica, etc. He published over 200 research papers. "Effects of Age, Node and Height in a Culm on Specific Gravity and Mechanical Properties of Bamboo", 1966, Published in Department of Forestry, National Taiwan University (NTU) Report 1, PP 1-45.
2. "The Effect of the Cutting Rotation of Bamboo on its Mechanical Properties", 1976, Published in *New Horizons in Construction Materials*, PP555-566, Evo Publishing Company, Inc., Pennsylvania, U.S.A..
3. "Study on the Structure of Bamboo Species Grown in Taiwan", 1976, Published in the Dept. of Forestry, N.T.U., Bulletin No. 16, PP1-79.
4. "Research and Development of the Production and Utilization of Bamboo in Taiwan, R.O.C." (The 17th IUFRO World Congress at Kyoto, Japan, September 6-17, 1981.)
5. "The Structural Variation of Leptomorph Type and Pachymorph Type Bamboo Species", 1986, Published in *Forest Products Industries* 5(2.):49-62.
6. "The Ultrastructure of Vascular Bundles of Some Taiwan Bamboo Species, 1987, Published in *Quart. Journ. Exp. For., N.T.U.* 1(1):21-44.
7. 3 research papers were presented in the 4th International Bamboo Workshop on Bamboo in Asia and Pacific, at Chiangmai, Thailand, November 27-30, 1991.
 - a) Anatomical Characteristics of Taiwan giant bamboo and Moso bamboo.
 - b) The ultrastructure of Taiwan giant bamboo and Moso bamboo.
 - c) Structural variability of vascular bundles of some exotic bamboo species.
8. "The drying shrinkage of 3 rattan specie grown in Taiwan." (IUFRO All-Division 5 Conference at Nancy, France. August 23-28, 1992)
9. "The Structural Variation of Recently Introduced Bamboo in Taiwan." (Symposium on International Bamboo Industrial Utilization at Beijing, China, December 7-10, 1992.)
10. After the Beijing symposium, Wu visited An-Chi Bamboo Garden, near Hangzhou, Zhejiang, China. Wu had collected 20 species involving 13 genera bamboo samples for his research work there. Later he had used those materials making several research achievements and published 7 related papers as follows:
 - a) "The Anatomical Properties of some Bamboo Species Grown in Mainland China (1), (2), (3)", 1995&1996, published in *Quart. Journ. Exp. For. National Taiwan University* 9(1): 93-119, 9(4): 53-70, 10(2):37-59.
 - b) "The Ultrastructure of Bamboos Grown in Mainland China (1), (2), (3), 1995&1996", published in the *Forest Products Industries* 14(4): 499-518, 15(1): 1-20, 15(2): 193-215.
 - c) "The Ultrastructure of Phyllostachys Genus Bamboos Grown in Mainland China, 1996", published in *Quart. Journ. Exp. For., National Taiwan University* 10(1):23.

11. "Movement of Water and Chemical Solution in Ma Bamboo and Moso Bamboos". (1993 FPRS Annual Meeting, at Clearwater, Florida, U.S.A. , June 21-23, 1993.)
12. "The wood Properties of Acacia Mangium and Acacia Auriculiformis Grown in Taiwan" ("International Symposium on the Utilization of Fast-Growing Trees (ISUFGT), at Nanjing, China, October 16-18, 1994.
13. Physical and Mechanical Properties of Malaysian Commercial Rattan Species, 1995, Published in Quart. Journ. For., N.T.U., 9(1):19-31.
14. 2 papers were presented in the 40th Anniversary of Japan Wood Research Society at Tokyo, Japan, April 4-9, 1995.
 - a) "Group analysis as applied to wood anatomy of Taxodiaceae members."
 - b) "A computer model developed for differentiating wood anatomical characteristics in members of Pinaceae. "
15. On November 26-28, 1996, Wu was invited to the "FORTROP'96: International Conference on Tropical Forestry in the 21st Century" at Bangkok, Thailand, and A statement of "Wood Utilization in the Tropics" as one of Conference Keynote Speakers. In this conference, Wu also have an honor as one of co-chairman with Prof. Walter Liese take a portion of congress.
16. " The Anatomical and Mechanical Properties of Bamboo Species" (IUFRO All Division 5 International Conference—Forest Products for sustainable Forestry, at Spokane, Washington, U.S.A., July 7-11, 1997.)
17. Anatomical and Mechanical Properties of 3 Thailand Bamboo Species, 1998, Published in the Forest Products Industries, 17 (1):19-31.
18. On early September, 1998, Wu visited "Bamboo Processing and Industrial Technology Training Center", at Moin near San Jose, Costa Rica that was supported by Taiwanese Government for promoting and assisting Costa Rica's farmers to raise up their income and techniques in cultivation and utilization of bamboos. And discussed some technical aids about their development in the future.

Dr. Wu joins us today, traveling all the way from Taiwan, to share his enthusiasm for bamboo with all the participants of the 9th World Bamboo Congress.

Masatoshi WATANABE (1933 -)



Masatoshi WATANABE graduated from the Kyoto College of Foreign Languages in 1955. He achieved qualification as Forestry Specialist by the Forestry Agency of the Japan Government ten years later and in 1986 received a Doctorate degree from National Kyushu University. His thesis title, “Basic studies on the management of bamboo forests based on ecological characteristics.” His central interest and specialty focus became bamboo forest ecology, silviculture and management.

Watanabe served as a technical official at Kyoto University from 1953-1991, and was the loyal assistant of Dr. Koichiro Ueda for 38 years. The World Bamboo Organization honored Dr. Ueda as the first Bamboo Pioneer at the 8th World Bamboo Congress, Bangkok in 2009, and subsequently honors Dr. Watanabe at this 9th WBC with the Ueda Lecture.

Since retiring from Kyoto University in 1991 to the present, Dr. Watanabe continues working as a specialist in the Japan Bamboo Society, as well as a specialist for the Rakusai Bamboo Park in Kyoto, lecturer for the Japanese government Forestry Agency, and since 2005 serves as Secretary General to the Japan Bamboo Association.

His international career included several fascinating opportunities. From 1970 – 1972, he was dispatched by the Japanese government to Thailand for technical cooperation as a bamboo expert, to Indonesia as a forestry expert in 1994 and 1995, to Chile as bamboo expert in 1996, and back again to Indonesia in 1997 as a bamboo expert



In November of 1992, Watanabe hosted a group of visitors from the American and European bamboo societies during the Third International Bamboo Congress. He generously guided and eagerly toured with this intrepid group of foreigners, crossing the bamboo bridge in friendship and spirit. He has continuously crossed over that bamboo bridge as researcher and scientist, participating as a speaker in the 7th World Bamboo Congress in Delhi, India in 2004X and attended the 8th World Bamboo Congress in Bangkok in 2009.

He has been a prolific writer, and has authored more than 150 papers. Those published in English, include:

Masatoshi, WATANABE & Prasarn BARMNGRASD : On the research works of bamboo forest in Thailand. 3rd Nat. Forestry Conf., Thailand 1970

Masatoshi WATANABE : Report of technical service and research work on silviculture and management of bamboo forest in Thailand. Overseas Tech. Coop. Agency, Tokyo. 96pp. 1970

Masatoshi WATANABE & Seiichi OOHATA : Studies on bamboo culm form (1). On *Phyllostachys bambusoides* Seib. et Zucc., J. Japan For. Soc., Vol. 62(1) : 9~16, 1980

Masatoshi WATANABE & Hajime HAMADA : How long is the flowering interval of bamboo? Bamboo Production & Utilization : 77~83, Proc. 17th IUFRO World Cong. in Kyoto, 1981

Masatoshi WATANABE, Koichiro UEDA, Ippei MANABE & Tatsuo AKAI : Flowering, seedling, germination, and flowering periodicity of *Phyllostachys pubescens*. J. Japan For. Soc., Vol.64(3)107~111, 1982

Masatoshi WATANABE : On the productivity of *Phyllostachys bambusoides* in recovering from flowering. J. Japan For. Soc., Vol.65(3) : 89~93, 1983

Takashige AOKI & Masatoshi WATANABE : Studies on the organization of *Phyllostachys bambusoides* stands. Bamboo Prod. & Util., Proc. P.5.04, 18th IUFRO World Cong., Yugoslavia : 37~41, 1986

Masatoshi WATANABE : A proposal on the life form of bamboos and ecological typification of bamboo forests. Bamboo Prod. & Util., Proc. P.5.04, 18th IUFRO World Cong., Yugoslavia : 37~41, 1986

Masatoshi WATANABE : Distribution of bamboos in the world. Bamboo Journal No.4 : 225~233, 1987

Masatoshi WATANABE, Masaharu INOUE & Tadao TAKANO : Discussion on the prediction of culm height in *Phyllostachys bambusoides* bamboos. Bamboo Journal No.7 : 27~38, 1990

Masatoshi WATANABE & Takashige AOKI : Ecological characteristics of *Phyllostachys bambusoides* stands. Abstracts of Vth INTECOL : 491, 1990

Masatoshi WATANABE & Takashige AOKI : Some productive aspects of *Phyllostachys bambusoides* stands. *Bamboo Journal* No. 8 : 1~8, 1990

Masatoshi WATANABE : Present status of bamboo industry in Japan. *Bamboo Journal* No.9 : 58~68, 1991

Masatoshi WATANABE : On the management of bamboo stands, with special reference to Japanese research. Constraints to production of bamboo and rattan, with special reference to planting materials and management of natural stands. Report of a consultation held 9~13 May 1994, Bangalore, India : 175~191, INBAR, 1994

Masatoshi WATANABE : Recent bamboo industry and research in Japan. International Workshop on Bamboo Research, 24~26 June 1994, Chu-Tou Forest Rec. Area, Exp. For. Nat. Taiwan Univ., Taiwan

Masatoshi WATANABE : Report of forest tending on after-care program for the trial plantation project in Benakat, South Sumatra. 43pp. Submitted to Indonesia Government, 1994

Masatoshi WATANABE, Agus Setyono, Dharmawan Pathi & Sairun : Final Report, Forest tending on after-care program for the trial plantation project in Benakat, South Sumatra. 66pp. Submitted to Indonesia Government, 1995

Masatoshi WATANABE, Claudio Zunino Aviles, Carlos Kahler G. & Carmen Gloria Quezada C. : Report on breeding control of *Quila*. 94pp., submitted to JICA & INFIR, 1996

Masatoshi WATANABE, Claudio Zunino Aviles, Carlos Kahler G. & Carmen Gloria Quezada C. : Ecological characteristics of *Chusquea quila* Kunth from central-south Chile. *Bamboo Journal* No.14 : 1~14, 1997

Masatoshi WATANABE : Report, Bamboo resources development program in Indonesia. Submitted to Ministry of Forestry, Indonesia. , 1997

Masatoshi WATANABE : On the above-ground biomass of four bamboo forests in Indonesia. *Bamboo Journal* No.16 : 22~32, 1999



We welcome him here today in Belgium for the 9th World Bamboo Congress as he presents the WBO Ueda Lecture, and honor him proudly as a recipient of the WBO Bamboo Pioneer Award.

9TH UEDA LECTURE

– AS PART OF THE WBO BAMBOO PIONEERS AWARD

Present Status of Bamboo in Japan

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Preface

On the occasion of the 9th World Bamboo Congress, I am very pleased to make this presentation entitled, “Present status of bamboo in Japan” and to express the greatest thanks for giving me this opportunity. Since I submitted a paper on the same subject at the 7th WBC held in New Delhi, India in 2004, the situation on the status in Japan has severely changed and the 9th WBC is an appropriate opportunity to review this status to the world.

Here I would like to present the great distress existing in bamboo circumstances in Japan, pointing out the problems and suggesting some counter plan for solving the problems.

1. Bamboo in Japan

The area of Japan is about 378 thousand km² between 20 and 45 degrees N latitude and consists of mainly five inlands of Hokkaido, Honshu, Shikoku, Kyushu and Okinawa and about 67% of Japanese territories are covered by forests.

According to the statistics in 2009 by the Japanese Forestry Agency, total bamboo cultivation area was recorded to be total about 57,400 ha with about 33,400 ha in culm cultivation and about 24,000 ha in shoot cultivation, as shown at Table 1. The distribution of bamboo cultivation area is typical; it is concentrated in the warm districts of southern Honshu, Shikoku and Kyushu islands, especially the most extensive in Kyushu accounting for approximately 77% of the total area. Almost all of the bamboo forests are private forests (not government owned).

Table 1 Cultivation area of bamboo in Japan

	Area (ha)	Rate (%)
Culm cultivation	33,440.3	58.3
Shoot cultivation	23,916.4	41.7
Total	57,356.7	100.0

Note: Statistic by Forestry Agency in 2009

The important bamboo species are *Phyllostachys pubescens*, *P. bambusoides* and *P. nigra* var. *henonis* and they are considered to be the Three Most Useful Bamboos in Japan; they are all monopodial.

Phyllostachys pubescens is the only exotic species and cultivated mainly for bamboo shoot production.

P. bambusoides is the best in physical quality for making arts and crafts of traditional culture,

construction materials, and so on. *P. nigra var. henonis* is also an important species for producing traditional handicrafts and other forms of utilization.

2. Present status of culm cultivation and production

At first, when looking at the yearly changes of culm cultivation area and culm production by the statistics of the Forestry Agency, the area decreases from about 90,000 ha in 1990 to 35,000 ha in 2009; only 38% at present when compared with that in 1990. The area of bamboo cultivation for culms decreased about 62% during 20 years, as shown at Fig.1 (A).

Culm production is in severe circumstances. When looking at the culm production in 1990, about 170 thousand tons were recorded, but in 2009 it was only at approximately 35 thousand tons, 15% of that in 1990. Culm production extremely decreased to 85% in 20 years, as shown at Fig.1 (B).

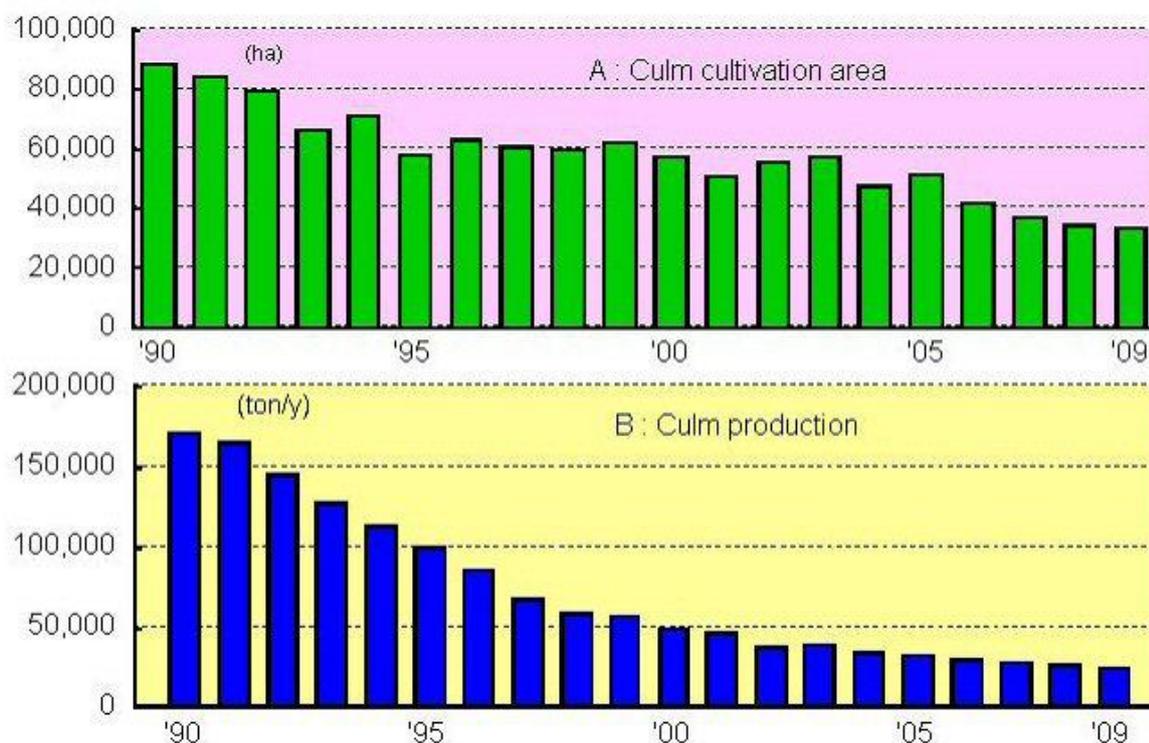


Fig.1 Yearly changes of culm cultivation area (A) and culm production (B)

The reason why such a downward trend occurred is due to the following reasons.

> Market decrease

Japanese housing is become much westernized. Traditional methods are becoming rare, so the use of bamboo materials in methods such as the muddy wall, is much less than in the past. There is a tendency of the Japanese people to not to have the opportunity to enjoy bamboo products and this may be due to the change of the traditional Japanese life style.

> Availability of substitute articles

In the past, Japanese people used many kinds of bamboo products, but now many kinds of substitute articles have appeared in the market, replacing bamboo products. Those are mainly products made of plastic materials. For example, the bamboo fences historically and typically constructed in Japanese gardens are also being replaced by plastic fences.

> Importation of bamboo products

According to the statistics by Japanese customs, many bamboo products are imported in large amounts, such as bamboo raw materials, bamboo charcoal, bamboo laminated materials, disposable chopsticks, bamboo skewers, baskets, bamboo textile goods, and so on. Thus, this import has great influence on the development of the present bamboo industry in Japan.

> Bamboo cultivation is labor intensive and very hard work

The field work for bamboo cultivation is very hard, because the use of machinery inside the groves is difficult, i.e. to remove branches, extract harvested culms, etc. These tasks require physical input and the younger generation is not interested.

> Aging skilled labor.

Farmers who manage bamboo stands are aging and many are over 60 years old. Despite the labor intensity, the resulting income is not high. As a result, younger generation farmers are not likely successors.

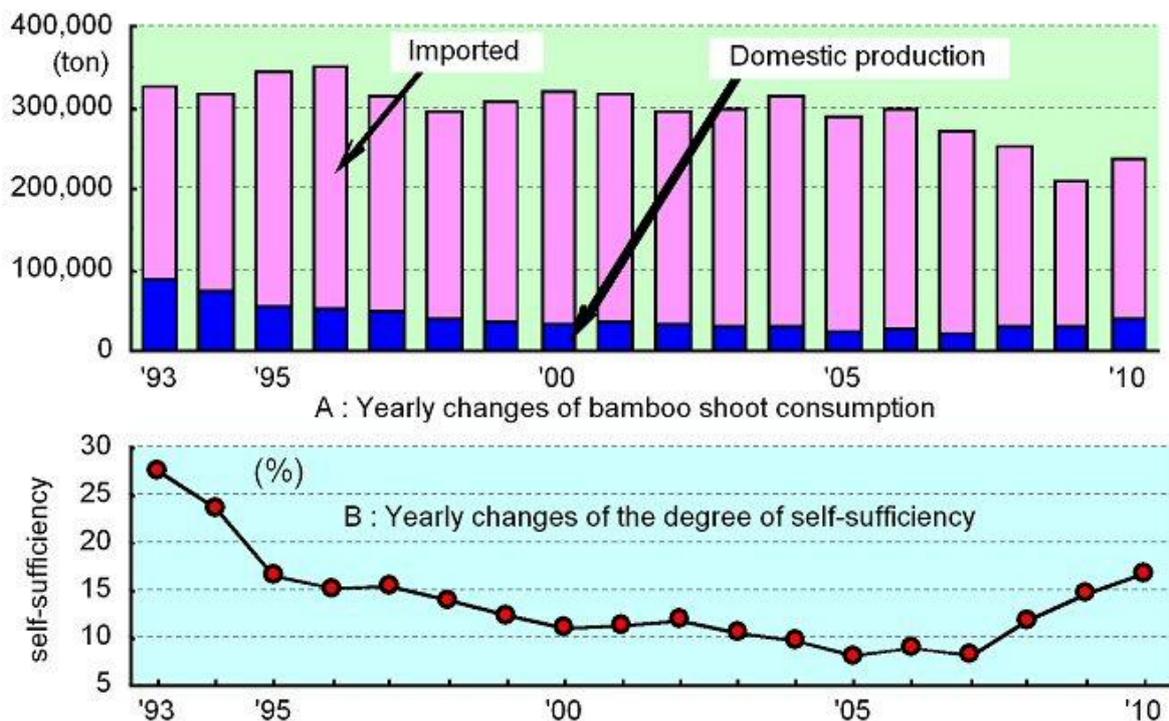


Fig.2 Yearly changes of domestic consumption of bamboo shoots (A) and the degree of self-sufficiency (B)

3. Present status of bamboo shoot production

As shown in Fig.2 (A), domestic production of bamboo shoots according to the statistics of the Forestry Agency was recorded as approximately 100 thousand tons in 1993, but it gradually decreased year by year and the lowest was recorded to be about 22 thousand tons in 2007. Recently, the statistics show the tendency of an increase after 2008. While total consumption of bamboo shoots is almost constantly moving at the level of 300 thousand tons since 1993, a somewhat decreasing trend can be

seen since 2009. A great difference can be recognized between domestic production and total consumption in Japan. According to the record of Japan Customs, this difference is made up by the import of bamboo shoots mainly from China.

When calculating the degree of self-sufficiency of bamboo shoot production in Japan, the total consumption in 1993 was about 327 thousand tons. This consumption was satisfied by 90 thousand tons of domestic production and 237 thousand tons of shoots imported from outside of Japan. Thus, the degree of self-sufficiency was about 28%. In 2005, the most severe condition was recorded, with only 8% of the total bamboo shoot consumption produced domestically. After 2006, the domestic percentage has increased to 17% in 2010.

When investigating the reason why such a trend can be seen in the bamboo shoot industry, the cause is only an actuality of the influence of import. In 1989 when the Japan Bamboo Association held the 30th National Bamboo Congress, a presenter sitting on the board of Japan Agricultural Cooperative (JA) excitingly emphasized that "we don't like to be a victim of the export of high technological products, like cars, electronic products and other high-tech products. Please help us politically" in the seminar. The year 1989 was also the beginning of the importation of a lot of bamboo shoots. He might have foreseen the present status of the bamboo shoot industry in Japan, however at that time we could not understand the meaning of his appeal and did not discuss countermeasures.

4. Present problems in Japan

1. Increase of non-managed bamboo forests

In 2003-2005, the Forestry Agency of the Japanese government investigated the area of bamboo forests by Landsat and conjectured the total to be about 247 thousand ha, as shown at Fig.3. The total areas of culm and shoot cultivation in 2003 were recorded to be about 80 thousand ha. Thus, the effective cultivation area of bamboo forests in 2003 was only 24% of the actual existing bamboo area. That means, approximately 167 thousand ha or 76% of the total bamboo forests in Japan were under non-managed practices in 2003. Presently, the percentage of non-managed bamboo forests has increased. The reason for this severe increase in non-managed forests is due to the decrease in culm production and the increase of bamboo shoot importation, as mentioned above.

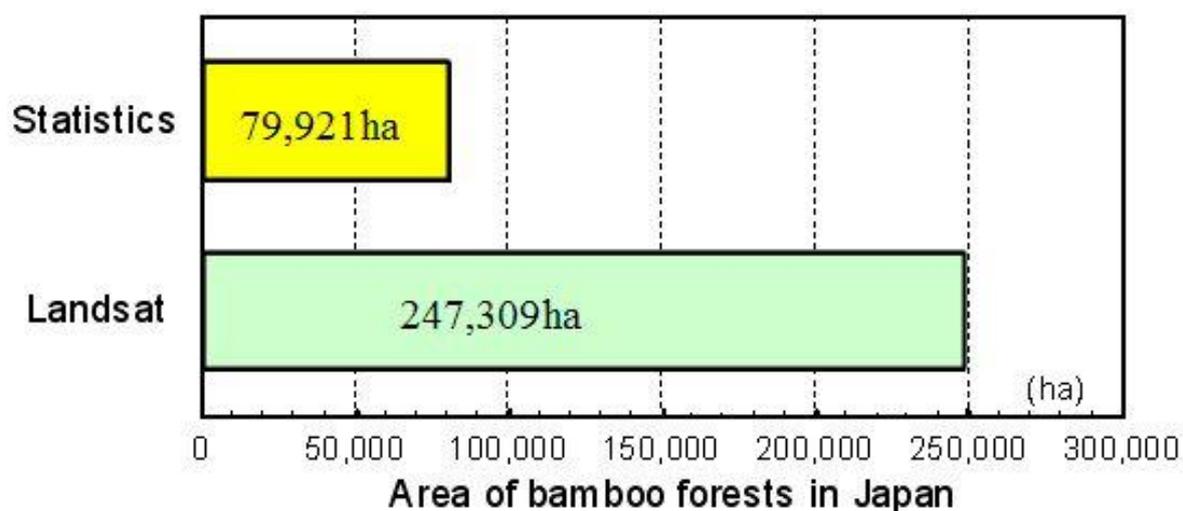


Fig.3 Bamboo areas by statistics and Landsat

Stand physiognomy of non-managed bamboo forests shows a really sad scene as the mortality of standing bamboo is high; fallen bamboos litter the forest and make it impossible to walk in the stand, just like a bamboo jungle. Stand conditions in three sites observed in 2009 are as shown in Fig.4.

Site A is high density with a total of 11,000 no/ha, including 8,600 no/ha of over 2-year-old bamboos, 300 no/ha of new bamboos and 2,100 no/ha dead bamboo. The rate of each bamboo for the total is 78% of 2-year-old bamboo, 3% new bamboo and 19% dead bamboo. While Site B and C are a little lower in stand densities than that of Site A, the stand densities are almost similar in statistics. The typical characteristic of non-managed bamboo forest is to see much mortality. To reach the maximum in stand density, new bamboo growth must be high, with an occupancy of over 2-year-old bamboo culms in high density. Non-managed bamboo forests results in very low culm productivity and the stand physiognomy results as a climax community (Ueda & Numata) in the monopodial bamboo forest.

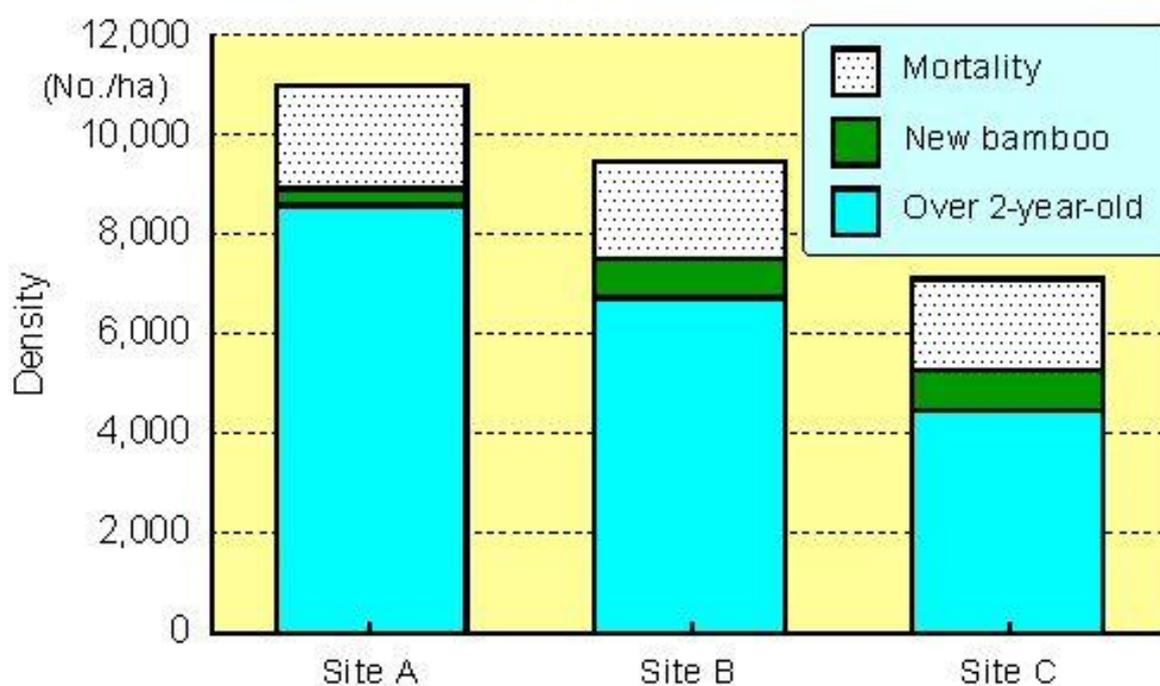


Fig. 4 Stand density of non-managed bamboo *P. pubescens* forests

Now, more than 70% of the total bamboo forests in Japan are under such a poor stand physiognomy and sadly, finely cultivated bamboo forests are not easy to find at the present.

2. Expansion of bamboo forests

Another problem is the expansion of bamboo growth into afforestation areas, woodlands near populated areas and various farms. For example, Fig.5 shows the expansion of *Phyllostachys pubescens* growing into the afforestation area of young Japanese cedar, *Cryptomeria japonica*, indicated by the red circle, where the red arrows show increasing expansion. Regrettably such a phenomenon is not rare; this bamboo expansion is observed everywhere in Japan at present.

The reason why such a problem is happening is very simple. Sadly, it can be said that almost no body likes to work in, or take care of, forestation sites, as the value of economical woods is down significantly. If it happened tens years ago, the owner or planter would work to remove any invaders as well as bamboos. In the case of Fig.5, such an expansion might not occur if the expanding bamboo was removed at an earlier stage as it began to grow into the area. The expansion of bamboo growth into tree forests is not due to the characteristics of bamboo, but because of the economic problem surrounding fundamental forestry in Japan.

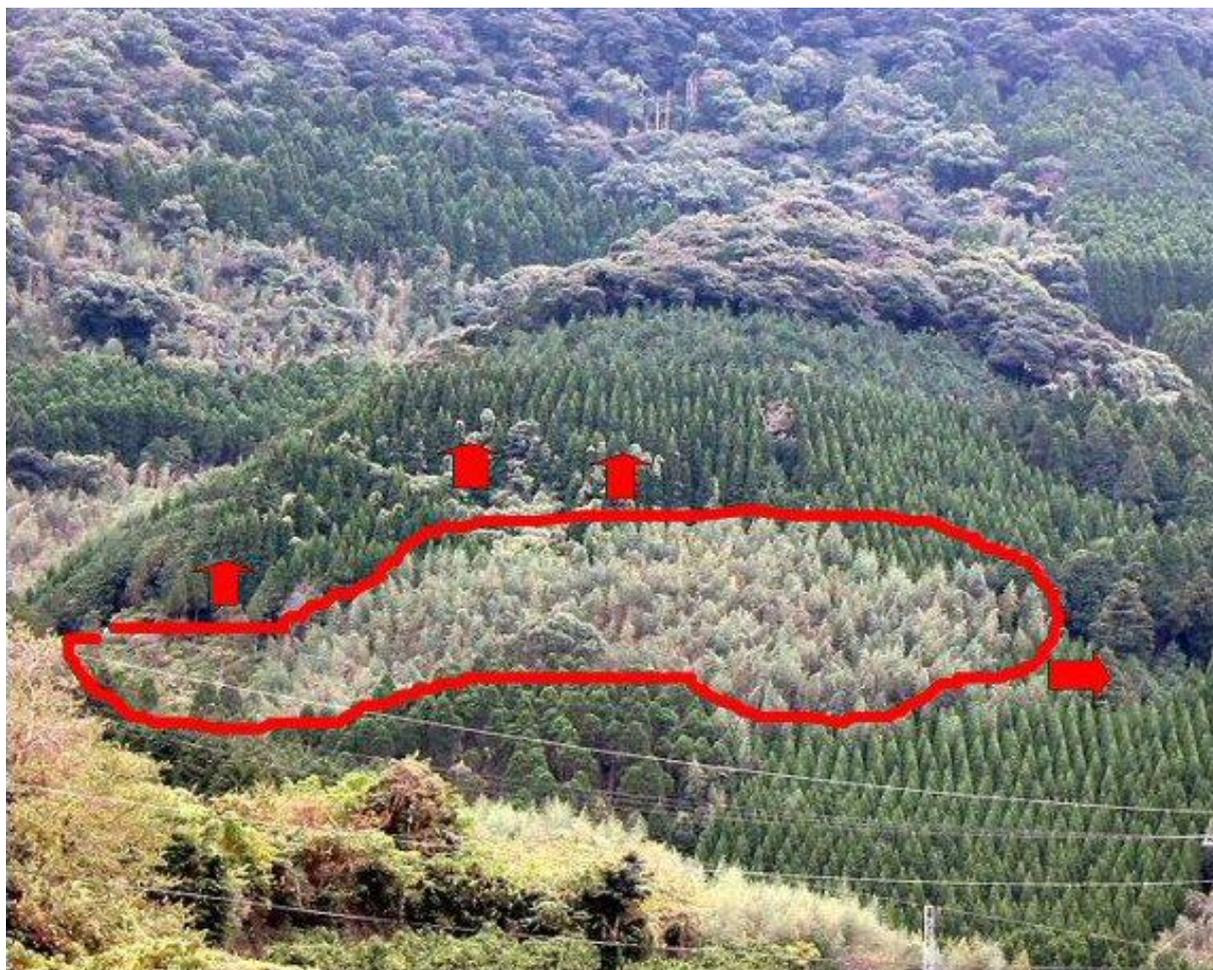


Fig.5 Expansion of *P. pubescens* to afforestation area

5. Utilization of bamboo and related problems in Japan

Before offering the countermeasure for the present problems, I would like to first explain the ways of utilization of bamboo in Japan as in Fig. 6.

Utilization of bamboo in Japan is basically divided first into two, as one is the cultural utilization and the other is the industrial utilization. Cultural utilization is defined as the products made by bamboo which are easily recognized, while industrial utilization is defined as the products made by bamboo which are more difficult to recognize.

The cultural utilization includes bamboo products made by traditional methods, such as tea-ceremony, flower arrangement, traditional housing and gardening, and then land preservation, creation of green

environment, etc. in broad meaning. When looking at the products in the cultural utilization, the demand for these has gradually decreased after the 1970's. This decline has also resulted in the increase of non-management of bamboo stands, as mentioned before.

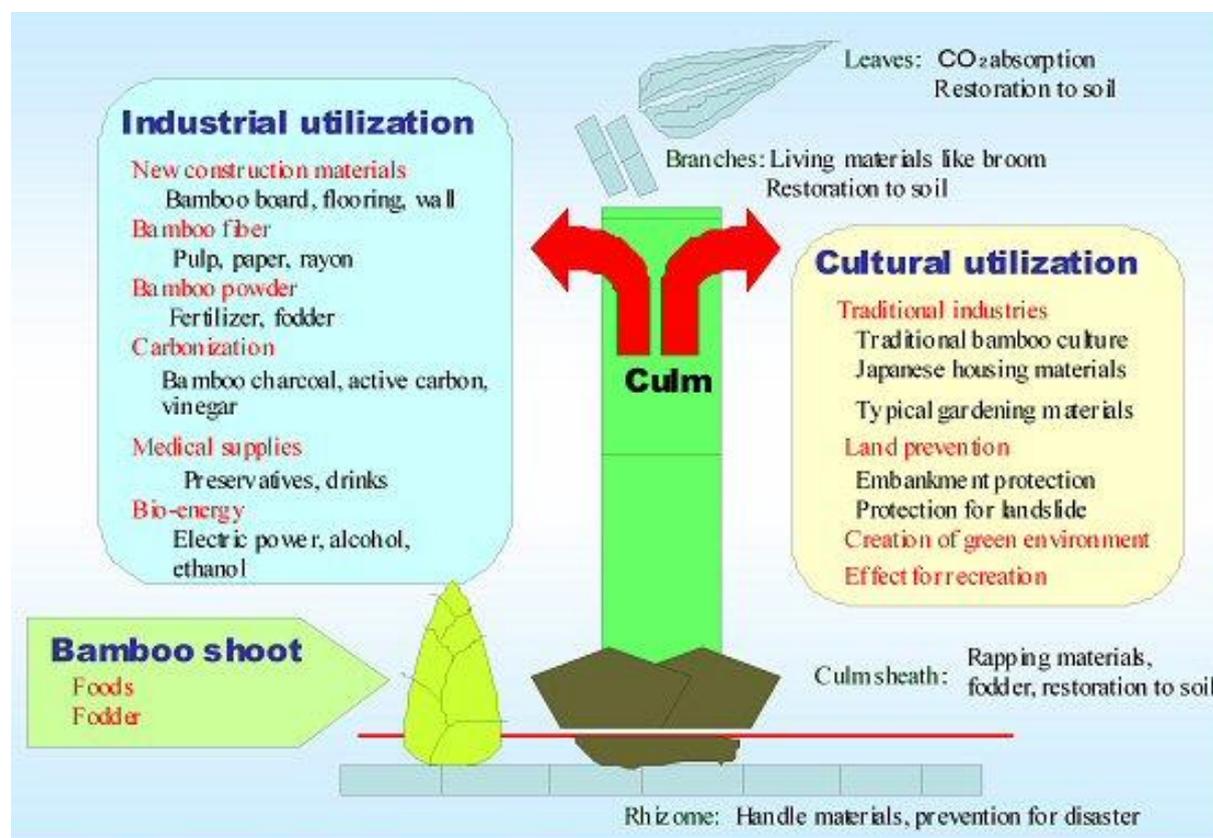


Fig.6 Utilization of bamboo in Japan

Industrial utilization of bamboo is the production of new construction materials like bamboo flooring, fiber products as paper and cloth, bamboo charcoal and vinegar, medical supplies as preservatives, and bio-energy as electric power, alcohol, ethanol, etc.; those which are produced by recent high technology. These achievements in technology have been fundamentally established by Japanese scientists. As we well know, the evolution of industrial utilization results in a great demand of bamboo materials and serves to solve the problem of non-managed stands to a shift to reasonable cultivation. Nevertheless, there exists a very difficult problem: production costs. According to the statistics provided by the customs department of Japan, many industrial bamboo products are imported from Asian countries, China in particular. Another problem is that the cultivation area of bamboo per one owner or one farmer is very small; a single cultivation area may be less than 0.1 ha on average. It may be more than ten owners or farmers, if there is the case of 10 ha of bamboo forest existing. So, it is also very difficult problem to draw the consensus for harvesting bamboos from all owners in order to produce bamboo raw materials in constant amounts for industrial utilization at present.

6. Counter measurement for solving present problems

1. On the problems of increasing non-managed forests

The main reason of the increase in non-managed bamboo forests is the decrease in demand of bamboo materials. However, an increase in bamboo supply is difficult to expect coming from cultural utilization because at present, the supply is kept at a constant level. The development of cultural utilization is stable with today's Japanese life. Of course, we need to endeavor to increase the demand for cultural uses, rather than accept substitute materials.

The key on how to solve the increase of non-managed bamboo forests is to increase the demand for bamboo products and this possibility should only be expected by the increase in the industrial utilization of bamboo. However, there are unsolved problems of "high production costs" and the reaction of large volumes needed for industrial utilization. So, I would like to propose a small scale plan. The development for large scale planning is very difficult when considering the above mentioned unsolved problems. For example, the balance between demand and supply of bamboo raw materials will be indeed satisfied by small communities like a village level, a village group, neighborhood associations, and such small scale communities. In the case of small scale factories, the production costs in these communities may be possible to manage economically by sharing the efforts in operations and get consensus by concerned area landowners for harvesting constant amounts of bamboo raw materials. At present, there are a few cases to be found in Japan, such as the utilization of bio-energy by bamboo chips, fodder by fine bamboo powder, sanitary products, fiber products, carbonized products, and so on.

Concerning bamboo shoot cultivation, the trend of import amounts seems to be decreasing recently. This is due to the fact that Japanese consumers are awakening to the security and safety concerns of imported foods. As a result, the domestic production of bamboo shoots seems to be increasing gradually in the recent past three years. We have to appeal for the security, safety, and health of our domestic shoots. By this appeal we can expect present non-managed bamboo forests to shift gradually towards increasing bamboo shoot cultivation.

2. On the problem of expansion of bamboo forests

This is not a subject of concern within the bamboo industry. When we look back to the past of tens years ago, the increasing supply of timber materials was a very important subject, so the afforested areas were carefully managed and tended very strictly. Of course, many plants which interfered with the growth of the planted trees were instantly removed, if such a case was found in the afforested area. But now the situation is changed. The necessary management, such as weeding, improvement cutting and selective thinning, is not satisfactorily conducted in the young forestation area, or in the country side forests which used to be very important forests for fuel wood before tens years ago.

The expansion of bamboo forests shows the result of the economic influence by trade liberalization. It is regrettable to say that this is a problem not easily solved by the hand of the bamboo industry associations in Japan.

Conclusion

I believe that Japan has achieved a level of high technology in the cultivation and utilization of bamboo in a global perspective. However, such a high technology cannot be manifested today, and presently the valuable bamboo resources of Japan are not utilized sufficiently. Despite this truth, I would like to emphasize that we Japanese never give up and we must find the best way to solve the various problems inhibiting our bamboo industry in the near future.

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The guadua bamboo forests in the Coffee region of Colombia: beyond of carbon sequestration

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Abstract

The species *Guadua angustifolia* (Guadua) is a woody bamboo that represents an important natural resource in Colombia and particularly in the coffee region. The guadua has been traditionally used by farmers to build products such as houses, furniture, handicrafts, agglomerates, veneers and flooring. These bamboo forests are almost the only existing forest cover left in this region between 900 and 2000 meters above sea level, because most of the forests have been eliminated a long time ago due to especially agriculture. As a result, small fragments of forest dominated by guadua are currently the remnants of natural forest in that area. Guadua forests are highly fragmented and most of the patches are not larger than 5 ha. However, these forests are an important refuge of biodiversity where more than 100 woody species have been identified. Besides, they provide habitat to about 69 birds species and 5 mammals (bats), which fulfil important ecological functions. In addition, guadua bamboo forest could be also used as referent for promoting strategies for climate change adaptation and mitigation. In this sense the possibilities of integrating guadua bamboo forests in the framework of a REDD+ initiative, different factors associated to this bamboo resource are analysed and incorporated in a structure defined by four blocks: scopes, level of reference, distribution and financing. Thereafter, it is possible to establish where it would be required to concentrate efforts whether the project is implemented. A capability for carbon sequestration of $109 \pm 54 \text{ t ha}^{-1}$ in average as well as the institutional and political development are remarkable and might contribute for easing REDD+ project implementation. Nevertheless, aspects related to monitoring and strategies for mainstreaming REDD+ initiatives as a priority within territorial, sector and institutional planning process must be consolidated.

Keywords

Adaptation, mitigation, biodiversity, REDD, ecosystems services, mainstreaming

Introduction

The total area covered by bamboo species is approximately 20 million ha around the world (Zhou et al. 1994). About 1200 species of bamboo have been registered (Londoño 1990), 440 native species in America (Hidalgo 2003) and 95 in Colombia (Londoño 1990). Bamboo is the most important non-timber forest product, which is in many countries the most important material for construction, especially in rural areas (Lobvikov et al. 2007). Although most bamboo stands correspond to small patches (Lobvikov et al. 2009), the resource represents an essential alternative for improving the livelihood of rural communities.

Bamboo species also provide raw materials for agriculture, pulp for paper, handicraft (Banik 1999; Hanumappa 1996). Shoots from some species are fit for human consumption (Razak 2001). Bamboo species also are the base for a wide range of industries. In India, for example, the industry is estimated to supply income to 0.5 million people, most of whom are employed by small-scale processing enterprises (Hanumappa 1996).

The species *guadua* (*Guadua angustifolia* Kunth) is a woody bamboo that represents an important natural resource in Colombia and particularly in the coffee region, where most of the forest were eliminated long time ago due especially to agriculture expansion. As a result, small fragments of forest dominated by *guadua* are currently the remnants of natural forest. Besides, commercial timber and that used for domestic applications comes especially from the natural forest located in the pacific coast (Camargo et al. 2007). Nevertheless, *guadua* is the most harvested species to obtain wood for different applications.

The *guadua* has been traditionally used by farmers to build products such as houses, furniture, handicrafts, agglomerates, veneers and flooring. After the last *guadua* inventory, 28000 ha were estimated in the coffee region (Kleinn and Morales 2006), most of them corresponding with natural stands. Information related to harvesting of *guadua* has not been officially registered after 2004. However, according to the number of harvest permissions registered by government institutions in charge of this control as well as the availability of *guadua* poles in timber stores, we know that volume of commercialisation is currently high.

The dynamics generated by changes of land use has contributed to define a particular landscape with remnants of forest. These forest, specially dominated by *guadua* are highly fragmented and most of the patches are not larger than 5 ha (Camargo and Cardona 2005), however, are an important refuge of biodiversity fulfil important ecological functions and can be used as stepping stones to develop strategies of ecosystems restoration. Besides, the characteristics of *guadua* forests associated with high values of biomass and their use by rural communities, in the context of climate change might be considered for developing strategies for mitigation and adaption.

The proper management of *guadua* forest is being promoted for government institutions. However, some problems associated with inadequate silvicultural practices, urban expansion and the little motivation of farmers because of incomes from these forests without incentives become low, are contributing with the illegal logging. In this context, alternatives for promoting a proper management, forest conservation and also include welfare for farmers are needed.

Initiatives as REDD (Reducing Emissions from Deforestation and Degradation) or REDD+ (REDD enhancement of carbon stocks) might be appropriate to improve conditions of *guadua* forests. After the Conference of the parties (COP 13) in Bali in December 2007, about 150 projects REDD+ have been planned with the aim of implementing a variety of intervention to reduce deforestation and forest degradation, as well as to promote the conservation and sustainable management of forest to enhance forest carbon stocks (Jagger et al. 2010).

At different scales (local to global), beyond carbon storage, forests are providing ecosystem services such as watershed protection, water flow regulation, nutrient recycling, rainfall generation and disease regulation (Parker et al. 2008). Therefore, these benefits can be considered in REDD+ initiatives and after of implementing a project, farmers could be beneficiated.

In order to enhance the importance of *guadua* bamboo forests beyond carbon sequestration and to establish the possibilities of integrating them in the framework of a REDD+ initiative, in this paper we describe the local initiatives that might contribute in this process as well as the main disadvantages and threats. Additionally the current state of these forests is analysed in terms of their fragmentation, the

potential for biodiversity conservation and carbon sequestration.

Methodology

Study area

This study was carried out at northwest of Colombia, states of Quindío, Risaralda, Caldas and Valle del Cauca. These states represent a part of the coffee region. The total influence area was about 5.766.397 ha, however for specific evaluations the area was smaller. For landscape analyses were included 58400 ha and for biomass estimation, the information came from 10 permanent plots located, 7 within natural guadua stands and 3, in guadua plantations of guadua established 8 years ago (Camargo and Arango 2011). These plots were selected from those sampled by Camargo (2006) in a previous study where growth, site quality, dendrometric and stand variables were evaluated. Destructive samples of biomass were carried out from culms (rhizome, branches and leaves included) belonging to the same clump. The maturity of culms also was considered, therefore in natural stands were included culms from 12 up to 96 months and in plantation from 12 up to 48 months.

The conditions of the total area corresponds to the natural distribution of guadua bamboo with elevations between 900 and 2000 m above sea level and. Most of the soils have good physical properties, belong to the order of Andisols and have been originated from volcanic ash. Topography is predominately crimped and climate is humid and warm with an annual precipitation of 2200 mm in average and mean temperature of 21°C.

Cover classification and fragmentation analyses

To describe the conditions of guadua forests in terms of forest fragmentations, information from Camargo and Cardona (2005) was used as reference. Therefore, cover classification come from Quick Bird images taken between January to July of 2003 and landscape pattern was defined by considering the structure and distribution of elements as patches, corridors and matrixes (Forman and Godron 1986). Forest fragments were used as a reference unit and the PATCH ANALYST extension of ARCVIEW GIS 3.3 was used for calculating landscape metrics and subsequently to determine landscape patterns (Elkie et al. 1999).

Biodiversity and guadua forests

The importance of guadua bamboo forest for biodiversity conservation was associated with results of fragmentation analyses, taking into account the probable relationship between both factors (Harvey et al. 2004). Also, results of different studies (ie. Calle and Piedrahita 2008; Ospina 2002; Fajardo et al. 2008) conducted on different groups of organisms within guadua forest were used for providing evidences with values of biodiversity represented by the number of species.

Guadua bamboo forest and climate change

In order to evaluate the possibilities of guadua forest of contributing with climate change mitigation and adaptation, we attempted to incorporate specific aspects of guadua forest within the structure for implementing REDD+ project.

In this sense, the approach suggested by Parker et al. (2008) was taken as a referent and the process to develop a framework supported on four basic building blocks (scopes, level of reference, distribution and financing) was followed. Therefore, aspects such as eligible land, carbon pools, and requirements of information, scale and financing were considered. Information on guadua forest related to each block was evaluated to determine available inputs for consolidating a project and also to elucidate gaps of information and possibilities of improving some aspects. Information was gathered from previous studies (ie. Camargo et al. 2007a; Moreno 2006a) and also from some of them currently ongoing.

Results and discussion

Landscape structure

According to Camargo and Cardona (2005) the average size of forests patch (MPS) is 7,9 ha and 67% of them were smaller than 5 ha. The landscape analysed has a fine texture, and is highly porous. That not means low connectivity; however a high quantity of edge and a stronger contrast. The wide range of MPS values has important implications because characteristics as the availability of resources will change with size, therefore exchange of species could be restricted toward those small fragments. As a consequence of this process could be isolation with negative consequences for some organisms (Diaz 1991). On the other hand, patches of guadua forests are not totally homogeneous and showed a different configuration depending on the dominance of species guadua. This condition was also considered by Camargo and Cardona (2005), who defined classes of patches according the dominance of guadua regarding to natural forest (Table 1).

Table 1. Classes of forest with guadua (*Guadua angustifolia* Kunth) . Coffee region of Colombia

Class	Example	Class	Example
Guadua forest (GF): Patches of guadua forest (<i>G. angustifolia</i>)		Association Guadua-Forest (AGB): Patches of forest where guadua and natural forests are equivalent	
Consociation Guadua-Forest (CGB): Patches of forest dominated by guadua (>75%)		Consociation Forest - Guadua (CBG): Patches of forest where guadua is < 25%	

Adapted from Camargo and Cardona (2005)

Camargo and Cardona (2005) found that guadua forests (GF) patches represent 68% of the total forest patches evaluated. Their average size (MPS) was significantly ($p < 0,05$) smaller than other classes, however GF have the longer total edge (TE), higher edge density (ED), average edge (MPE) and mean ratio area-perimeter (MPAR). Therefore, areas dominated by GF in the study zone are more fragmented and have lower connectivity. In contrast patches with CBG and AGB which have a tendency to be larger and have less edge (Table 2).

Table 2. Landscape metrics calculated by classes of patches (Adapted from Camargo and Cardona 2005)

Metric	GF	CGB	ABG	CBG
TLA* (ha)	2521	646	247	653
MPS (ha)	4,46 ^b	20,19 ^{ab}	22,52 ^{ab}	31,13 ^a
TE (Km)	1004 ^b	171 ^{ab}	56 ^{ab}	146 ^a
ED (m/ha)	153 ^b	26 ^{ab}	8,5 ^{ab}	22,2 ^a
MPE (m)	1774 ^b	5343 ^{ab}	5097 ^{ab}	6949 ^a
MSI	2,34	3,29	3,04	3,37
AWMSI	3,19	4,25	3,77	4,71
MPAR (m/ha)	478 ^a	343 ^b	295 ^b	302 ^b

*Different letters between classes represent significant differences ($p < 0,05$). * It was not statistically compared. TLA= total area; MPS= average size of patches; TE= total edge, ED= edge density; MPE= average edge; MSI= shape index; AWMSI= average weighted shape index; MPAR= mean ratio area-perimeter. GF = Guadua forest; CGB= Consociation Guadua-Forest; AGB = Association Guadua-Forest; CBG = Consociation Forest –Guadua.*

Camargo and Cardona (2005), also use some metrics to identify the degree of patchiness. The juxtaposition index (IJI), which measures the degree of patches dispersion which is expressed in percentage with values between 0 (when adjacencies are uneven and getting connectivity is complicated) and 100 when patches are equally adjacent (Elkie et al. 1999). The proximity index (MPI) that gives values about 0 when edges of other patches are distant while value increases patches of the same class are closer (Gustafson and Parker 1992) and the nearest neighbour distance (M) which represents the average distance to patches of the same class (Elkie et al. 1999).

Concerning to the above mentioned indexes, Camargo and Cardona (2005) identified two tendencies where patches of GF seem to be closer among patches of the same class according to values of MPI and M. While patches of other classes CGB; CBG and ABG tend to be dispersed (Figure 1).

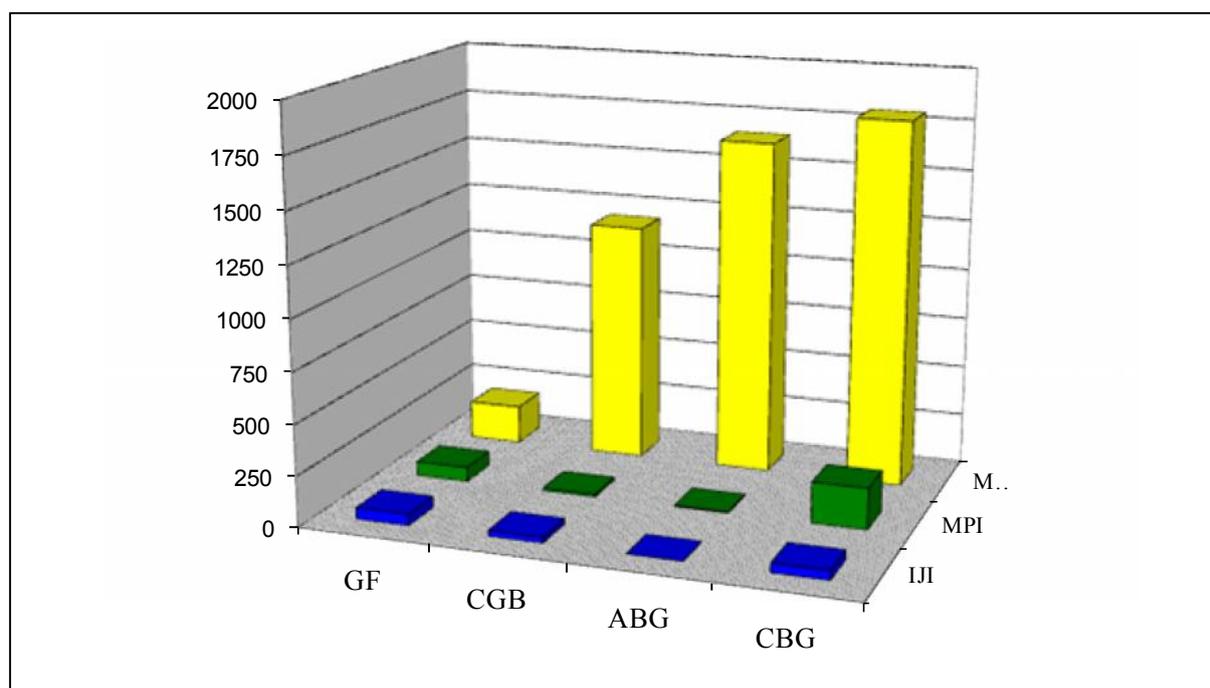


Figure 1. Dispersion metrics applied to classes of patches. GF = Guadua forest; CGB= Consociation Guadua-Forest; AGB = Association Guadua-Forest; CBG = Consociation Forest –Guadua. Juxtaposition index (IJI); proximity index (MPI) and nearest neighbour distance (MNN). Adapted from Camargo and Cardona (2005).

In terms of biodiversity conservation, small patches of forest can be considered a disadvantage because the proportion of undisturbed habitat is reduced (Elkie et al. 1999; Murcia 1995; Díaz 1991). Also dispersion of patches affects the moving of species and particularly when adjacent covers are very contrasting become barriers for many species (Laurance and Vasconelos 2004). However, if distances among patches are shorter it could be an advantage to promote the connectivity could be easier.

According to Susan and Laurence (2004) connectivity can be improved by corridors and a wide range of species could live within them depending on composition and structure. Therefore corridors should be as large as possible and with a complex structure for getting connectivity through different levels (canopy, overstory).

Guadua forests and biodiversity

The different classes or categories of forest previously defined according to the guadua dominance show that the composition and therefore plant biodiversity can highly vary among patches of guadua forest. Nevertheless, there are enough evidences that these forests are an important refuge of plant biodiversity. Besides, they provide habitat to about 50 species of birds and 5 of mammals (bats), which fulfil important ecological functions (CIEBREG 2008). In addition, some specific studies on richness

of vegetation species are referred in Calle and Piedrahita (2008) and Ospina (2002) and birds in Fajardo et al. (2008).

Within guadua forests located in the states of Quindío, Valle del Cauca, Risaralda and Caldas Ospina (2002) found a total of 26 families and 63 species (diameter at breast height larger than 10 cm) among palms, shrubs and trees. About 77% of families were represented by two or less species. On the other hand were found 182 species with diameter at breast height lesser than 10 cm. Most of these species are heliophyte which achieve their establishment within guadua forests; however few of them go above of the canopy. This author emphasises on the worth of guadua forests as remnants of habitat that contribute with the conservation of biodiversity and also with functions of flow regulating as riparian ecosystems.

In the low lands of La Vieja river basin, which is also part of the coffee region, Calle and Piedrahita (2008) determined that about the 70% of plants biodiversity are confined in fragments of forest with different levels of connectivity and surrounded by other land-uses highly contrasting in terms of structure and composition. Likewise, Calle and Mendez (2009) registered within plots of 1000 m² located within guadua forest up to 236 individuals and 9 different species. These results show the importance of guadua forests for plant biodiversity, especially because of at level of landscape, the matrix is dominated by grasslands most of them without trees and with low values of biodiversity.

Biodiversity of guadua forest also is represented by other groups of organisms. Fajardo et al. (2009), also in the low lands of La Vieja river basin, found that fragments of forest have the higher richness and diversity of birds in comparison with other land-uses. Fragments of guadua forest with 69 species of birds were one of the covers more diverse. Perez-Rojas et al. (2009), also registered the importance of guadua forests as habitat of bat species. They found within guadua forest 5 species of bats, which are associated with functional groups that contribute with ecological functions as seed dispersion and pollination. Both groups of organisms assessed represent the importance of these forests providing elements of interest for biodiversity conservation and therefore one more argument to promote a proper management.

Guadua bamboo forest and REDD+

About 28000 ha estimated (Kleinn and Morales 2006) of guadua forests of the Colombian coffee region are considered the target of this REDD+ proposal. As it was above described these forests have a spatial distribution pattern highly fragmented with most of patches lesser than 5 ha (Camargo and Cardona 2005) and located mainly along of the rivers or streams as riparian arrangement.

Although the species guadua is adapted to different site conditions, there are special environment which favour its growth and optimal development (Castaño and Moreno 2004). Guadua grows best between 900 and 1600 m above sea level, at temperatures between 20 and 26°C, precipitation between 1500 mm and 2500 mm per year, and in slightly acidic soils (Cruz 2009).

The growth patterns of the guadua and trees are completely different; therefore bamboo inventory and mensuration should be conducted by using different criterions (Camargo 2006). For reaching an adequate management of this resource basic information on the dendrometric attributes of culms, on stand variables as well as on stand management options is quite relevant. Also aspects such as stand productivity, behaviour in different environments and stand management should be considered (Camargo 2006, García 2004; Hincapie and Penagos 1994).

Because of characteristics of guadua culms, logging and processing is usually conducted by using a machete and just recently chainsaws are being used for conducting out harvest in some forest. Harvesting of guadua forest consists of the extraction of a fraction of mature culms, it means that a significant number of standing culms with different stages of maturity remain after harvest in guadua forests. When harvesting, a portion of mature and over-mature guadua culms are cut, then each one is divided usually in four pieces according to the possibilities of market.

Culms of guadua with mean diameter of 12 cm, as well as average length of 21 meters are daily

harvested. Although culms are hollow these can be used, however for some specific cases are spliced to obtain other kind of products. Between 2000 and 2004 roughly 2,420,000 culms of guadua were logged from 2,557 ha (Moreno 2006). This means that about 90 % of guadua forests were not harvested in this period of time. However, guadua forests have been significant for consolidating enterprises (Held 2005) and have improved the possibilities for expanding markets to Europe (Becker 2004).

In order to define the level of reference for a REDD+ approach, it is useful to determine historical trends of factors influencing forest cover in the coffee region of Colombia. Because the existing forest area and its level of degradation is the consequence of the dynamics in land use and land change along the time.

In the context of the country Colombia between 1960 and 1995 pasturelands changed from 14.6 to 35.5 million ha, whereas natural forests and agriculture declined from 94.6 to 72.4 million ha (IAvH et al. 1998). Currently, the country has 40.6 million ha of permanent grazing lands and an annual deforestation rate of 300,000 ha, which represent the double of the period between 2004 and 2009 (Jarvis et al. 2010).

In the coffee region, forest cover has been gradually eliminated first for establishing especially coffee plantations and then for pastures. Traditionally coffee plantations have less negative impacts on environment, because were designed as agroforestry systems. However, during the last 20 years, trees have been removed and plantations without trees are now predominant. Besides, the area of pastures has rapidly increased and currently is the larger land use in this region. In addition, the urban expansion process is also contributing with the pressure exercised on these forests. Cities of the coffee region have defined plans of growing towards rural areas where guadua forests are located. Hence the land price is increasing and this process continues more and more to accelerate. Figures 2 and 3 show images of how guadua forests are influenced by both agriculture and urban expansion.



Figure 2. Evidences of soil degradation associated to cattle (left) and agriculture (right) in the coffee region of Colombia.



Figure 3. Urban expansion. Cities of the coffee region are rapidly growing out toward rural areas where guadua bamboo forests are located.

Fragmentation of guadua bamboo forests has implications for silviculture. Whether market increases, larger area of guadua forests would be required to meet the demands of products. In addition, a number of farmers are not managing the small guadua areas properly to avoid costs of forest planning and technical assistance. Although, most of the culms harvested are used for domestic applications and the intensity of harvesting is usually low, the silvicultural practices to obtain these are inadequate. Therefore, domestic harvest sometimes causes damages and contributes to increased susceptibility of guadua stands as well as to a decreased productivity and quality.

For guadua bamboo forests, there is not adequate information on illegal logging. Nevertheless, institutions in charge of forests control have reported that a significant amount of guadua culms are illegally harvested and commercialised (ie. Moreno 2006a). Problems in control of illegal logging are also a consequence of difficulties to monitor the large number of small forest patches distributed along the coffee region and due to the fact that logged culms are used mainly for domestic purposes.

Above, were described those aspects related with possibilities of degradation of guadua forests. This information is relevant in the context of a REDD initiative because it permits to show which changes can be achieved and specifically that could be improved. Thereafter, it is also important to define how these forests can contribute with climate change mitigation.

Carbon sequestration has been preliminary estimated in natural guadua forests and plantations (Camargo and Arango 2010). Results are separately shown because plantations are still growing and have different characteristics such as higher density of culms per hectare and smaller sizes (diameter and length). Total carbon stored by natural guadua stands with an average density of 4050 culms per ha is of $126 \pm 41,7 \text{ t ha}^{-1}$ (Figure 6) with about 85% corresponding to aboveground biomass (culms, branches and leaves) and 15% to belowground biomass (rizhome). For plantations with an average density of 7700 culms per ha, the total carbon stored is of $24,6 \pm 5 \text{ t ha}^{-1}$ (Figure 7) with 86% of aboveground biomass and 14% of belowground biomass. It is also important to remark that soil carbon under guadua stands and bamboo plantation measured at 0,5 m of depth, was estimated in $544 \pm 125 \text{ t ha}^{-1}$ on average. Allocation of biomass in different compartments of is shown in Figure 4 and 5. The high differences between carbon values of plantations and natural stands are associated with the dimensions of the culms, whereas in natural stands the average diameter is of 11 cm ($\pm 1,9$) within plantation is of 6,7 cm ($\pm 1,9$). However plantations are still growing and therefore biomass will become higher.

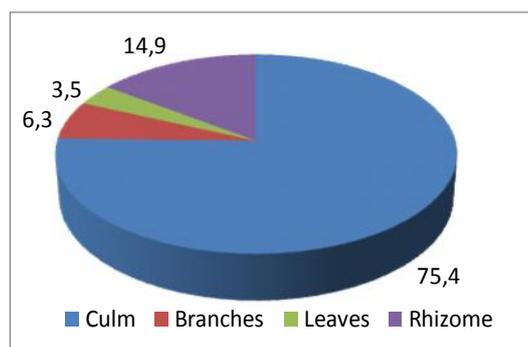


Figure 4. Distribution of carbon stored (%) by compartments of *Guadua angustifolia* Kunth within natural stands. Coffee region of Colombia.

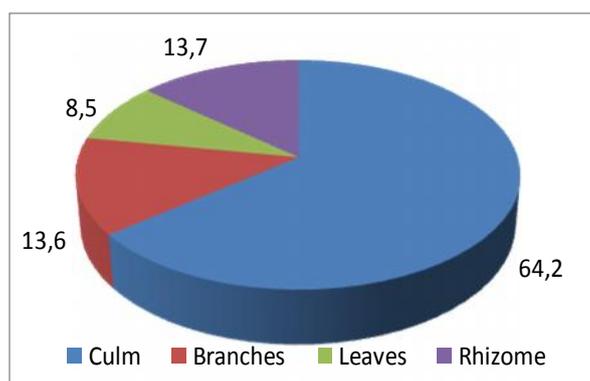


Figure 5. Distribution of carbon stored (%) by compartments of *Guadua angustifolia* in a plantation, eight years after established. Coffee region of Colombia.

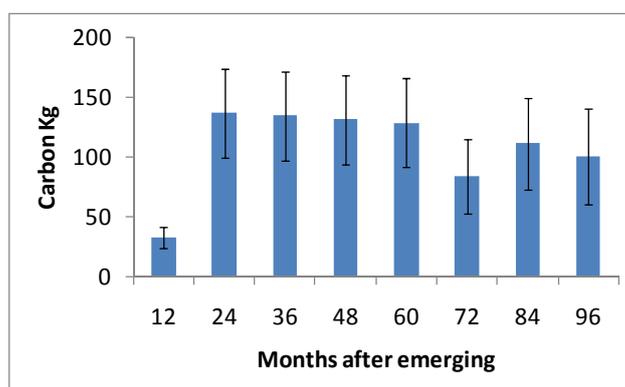


Figure 6. Total carbon stored (Kg) by an entire culm (cum, leaves, branches and rhizome) of *Guadua angustifolia* Kunth within natural stands according to its state of development.

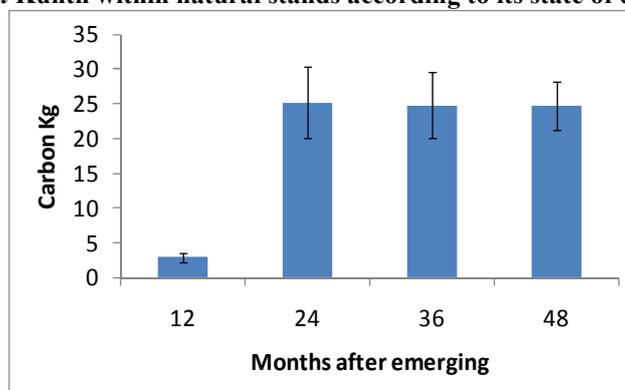


Figure 7. Total carbon stored (Kg) by an entire culm (cum, leaves, branches and rhizome) of *Guadua angustifolia* Kunth within a plantation eight years after established.

Apparently the allocation of biomass is similar between natural stands and plantations. However, biomass in branches and leaves is higher in plantations, probably because photosynthesis is optimised for growing and additionally there is more available space for the development of lateral branches and leaves. Changes in biomass allocation depending on availability of light and age of species have been reported by some authors (ie. Anten and Hirose 1998; King 2003). Also for bamboo species, allocation of biomass and nutrients can vary in accordance with the level of disturbance of the stands and the total density of culms (Kleinhenz and Midmore 2001). According to Camargo (2006) the leaf area of guadua clumps within plantations increases fast during the first years after establishing. This increment tends to decrease when branches of culms reach those from other clumps. Increment of leaf area is associated with the emission of new culms which represent also increase of total area occupied by each clump.

The decreasing of biomass after 5 years is related to the loss of leaves and branches of older culms. In

addition, they usually are also broken by wind. As a consequence about 10% of biomass corresponding to leaves and branches cannot be included at this stage of maturity. If guadua culms are left in the forest, after 10 or 12 years they naturally die, consequently become emissions of CO₂. This is a significant aspect that should be considered for promoting an adequate silvicultural management, given that whether culms are harvested CO₂ might be stored in subsequent products. However, proper treatments to guarantee durability should be applied on these.

Protocols for monitoring carbon within guadua stands (natural and plantations) are being assessed (Camargo and Arango 2010) and there are approaches which also could be applied for indirectly estimates of biomass (Camargo and Kleinn 2010). In addition approaches for sampling and plot design are available and permit appropriate estimates when inventories are conducted out. Plot design has been evaluated by Camargo (2006); Schumacher (2006) and Rijal (2006). Also approaches for guadua inventory can also be observed in Kleinn and Morales (2006) and Camargo et al. (2008).

Possibilities of guadua forests as refuge of biodiversity were above described. In addition, guadua bamboo forests are important as stepping stones for processes of ecological restoration. Hence strategies orientated to improve their connectivity can significantly contribute to reduce the contrast with respect to predominant matrix of pastures. Strategies therefore, should not consider only the specific management of guadua forest but also to promote alternatives at landscape level which can integrate agroforestry systems with different arrangements (Harvey et al. 2004) for improving the connectivity between fragments of forests and to guarantee ecological functions. Figure 8 shows how fragments of guadua forest are connected with living fences of *Gliricidia sepium* which contribute to improve ecological conditions for a transformed landscape.



Figure 8. Guadua bamboo forests connected by living fences of *Gliricidia sepium*. Some of living fences are embedded in red lines.

In order to consolidate the potential of guadua forests for facing climate change, their capability of carbon sequestration and the other ecological functions associated with them are the base for developing strategies. These forests really contribute to develop agroecosystems less vulnerable to changes. Besides, if some strategies as agroforestry systems are also integrated by farmers, they can have better possibilities of building farming systems less vulnerable and more resilient. Nevertheless, these aspects ought to be complemented with actions from the political and financial side.

Aspects related with how the benefits should be distributed and financing are simultaneously analysed because both are strongly related. To define how the benefits of reductions will be allocated, which distribution mechanism could be used, it is also necessary to know where the money might come from. The implementation of REDD+ projects requires also a political framework which provides a proper legislation, institutional strengthening and possibilities of financing. Therefore some political

initiatives supported by institutions and projects previously conducted out, may be linkage or taken as reference for the implementation of a REDD+ project.

To face the current situation of guadua forests and to contribute to their sustainable management, different strategies have been promoted. This process has been led by government institutions in charge of environmental control so called Corporaciones Autónomas Regionales (CARs) and throughout different projects. Also, the technological and scientific support of universities has been significant.

As a result of the above mentioned process, have been defined lineaments for an adequate management of guadua forests. One of the outcomes is the Unified Norm (Norma Unificada para el manejo sostenible de guaduales naturales), which defined the guidelines for an adequate management in the framework of the current legislation. In addition, the Terms of Reference for the Management and Harvesting of Guadua Stands (TRMHGS) was also defined as a mechanism to apply the Unified Norm.

The Unified Norm and the TRMHGS as well, aim to achieve the sustainable management of guadua forests. Therefore, those guadua forests, which fulfil the requests of the Unified Norm and the TRMHGS, are registered as those with sustainable management. After that, if good planning and management is evidenced by CARs, farmers can receive incentives such as the reduction of taxes and the provision of technical assistance. This instrument is an important mechanism that could be useful for developing schemes of payments or incentives, which would be focussed on farmers who are performing good practices. In this case, institutions would be in charge not only of control but also of managing mechanisms to distribute benefits to farmers.

On the other hand, the planning of guadua forests has been contributed with the definition of suitable area for establishing guadua plantations and the consolidation of Units Forest Management (UFM). According to Camargo et al. (2007a), about 124000 ha have a high suitability for establishing guadua plantations for commercial aims. It was determined after the evaluation of 25 biophysical and socioeconomic variables. It means high possibilities of establishing guadua plantations with possibilities for commercial outputs in a relative short time (eight years). Additionally, incentives associated with ecological restoration and carbon sequestration, could be also obtained.

The UFM represents a set of conditions joined to a territory where guadua forests, farmers, market of products from guadua forests and the adequate infrastructure, are together. In the coffee region were estimated about 87000 ha associated to UFM of high productivity or with the better conditions for promoting alternatives associated to the management of forests (Camargo et al. 2007a).

UFM also have been useful to develop initiatives of forest management by groups of farmers, especially of those with small (but closed) patches of guadua forests in their farms. As a consequence costs of management and technical assistance as well as labours are considerably reduced. Therefore contributions for increasing the feasibility of obtaining benefits from forest even for farmers with small patches are now reachable and are significant for improving the organization of farmers, which is also convenient for development schemes for the distribution of benefits.

Camargo et al. (2007a) also did estimates of the potential of guadua forests for carbon storing at regional level and they give values of CO₂ stored for each UFM. In this study, the biomass estimation was done indirectly by using data of culm volume and culm density (wood density) registered by Camargo (2006). Additionally, these estimates are compared with information extrapolated from measurements done directly with destructive sampling (Arango 2011). When values of carbon are extrapolated to the total area covered by these forests, the real potential of these forests for carbon sequestration is elucidated. In Table 3, are presented values for two of the states analysed, Risaralda and Quindío.

Table 3. Average of CO₂ stored by guadua forests in two states (Risaralda and Quindío) of the coffee region of Colombia according to Units of Forest Management (UFM).

Risaralda State							
UFM	Area/ ha	Average Culms / ha	CO ₂ t / ha Study ^a		CO ₂ t / ha Total* Study ^b	CO ₂ t Total* area Study ^a	CO ₂ t Total* area Study ^b
			Biomass	Total*			
High	1354	8233	943,2	1461	929	1978600	1257866
Moderate	836	8883	1017,6	1536	907	1283845	758252
Low	45	5500	630,1	1148	760	51669	34200
Total	2235	7539	863,6	1382	865	3088100	1934020
Quindío State							
UFM	Area/ ha	Average Culms / ha	CO ₂ / ha Study ^a		CO ₂ / ha Total Study ^b	CO ₂ Total area Study ^a	CO ₂ Total area Study ^b
			Biomass	Total			
High	2806	5090	583	1101	727	3089938	2041084
Moderate	1061	5133	588	1106	646	1173579	685512
Low	10	9416	1079	1597	788	15970	7880
Total	3867	6546	750	1268	2162	4903463	8360067

UFM= Units of forest management ; Study a= Arango (2011); Study b= Camargo et al. (2007a); * = values include carbon in soils and biomass

The values of CO₂ stored are remarkable compared with other types of forest covers. For example in Amazonian ecosystems Callo-Concha et al. (2002) found values of total carbon stored (biomass and soil) for primary forest of 465,8 t Cha⁻¹ and for secondary forests of 181,02 t Cha⁻¹. The differences in estimates for guadua bamboo forests are associated with the methods applied in each case for estimating the CO₂ stored and also with the precisions of the model used to determine culm volume. Therefore to standardise approaches and developing more precise models should be a priority. However, it is just a piece of the whole structure that should be constructed for consolidating a real strategy which permit consolidates the bases for a REDD+ initiative.

The political framework that now exists in Colombia and the promotion of forest governance during the last years should be seen as an opportunity. Many of the instruments developed and used sometimes for controlling illegal logging, can be also useful for improving organisation of farmers, their training, provision of technical assistance and the access to incentives. Institutions in charge of leading these projects have possibilities of enhance the possibilities and probably also better possibilities to promote the sustainable management of forests in the coffee region.

Conclusion

Guadua forests are highly fragmented, this evidenced through different metrics utilised by landscape analyses. However, most of the patches are close to others and this condition ease the possibilities of promoting alternatives addressed to improve their connectivity. When distances increases among patches, their size also increase, therefore the drawbacks associated with dispersion can be compensated by a larger size. Thus, patches of guadua forests can be always seen as a possibility for biodiversity conservation and stepping stones for process of ecological restoration.

Biodiversity registered within guadua forests shows their relevance as habitat of different species that contribute with different ecological functions. A good example is the richness of birds which can be higher in guadua bamboo forest than in a secondary forest in the Colombian coffee region (Fajardo et al. 2009). The high contrast that represents the nearer land cover as grasslands and other agricultural

uses, can affect some conditions because of the edge effect. On the contrary, ecological functions provide by organism found within these forests might generate benefits for the nearby land uses.

The capability for carbon sequestration of $109 \pm 54 \text{ t ha}^{-1}$ in average as well as the institutional and political development are remarkable and might contribute for easing REDD+ project implementation. Soil with about $544 \pm 125 \text{ t ha}^{-1}$ at 0,5 m of depth is an important part of carbon pools and should be seen with more interest as an significant carbon sink. The proportion of soil carbon regarded to biomass carbon, has been defined as higher for different authors, ie. Lal et al. (1995) suggests that carbon can be three times more than in living organisms which is consistent with results presented here.

Aspects related to monitoring carbon and strategies for mainstreaming REDD+ initiatives as a priority within territorial, economical sectors and institutional planning process must be strengthened. Different approaches for bamboo inventory and carbon monitoring should be consolidated and standardised and additionally must be promoted to be included in a national strategic for carbon estimation led in Colombia by Ideam.

The implemented model for forest planning is useful to define land suitability for commercial guadua plantations and also UFM. Nowadays, government institutions can lead the planning of guadua stands based on this model. Besides, consolidation of UFM is an alternative against drawbacks relate to fragmented pattern of guadua bamboo forests and also for avoiding their degradation.

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References

- Anten, N. P. R. and Hirose, T. (1998). Biomass allocation and light partitioning among dominant and subordinate individuals in *Xanthium canadense* stands. *Annals of Botany* (82): 665–673.
- Arango, A.M. (2011). *Posibilidades de la Guadua para la Mitigación del Cambio Climático. Caso: Eje Cafetero Colombiano*. Trabajo de Grado Pregrado. Facultad de Ciencias Ambientales Universidad Tecnológica de Pereira. 113p.
- Banik, R. L. (1999). *Domestication and improvement of bamboos*. Chittagong, Bangladesh: Bangladesh Forest Research Institute. 15p.
- Becker, M. (2004). Bamboo markets in Western Europe: Perspectives for guadua products. In: *Proceedings International Symposium Guadua 2004*. Pereira, Colombia.
- Calle, Z. and Piedrahita, L. (2008). Conservación de flora amenazada en fincas ganaderas de la cuenca media del río La Vieja. En: *Ganadería del futuro. Investigación para el desarrollo*. 148- 169 p Cali, Colombia: Fundación CIPAV.
- Calle, Z.; Méndez, L.E. (2009). Estructura y Composición de la Vegetación Arbórea en el Agropaisaje del Rio la Vieja. In: Rodríguez, J.M.; Camargo, J.C.; Niño, J.; Pineda A.M.; Arias, L.M.; (Eds). (171-182 p) *Valoración de la Biodiversidad del Eje Cafetero*. Pereira, Colombia: CIEBREG.
- Callo-Concha, D.; Krishnamurthy, L.; Alegre, J. 2002. Secuestro de carbono pro sistemas agroforestales amazónicos. *Revista Chapingo*. (8) 2: 1001-106.
- Camargo, J C. (2006). *Growth and productivity of the bamboo species Guadua angustifolia Kunth in the coffee region of Colombia*. Göttingen, Germany: Cuvillier Verlag.
- Camargo, J.C., Cardona, G. (2005). *Análisis de fragmentos de bosque y guaduales. Enfoques silvopastoriles integrados para el manejo de ecosistemas*. Pereira, Colombia: CIPAV-CATIE-BANCO MUNDIAL-GEF-LEAD. Unpublished project report (38p).
- Camargo, J. C.; Gaviria, J. and Cardona, H. (2007). *Sistemas Silvopastoriles con Árboles Maderables dentro de Pasturas: Estrategias para su Establecimiento*. Pereira, Colombia: GATA, CIEBREG. Universidad Tecnológica de Pereira, Colciencias. 108p.
- Camargo, J. C.; Dossman, M. A.; Cardona, G.; García, J. H. and Arias, L. M. (2007). *Zonificación detallada del recurso guadua en el Eje Cafetero, Tolima y Valle del Cauca: Municipios piloto del proyecto Manejo Sostenible de Bosques en Colombia (Guía metodológica y resultados)*. Pereira, Colombia: Ministerio de Ambiente Vivienda y Desarrollo Territorial, Universidad Tecnológica de Pereira, Corporaciones Autónomas Regionales del Tolima, Quindío, Valle del Cauca y Risaralda. 144p.
- Camargo, J. C. and Arango, A. M. (2010). *Estimación del contenido de carbono en guaduales naturales y plantados de la zona cafetera de Colombia*. Pereira, Colombia: Tecnología para definir la madurez del culmo de *Guadua angustifolia* Kunth: una contribución al desarrollo forestal del Eje Cafetero Colombiano. Unpublished Project Report. 15p.
- Camargo, J.C.; Morales, T.; García, J.H. (2008). *Mensura e Inventario forestal para la Planificación y Manejo Sostenible de Bosques de Guadua*. POSTERGRAPH. Pereira: Universidad Tecnológica de Pereira, Grupo Gestión de Agroecosistemas Tropicales Andinos. 90p.
- Camargo, J.C.; Kleinn, C. (2010). Length curves and volume functions for Guadua bamboo (*Guadua angustifolia* Kunth) for the coffee region of Colombia. *European Journal of Forest Research*. 129 (6): 1231-1222 p.
- Castaño, F and Moreno, R. D. (2004). *Guadua para todos. Cultivo y Aprovechamiento*. Bogotá: Proyecto Manejo Sostenible de Bosques de Colombia.190p.
- CIEBREG. (2008). Informe anual proyecto “*Valoración de los Bienes y Servicios de la biodiversidad para el desarrollo sostenible de paisajes rurales colombianos: Complejo Ecorregional Andes del Norte*”. Universidad Tecnológica de Pereira, Pontificia Universidad Javeriana, Fundación CIPAV, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.
- Cruz, H. (2009). *Bambú –Guadua. Guadua angustifolia* Kunth. Bosques naturales en Colombia. Plantaciones comerciales en México: Pereira, Colombia. 690p.
- Diaz, N. (1991). *Forest landscape analysis and design. A process for developing and implementing land management objectives for landscape patterns*. United States Department of Agriculture: Forest Service Pacific Northwest Region. 63p.
- Elkie, P.C.; Rempel, R. S.; Carr, A.P. (1999). *Patch Analyst User’s Manual: A tool for quantifying*

- landscape structure*. Queen's, Ontario, Canadá. 22p.
- Fajardo, N.D.; González, R. J. and Neira, L. A. (2008). Sistemas ganaderos amigos de las aves. En: *Ganadería del futuro. Investigación para el desarrollo*. Cali, Colombia: Fundación CIPAV. 171-203p.
- Fajardo, D.; Gonzales, J.; Neira, J.; Chará, J.; Murgueitio, E. (2009). Influencia de Sistemas Silvopastoriles en la Diversidad de aves en la Cuenca del Rio La Vieja. *Recursos Naturales y Ambiente (CATIE)*. 58: 9-16 p.
- Forman, R. T. T. and Godron, M. (1986). *Landscape Ecology*. New York: John Wiley. 619 p.
- García, J H. (2004). *Definición de áreas óptimas de calidad de guadua (Guadua angustifolia Kunth), orientada a satisfacer las necesidades del mercado*. Master thesis. Facultad de Ingeniería Industrial, Universidad Tecnológica de Pereira. 110p.
- Gustafson, E. J. and Parker. G. R. (1992). Relationships between landcover proportion and indices of landscape spatial pattern. *Landscape Ecology* 7:101-110 p.
- Hanumappa, H.G. (1996). *Agarbathi: A bamboo-base industry in India*. Bangalore, India: Institute of Social and Economic Change. INBAR, Working Paper 9. 22p.
- Harvey, C.; Nigel, J.; Tucker, Estrada, A. (2004). Live fences, isolated trees and windbreaks: tools for conserving biodiversity in fragmented tropical landscapes. In: Götz S., Fonseca G., Harvey, C., Gascon, C., Vasconcelos, H., Izac, A.M. (Eds) *Agroforestry and biodiversity Conservation in Tropical Landscapes* (261-283p). Washington: Island Press.
- Held, C. (2005). *Promotion of innovations in forest based small and medium size enterprises of developing countries*. An actor-oriented analysis of the Colombian bamboo sector. Zugl.:Freiburg: Uni., Diss, 2004. 223p.
- Hidalgo, O. (2003). *Bamboo, the gift of the gods*. Ed. Hidalgo O. Bogotá, Colombia. 553p.
- Hincapié, A. N. and Penagos. J. I. (1994). *Aplicación de métodos multivariados de componentes principales y medidas repetidas para la evaluación ecológica de los bosques de Guadua angustifolia de la jurisdicción de CVC bajo diferentes condiciones de sitio*. Cali. Colombia: Universidad del Valle, Corporación Autónoma Regional del Valle del Cauca CVC. 318 p.
- Instituto Alexander von Humboldt, PNUMA, Ministerio del Medio Ambiente. (1998). Informe nacional sobre el estado de la biodiversidad 1997. In: Chaves. M.E., Arango. N. (Eds.) *Causas de pérdida de biodiversidad*. Tomo II: Bogotá, Colombia.
- Jagger, P. ;Sills, E. ; Lawlor, L.and Sunderlin, W. (2010). *A guide to learning about livelihood impacts of REDD+ projects*. Occasional paper 56. Bogor, Indonesia: CIFOR.108p.
- Jarvis, A.; Touval, J. L.; Castro, M.; Sotomayor, L.; Hyman, G.G. (2010). Assessment of threats to ecosystems in South America. *J. Nat. Conserv.* 18 (3), 180–188 p.
- King, D. A. (2003). Allocation of above-ground growth is related to light in temperate deciduous saplings. *Functional Ecology* 17: 482–488 p.
- Kleinhenz, V. and Midmore, D. J. (2001). Aspects of bamboo agronomy. *Advances in agronomy*. 74: 79-145 p.
- Kleinn, C. and Morales, D. (2006). An inventory of Guadua (*Guadua angustifolia*) bamboo in the Coffee Region of Colombia. *European Journal of Forest Research* 125 (4): 361-368 p.
- Lal, R.; Kimble. J.; Levine. E.; and Whitman. C. (1995). World soils and greenhouse effect: An overview. In: *Soils and global change*. Adv. Soil Sci., Lewis Publishers, Boca Raton: 1-8.
- Laurance, W. F.; Vasconcelos, H. L. (2004). Ecological effects of habitat fragmentation in the tropics. In: Götz. S.; Fonseca. G.; Harvey, C.; Gascon. C.; Vasconcelos, H.; Izac. A.M. (Eds.) *Agroforestry and biodiversity Conservation in Tropical Landscapes*. Washington: Island Press. 33-49 p.
- Lobovikov, M.; Lou, Y.; Schoene, D.; Widenoja, R. (2009). The poor man's carbon sink, FAO, *Non-Wood Forest Products Working Document* N° 8. 72p.
- Lobovikov, M.; Paudel, S.; Piazza, M.; Ren, H.; Wu, J. (2007). World Bamboo Resources. FAO, *Non-Wood Forest Products* 18. 87p.
- Londoño, X. (1990). Aspectos sobre la distribución y ecología de los bambúes de Colombia (Poaceae: Bambusoideae). *Caldasia* 16 (77): 139-153 p.
- Murcia, C. (1995). Edge effects in fragmented forest: Implications for conservation. *Tree*. (10) 2: 50-62 p.
- Moreno, R. D. (2006). *Estadísticas Forestales para La Guadua en el Eje Cafetero Tolima y Valle del*

- Cauca. Proyecto Manejo Sostenible de Bosques en Colombia*. Pereira: Programa Ambiental de la Agencia de Cooperación Alemana GTZ, Ministerio de Ambiente, Vivienda y Desarrollo Territorial, Corporación Autónoma regional de Risaralda CARDER. 53p.
- Moreno, R. D. (2006a). *Lineamientos generales para la conformación y operación de núcleos forestales productivos para la guadua*. Proyecto Manejo Sostenible de bosques para Colombia. Programa Ambiental GTZ. 64p.
- Ospina, R. (2002). Factores que determinan las características florísticas estructurales de los fragmentos dominados por *Guadua angustifolia* Kunth en el Eje Cafetero Colombiano y su relación con los aprovechamientos de Guadua. Turrialba, Costa Rica. Thesis MSc. CATIE. 71p.
- Parker, C.; Mitchell, A. ; Trivedi, M. and Mardas, N. (2008). *The Little REDD Book. A guide to governmental and non-governmental proposals for reducing emissions from deforestation and degradation*. Oxford: Global Canopy Foundation, Global Canopy Programme, John Krebs Field Station, 60p.
- Perez-Rojas, J.; Sánchez Lalinde, C.; Cortes Delgado, N. (2009). Murciélagos Asociados a Sistemas Naturales y Transformados en la Ecorregión Eje Cafetero. In: Rodríguez, J.M.; Camargo, J.C.; Niño. J.; Pineda A.M.; Arias, L.M.; (Eds). *Valoración de la Biodiversidad del Eje Cafetero*. 157-167p Pereira, Colombia: CIEBREG.
- Razak, ABD. ; (2001). Bamboos growth assessment related to soil suitability. *J. Bamboo and Rattan*. 1 (1): 71-76.
- Rijal, B.; (2006). *Methodological Assessment of Sample Based Bamboo Management Inventory in Colombia*. Thesis produced to fulfill a partial requirement of M. Sc. Forestry. Göttingen, Germany. 79p.
- Schumacher, N. (2006). *Spatial distribution of the bamboo species Guadua angustifolia Kunth in the coffee region of Colombia*. Thesis M. Sc. Forestry. Göttingen, University, Germany. 93p.
- Susan, G.; Laurance, W.F. (2004). Landscape connectivity and biological corridors. In: Götz. S.; Fonseca. G.; Harvey. C.; Gascon. C.; Vasconcelos. H. ; Izac. A.M.; *Agroforestry and biodiversity Conservation in Tropical Landscapes*. Washington: Island Press. 50-63p.
- Zhou, S.L.; Ma, N.X.; FU, M.Y. (1994). *A compendium of chinese bamboo*. Beijing: Forestry Publishing House.

Effects of low temperature stress on antioxidase activity, osmoregulation substance and membrane lipid fatty acids of *Dendrocalamus latiflorus* seedlings

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Abstract

An indoor low temperature experiment was conducted to study the variations of membrane permeability, malondialdehyde, soluble protein and soluble sugar contents, superoxide dismutase (SOD) and peroxidase (POD) activities and membrane lipid fatty acid content in leaves and roots of *Dendrocalamus latiflorus* seedlings under different levels of cold stress. The differences in the response characteristics and related physiological indices were also analyzed. Results showed that after low temperature pretreatment (8 °C) for 15 days, the soluble protein, soluble sugar contents and POD activities in leaves as well as soluble sugar contents and POD activities in roots were increased obviously. After cold-hardening (-2 °C) for 72 h, the soluble protein, soluble sugar contents and SOD, POD activities in leaves and SOD, POD activities and ratio of membrane lipid unsaturated fatty acid in roots of *D. latiflorus* seedlings with pretreatment of cold-hardening were obviously higher than those of *D. latiflorus* seedlings without pre-treatment of cold-hardening. Membrane permeability in leaves of *D. latiflorus* seedlings with pretreatment of cold-hardening was obviously lower than that of *D. latiflorus* seedlings without pretreatment of cold-hardening. But the level of membrane lipid peroxidation in leaves of *D. latiflorus* seedlings with pre-treatment of cold-hardening was significantly greater than that before cold-hardening. While the level of membrane permeability and lipid peroxidation in roots of *D. latiflorus* seedlings with pre-treatment had no significant change after cold-hardening. Overall, low temperature stress had serious effects on the physiological characteristics of *D. latiflorus*. Leaves of *D. latiflorus* had higher soluble protein, soluble sugar contents and POD activities to avoid low temperature injuries while roots had higher SOD, POD activities and higher membrane lipid unsaturated fatty acid content to avoid membrane lipid peroxidation and membrane injuries.

Keywords

Dendrocalamus latiflorus, low temperature stress, antioxidase, lipid fatty acids

Introduction

Dendrocalamus latiflorus (Munro) is a perennial bamboo (family *Gramineae*, subfamily *Bambusoideae*), distributed mainly in Fujian, Taiwan, Guangdong, Guangxi, Hainan, Guizhou, Sichuan, Chongqing and Yunnan provinces in China. It is an important large sympodial bamboo species for shoot and timber, characterized by its high quality and fast-growth. *D. latiflorus* is a typical philotherm and sensitive to low temperature. It is easily to be injured by chilliness with the average temperature below 6 °C and extreme low temperature below -2 °C in January. As global climate change, a massive ice and snow storm occurred in early 2008 in south China and caused extensive damage to bamboo. Of all bamboos the species belonging to sympodial rhizome type suffered the heaviest frozen injury while those of monopodial rhizome type had the lightest damages (Zhou B. *et al.*, 2011). So it is important to explore the physiological and biochemical mechanisms of cold-tolerance formation in sympodial bamboos, identify the physiological characteristics and parts that are most closely related to cold-tolerance.

Currently cold-tolerance research of bamboo is only restricted to direct field sampling and measurement, while few reports have studied the mechanism of cold-tolerance formation in bamboos under controlled low temperature (Liu G. *et al.*, 2006; Liu Q. *et al.*, 2006). In this study, we used *Dendrocalamus latiflorus* seedlings from tissue culture and investigated the changes of physiological characteristics in leaves and roots at low-temperature through indoor low-temperature induced treatment methods. The results provide a basis for the future selection of cold-tolerance bamboo species.

Materials and methods

Experimental Materials

One-year old *Dendrocalamus latiflorus* monoclonal tissue culture seedlings were provided by the Seedling Center of Yong'an Forestry Ltd. Co, China. 24 seedlings were planted in 15 cm x 15cm x 15 cm pots, with one seedling per pot (with medium peat soil: riversand=3:1). All seedlings were maintained under the same growth conditions and routine management during the experiments.

Low temperature treatment

Low-temperature environment was simulated by light incubator and temperature control refrigerator in laboratory. All *Dendrocalamus latiflorus* seedlings were placed in the light incubator for one month (culture conditions: temperature 25 °C; relative humidity: 75%; light: 12 hours per day; light intensity: 4000lx). Then half of the seedlings (12 pots) were continuously grown in light incubator for 15 days (with same culture conditions as above, recorded as CK), half (12 pots) were placed in low-temperature light incubator for 15 days (day and night temperature was 8 °C; the remaining culture conditions were the same, recorded as C1). Then the control (CK) and cold-tolerance treated (C1) seedlings both were moved into temperature-controlled refrigerator (under darkness). Cooling rate was controlled to 2 °C per hour until reaching the target temperature of -2 °C. Seedlings were treated for 72 hours (recorded as CK 'and C1', respectively); leaves and roots samples were collected from CK, CK ', C1, C1' (root samples were washed with the corresponding temperature of pure water to remove the soil, and -2 °C treatment was rinsed with ice water). Control and treatment both had three repeats, each containing four seedlings, the sample were frozen in liquid nitrogen, wrapped tightly with aluminum foil, and stored at -80 °C as test materials to measure physical and chemical characteristics.

Measurement of physical and chemical characteristics

Characteristics were measured according to The guide of modern plant physiology experiments (Editorial Board of Shanghai institute for plant physiology, CAS, 2004), included electrolyte leakage, malondialdehyde (MDA) content, superoxide dismutase (SOD) and peroxidase (POD) activity, soluble sugar and soluble protein content. Unsaturated fatty acid content in membrane lipid was measured using Soviet method (Soviet *et al.*, 1980).

Results

Effects of low temperature on membrane permeability and lipid peroxidation of *Dendrocalamus latiflorus* seedlings

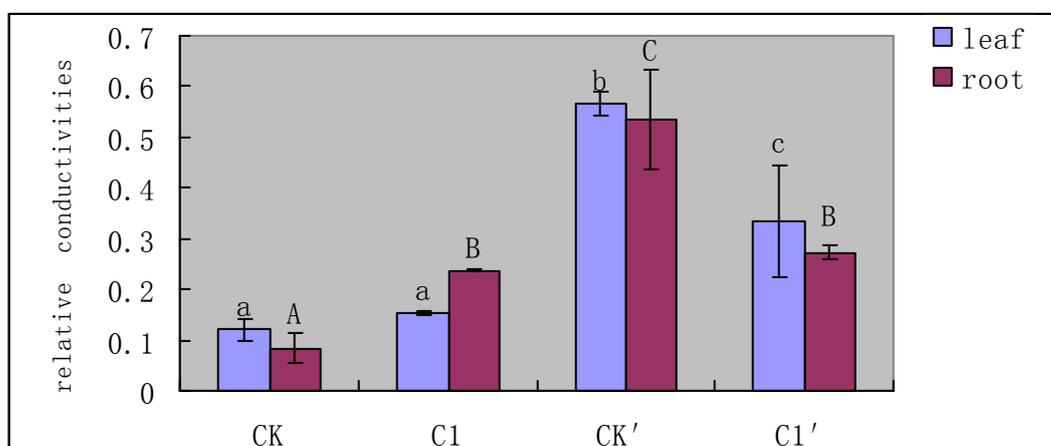


Figure 1 Effects of low temperature on relative conductivities in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n= 3). Different letters designate significant difference at $p < 0.05$.

After pretreatment of cold-hardening, the plasma membrane permeability of treated *Dendrocalamus latiflorus* (C1) leaves had no significant difference with the control (CK), but the membrane permeability in C1 roots was significantly higher than the control (Figure 1). After -2°C cold treatment, the membrane permeability of control (CK') leaves and roots had significantly increased. Membrane permeability increased significant in leaves in C1' group, but not in roots. In addition, in pretreatment group (C1'), the membrane permeability in both leaves and roots was significantly lower than control (CK').

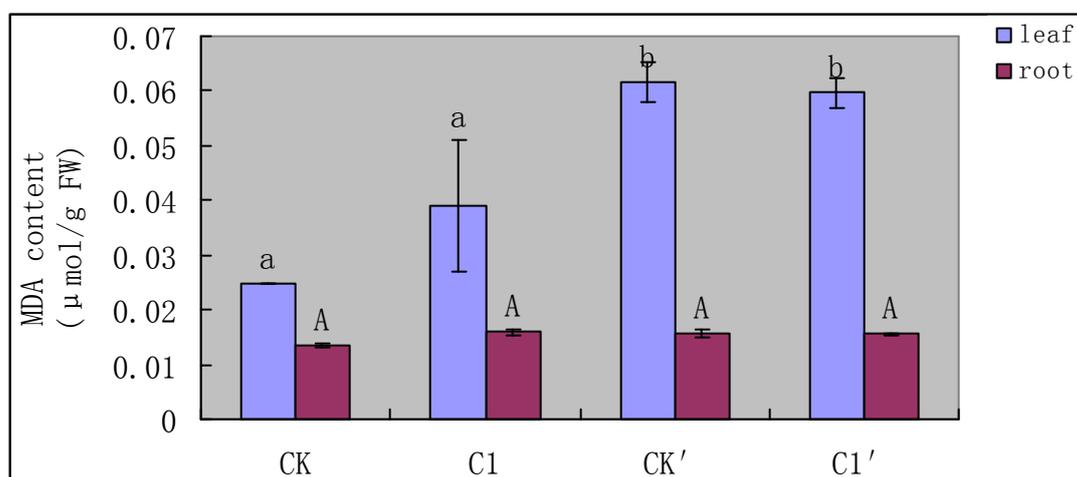


Figure 2. Effects of low temperature treatment on MDA content in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n= 3). Different letters designate significant difference at $p < 0.05$.

After pretreatment of cold-hardening, the MDA content in *Dendrocalamus latiflorus* seedling (C1) leaves was slightly higher than control (CK), but the difference was not significant (Figure 2). After -2°C cold-tolerance treatment, MDA content in control (CK') and pretreatment (C1') *Dendrocalamus latiflorus* seedlings were both significantly increased, but the MDA showed no significant difference in control (CK') and cold-tolerance treatment group (C1'). The MDA content of *Dendrocalamus latiflorus* roots in pretreatment group and -2°C treatment group was not significantly different before

and after treatment, and was significantly lower than membrane lipid peroxidation of leaves at the same stage. The results showed that as the treatment temperature decreased, the level of lipid peroxidation in *Dendrocalamus latiflorus* leaves increased, while the lipid peroxidation level in roots was not correlated with the temperature.

Effects of low temperature on soluble protein and soluble sugar content in *Dendrocalamus latiflorus* seedlings

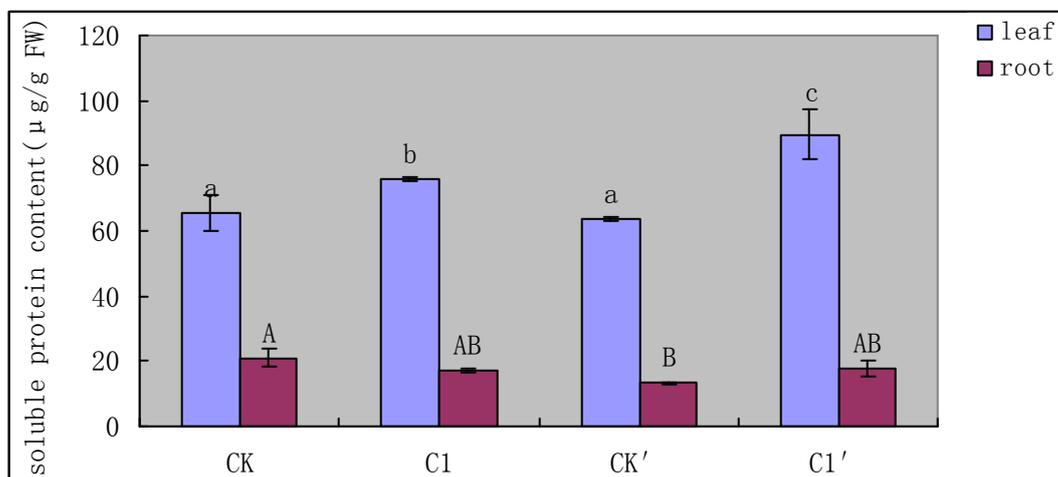


Figure 3 Effects of low temperature treatment on soluble protein content in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n=3). Different letters designate significant difference at $p < 0.05$.

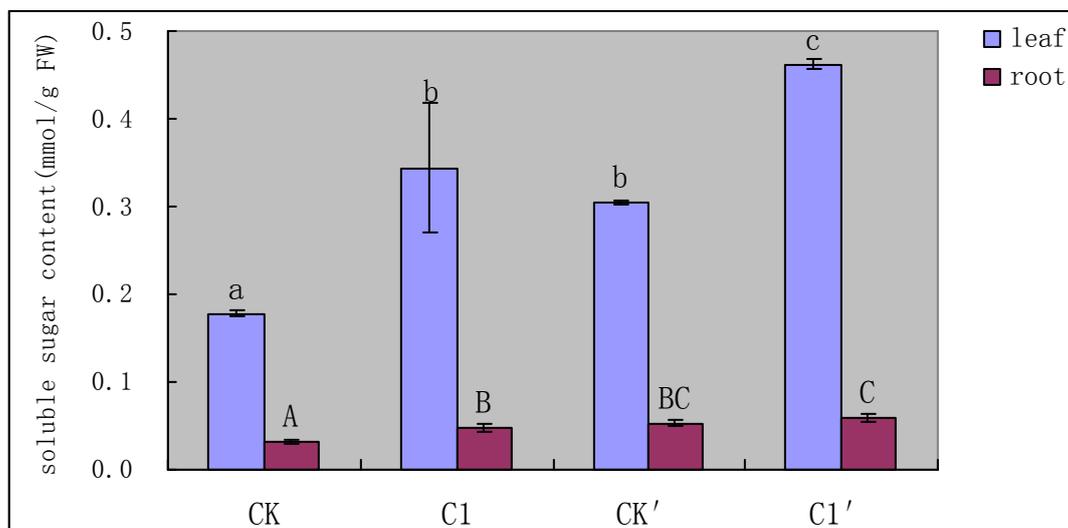


Figure 4 Effects of low temperature treatment on soluble sugar content in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n=3). Different letters designate significant difference at $p < 0.05$.

After pretreatment of cold-hardening, soluble protein and soluble sugar contents in *Dendrocalamus latiflorus* (C1) leaves all significantly increased compared to control (CK). After -2°C low temperature treatment, soluble protein and soluble sugar contents in *Dendrocalamus latiflorus* (C1') leaves were still significantly higher than those before treatment (C1), being also significantly higher than control (CK'). After -2°C low temperature treatment, the soluble protein and soluble sugar contents in *Dendrocalamus latiflorus* (C1') roots had no significant differences compared with the control (CK') (figures 3 and 4).

Effects of low temperature on antioxidant enzyme activity in *Dendrocalamus latiflorus* seedlings

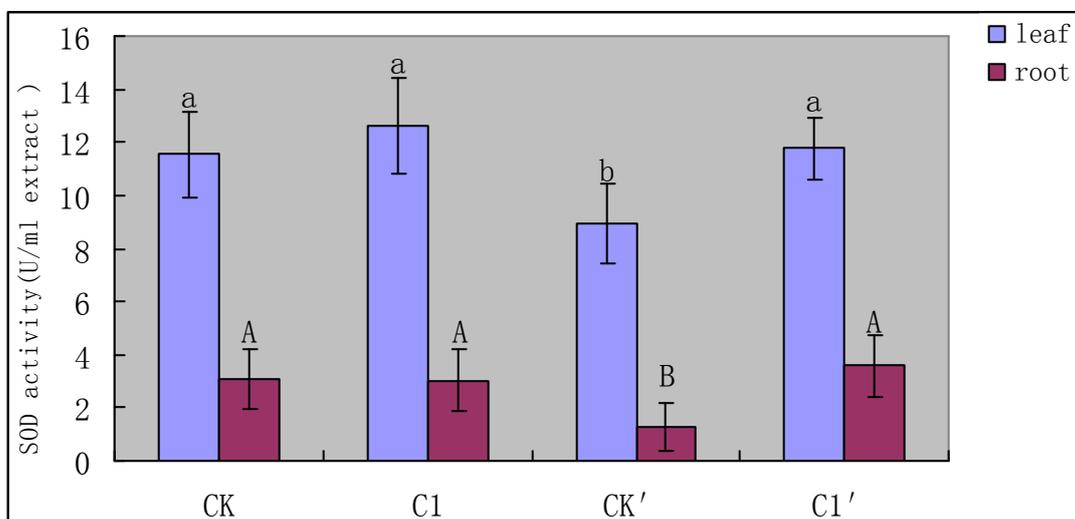


Figure 5 Effects of low temperature treatment on SOD activity in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n = 3). Different letters designate significant difference at $p < 0.05$.

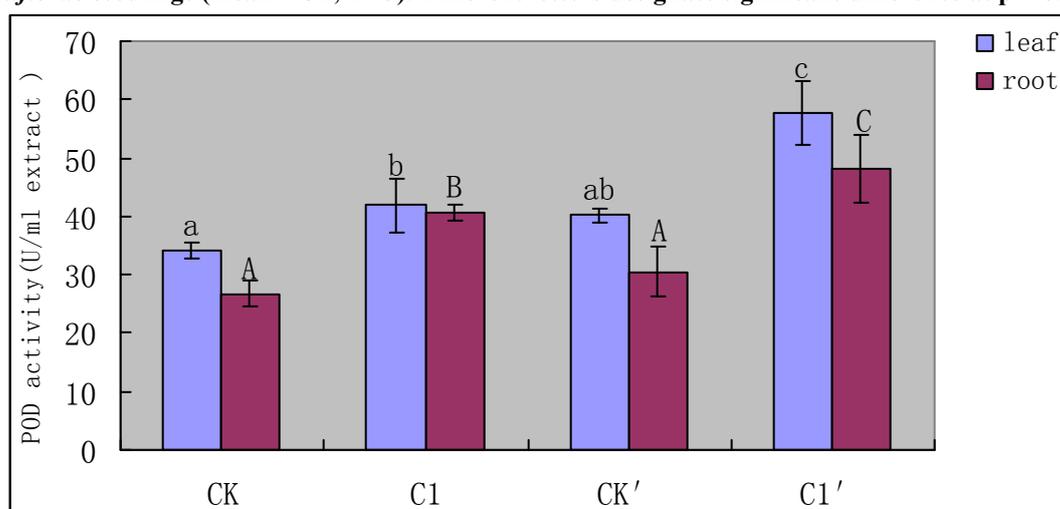


Figure 6 Effects of low temperature treatment on POD activity in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n = 3). Different letters designate significant difference at $p < 0.05$.

After pretreatment of cold-hardening, the SOD activity of *Dendrocalamus latiflorus* (C1) leaves increased by 9.3% comparing to that in control (CK), After -2°C low temperature treatment, the SOD activity of *Dendrocalamus latiflorus* leaves in the treatment group (C1') decreased by 6.6% compared to that before treatment, but the difference was not significant, while the SOD activity in control *Dendrocalamus latiflorus* leaves significantly decreased by 22.4%. The SOD activity changes in *Dendrocalamus latiflorus* roots showed similar trend as in leaves. After pretreatment (8°C), the SOD activity changes in *Dendrocalamus latiflorus* roots had no significant change before and after -2°C low temperature treatment, without pretreatment (8°C) pretreatment, the SOD activity changes in roots of *Dendrocalamus latiflorus* seedlings had significantly decreased after -2°C low temperature treatment (figures 5).

After pretreatment of cold-hardening, the POD activity in *Dendrocalamus latiflorus* (C1) leaves and roots significantly increased. After -2°C low temperature treatment, the POD activity of *Dendrocalamus latiflorus* (C1) leaves and roots in CK group were significantly higher than that in

CK' group; they were also higher than before the pretreatment (C1). The results show that the treated that the POD activity in *Dendrocalamus latiflorus* (C1) leaves and roots after pretreatment, was significantly higher than that without low temperature treatment, indicating that the POD activity in *Dendrocalamus latiflorus* seedlings (C1) is more sensitive to low temperature conditions, and POD activity can be induced by low temperature and significantly increase (figures 6).

Effects of low temperature on relative content of membrane lipid fatty acids

Table 1 Effects of low temperature treatment on relative content of membrane fatty acid in leaves and roots of *Dendrocalamus latiflorus* seedlings (mean \pm SE, n= 3).

Part	Treatment	Palmitic acid C16:0(%)	Stearic acid C18:0(%)	Oleic acid C18:1 (%)	Linoleic acid C18:2(%)	Linolenic acid C18:3(%)	Total unsaturated fatty acid
leaf	CK	14.33 \pm 0.39	1.90 \pm 0.13	2.63 \pm 0.70	9.15 \pm 0.36	69.98 \pm 0.56	81.76 \pm 0.97a
	C1	15.37 \pm 0.61	2.07 \pm 0.02	2.52 \pm 0.75	8.15 \pm 0.21	71.88 \pm 0.10	82.55 \pm 0.79ab
	CK'	13.84 \pm 0.34	1.90 \pm 0.11	2.22 \pm 0.12	8.44 \pm 0.18	73.04 \pm 0.95	83.7 \pm 0.97b
	C1'	14.85 \pm 0.19	2.09 \pm 0.03	2.28 \pm 0.22	6.55 \pm 0.10	74.22 \pm 0.15	83.05 \pm 0.28ab
	CK	25.23 \pm 0.82	4.42 \pm 0.01	4.50 \pm 0.01	44.29 \pm 0.95	20.78 \pm 0.13	69.57 \pm 0.96A
root	C1	25.63 \pm 0.91	3.14 \pm 0.41	3.00 \pm 0.23	50.34 \pm 1.10	17.03 \pm 0.26	70.37 \pm 1.15A
	CK'	28.70 \pm 0.10	3.93 \pm 0.08	4.86 \pm 0.09	44.49 \pm 0.44	16.03 \pm 0.81	65.38 \pm 0.93B
	C1'	24.90 \pm 0.20	3.70 \pm 0.03	1.10 \pm 0.01	52.41 \pm 0.05	16.77 \pm 0.02	70.28 \pm 0.05A

Analysis of membrane lipid fatty acids in *Dendrocalamus latiflorus* leaves and roots (Table 1) showed that the membrane lipid fatty acid composition was similar. Membrane lipid fatty acids in *Dendrocalamus latiflorus* leaves and roots mainly consists of five components, palmitic acid (16:0, e.g. 16:0 means there are 16 carbons and 0 double bounds in the carbon chain of the fatty acid), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), and some arachidic acid (20:0), whose content is less than 2%. The five major fatty acids account for about 98% of total membrane lipid fatty acid. In *Dendrocalamus latiflorus* seedlings, the relative contents of membrane lipid fatty acids were significantly different. The saturated fatty acids in leaf membrane lipid are mainly palmitic acid, whereas linolenic acid has the highest relative content in unsaturated fatty acids of leaf membrane lipid. The saturated fatty acids in root membrane lipid are mainly palmitic acid, while linoleic acid has the highest relative content in unsaturated fatty acids of root membrane lipid.

As the treatment temperature decreased, the ratio of membrane lipid fatty acids in *Dendrocalamus latiflorus* leaves and roots showed big differences. After cold-hardening, the relative content of *Dendrocalamus latiflorus* unsaturated fatty acids in leaf and root membrane lipid of seedlings (C1) increased by 0.97% and 1.1% respectively. After -2°C low temperature treatment, unsaturated fatty acids in *Dendrocalamus latiflorus* leaves showed no significant difference between C1' and CK'. However, the unsaturated fatty acid content in *Dendrocalamus latiflorus* (CK') roots significantly decreased.

Discussion

Cold-sensitive plants can improve their cold-tolerance through cold acclimation (Kuk *et al.* 2003). 0-10 degree low temperature from late autumn to early winter is the external ecological factor that produces cold-tolerance in temperate plants (Kunihiro K *et al.*, 1992; Guo H *et al.*, 2005). The physiological changes during cold-tolerance, such as the increased content of sugar and other carbohydrates as well as the accumulation of proline and soluble protein are the basis for the formation of cold-tolerance. Its physiological role is to reduce cell freezing point, stabilize intracellular water, prevent lipid peroxidation, stabilize protein structure and maintain cell membrane structure (Gao C *et al.* 2006; Sasaki *et al.* 1996). Malondialdehyde (MDA) is a membrane lipid peroxidation product in plants. Its content reflects the level of cellular reactive oxygen induced lipid peroxidation that causes plant cell injury. The results show that under -2°C cold treatment, the *Dendrocalamus latiflorus* seedlings present significant differences depending on whether they had or had not been submitted to an 8°C pretreatment. After -2°C cold treatment, the membrane permeability and lipid peroxide levels in

roots were both lower than in leaves, indicating the stronger cold-tolerance of roots than leaves. As the processing temperature decreased, the soluble protein and soluble sugar accumulated in leaves of *Dendrocalamus latiflorus* seedlings. This may be an important physiological and biochemical basis to enhance its cold-tolerance. The soluble sugar contents slightly increased in roots. This also showed that low temperature pre-treatment cannot effectively reduce the levels of lipid peroxidation in *Dendrocalamus latiflorus* leaves, and in each treatment temperature, the *Dendrocalamus latiflorus* roots had lower levels of lipid peroxidation and had higher cold-tolerance than leaves. Vessels in plant xylem widely emerged cavitation and embolism when being subjected to drought and low temperature which reflects to some degree plant drought or cold resistance capacity. As for the resistance capacity of recovering from vessel embolism plays an important role. This remains to be further studied.

Biomembrane is the original site subject to cold injury in plants. The critical temperature causes membrane lipid phase transition, changing a lipid from liquid crystal phase into a gel phase, resulting in damage and changes of membrane function (Lyons *et al.* 1964). For the outside temperature, biomembrane itself can respond and change membrane lipid composition, structure and status, to improve membrane fluidity (Williams *et al.* 1988 Kasamo *et al.* 1992 Palta *et al.* 1993). The content and changes of unsaturated fatty acids in bamboo species with weak cold-tolerance was relatively low during wintering, with cold-tolerance being a key process for the accumulation and improvement of cold-tolerance in sympodial bamboo species. However, this experiment shows that a sudden dramatic cooling is not conducive to increase of unsaturated fatty acid content in organs. The relative content of unsaturated fatty acids in *Dendrocalamus latiflorus* roots were both lower than in leaves, but the cold-tolerance of roots is stronger than in leaves. In the chilling conditions. There was a close relationship between the increase of unsaturated fatty acids and the addition of lipid peroxidation and both of them were relative to the cold adaptation in rice (Wang P *et al.* 2006). The unsaturated fatty acids may be an indicator of chilling resistant. The changes of fatty acid composition and protection system on membranes may be the key factor of cold adaptation especially the latter.

Conclusion

In summary, as the treatment temperature decreased, the membrane permeability increased in *Dendrocalamus latiflorus* leaves, the lipid peroxidation products (MDA) augmented. Leaves of *D. latiflorus* had higher soluble protein, soluble sugar contents and POD activities to avoid low temperature injuries while roots had higher SOD, POD activities and higher membrane lipid unsaturated fatty acid content to avoid membrane lipid peroxidation and membrane injuries.

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Reference

- Editorial Board of Shanghai institute for plant physiology, CAS, Editorial Board of the Shanghai Society for plant physiology (2004). The guide of modern plant physiology experiments. Science Press, Beijing. 127,223,302-316. (in Chinese)
- Gao C, Hu J, Zheng J, *et al.* (2006). Antioxidant enzyme activities and proline content in maize seedling and their relationships to cold endurance. Chinese Journal of Applied Ecology, 17(6), 1045-1050. in Chinese with English abstract
- Guo H, Li Q, Gao S, *et al.* (2005). Preliminary study on cold-resistance of *Euonymus radicans* 'Emerald Gold'. Bulletin of botanical research, 25(2), 219-225. in Chinese with English abstract
- Kasamo K, Kagita F, Yamanishi H *et al.* 1992. Low temperature-induced changes in the

- thermotropic properties and fatty acid composition of the plasma membrane and tonoplast of cultured rice (*Oryza sativa* L.) Cells *Plant and Cell Physiology* **33** 609-616.
- Kuk YI Lee JH Kim HY *et al* 2003 Relationships of cold acclimation and antioxidative enzymes with chilling tolerance in cucumber (*Cucumis sativus* L.) *Journal of the American Society for Horticultural Science* **128** 661-666.
- Liu G, Luan Y, Zhang Y (2006).A study on cold resistance of several bamboo species under natural condition *Journal of Bamboo Research*,25(2),10-14. in Chinese with English abstract
- Liu Q, Zhang X, Zhou J, *et al* (2006).Advances in research on the cold endurance of bamboo in china *World Forestry Research*,19(5),59-62. in Chinese with English abstract
- Lyons JM Wheaton TA Pratt HK 1964 Relationship between the physical nature of mitochondrial membranes and chilling sensitivity in plants *Plant Physiology* **39** 262-268.
- Palta JP Whitaker BD Weiss LS, *et al* 1993 Plasma membrane lipids associated with genetic variability in freezing tolerance and cold acclimation of *Solanum* species *Plant Physiology* **103** 793-803.
- Sasaki H Ichimura K Oda M 1996 Changes in sugar content during cold acclimation and deacclimation of cabbage seedlings *Annals of Botany* **78** 365-369.
- Soviet, Wang WY, Li JS (1980). Analysis of plant lipid and fatty acid—TLC-GLC *Plant Physiology Communications*,3, 54-60. in Chinese
- Wang P, Zhang CJ, Chen GX, *et al* (2006).Effects of Low Temperature on Fatty Acid Composition of Thylakoid Membranes and Lipid Peroxidation in Leaves of Rice Seedlings *Chinese J Rice Sci*,20(4),401-405. in Chinese with English abstract
- Williams JP Khan MU Mitchell K, *et al* 1988 The effect of temperature on the level and biosynthesis of unsaturated fatty acids in diacylglycerols of *Brassica napus* leaves *Plant Physiology* **87** 904-910.
- Zhou B, Li Z, Wang X, *et al*.2011. Impact of the 2008 ice storm on moso bamboo plantations in southeast China. *J. Geophys. Res.*, 116, G00H06.

Biomass production and carbon sequestration potential of various Bamboo species in the Mid Himalayan region of India

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Abstract

Carbon sequestration potential of five bamboo species namely *Dendrocalamus strictus*, *Bambusa vulgaris*, *B. multiplex*, *B. bambos* and *Phyllostachys nigra* was evaluated in the mid Himalayan region of India. Estimation of above ground biomass of all these five species was done using a linear regression model. Three independent variables, length of culm, girth to height at 1m & at 1.5m were taken in the linear regression model. Above ground biomass was estimated on a fresh and dry weight basis. Out of five species, highest biomass on a fresh weight basis was found in *Dendrocalamus strictus* (106.49 t ha⁻¹) but on a dry weight basis *Phyllostachys nigra* showed the maximum (89.76 t ha⁻¹) biomass production. In *B. bambos* biomass was highest on per culm basis but culm density was low in this mid-Himalayan region. Maximum Carbon sequestration potential was observed in *P. nigra* (44.88 t ha⁻¹) in the present study.

Keywords

Dendrocalamus strictus, *Bambusa* spp., *Phyllostachys nigra*, Biomass, carbon sequestration

Introduction

Bamboo's fast growing attribute makes it a very useful resource to capture and sequester atmospheric carbon and consequently mitigate climate change, in a similar way that tree does. The unique growing capacity makes bamboo a valuable sink for carbon storage. Bamboo is a versatile non-timber plant and produces substantial biomass. As per the conclusion from various studies bamboo biomass and carbon production may be 7-30 % higher as compared to the fast growing wood (INBAR 2009). There are about 100 genera and 1000 species of sympodial and monopodial bamboos, distributed throughout the tropics, subtropics and temperate zones of the world. Among the 130 wild and cultivated bamboo species occurring in India (Sharma 1987), some are distributed throughout the Himalayas, with a variety of different genera adapted to different ecological zones. In the Siwalik hill region which is hot and dry, a limited range of species occurs, such as *Dendrocalamus strictus* and *Bambusa bambos*. At higher altitudes, bamboos in the genera *Arundinaria*, *Thamnocalamus* & *Himalayacalamus* are common.

In India and abroad, many studies on net production and carbon cycling in various bamboos have been conducted (Shanmughavel & Francis 2001; Singh et al. 2004; Kumar et al. 2005; Isagi et al. 1997; Yen et al. 2010; Nath & Das 2011) but very few in the Himalayan region. In the central Himalayan region a study was conducted on *Thamnocalamus spathiflorus* (Saxena et al. 2001) and in the mid-Himalayan region on *Dendrocalamus asper* (Agarwal & Purwar 2009). The Himalayas are extremely vulnerable to climate change, hence 'Sustaining the Himalayan Ecosystem' is one of the missions under the National Action Plan on Climate Change.

The purpose of this study was to analyze the potential of various species of bamboo to sequester carbon in terms of above ground biomass production in the mid-Himalayan region. Five species, namely *Dendrocalamus strictus*, *Bambusa vulgaris*, *B. multiplex*, *B. bambos* and *Phyllostachys nigra* were selected out of which four are sympodial and *P. nigra* is a monopodial species.

Materials and Methods

The study was conducted over the period 2006 -2011 at the Agriculture Research Station, Majhera Garampani, Nainital, Uttarakhand, India. Altitude, latitude and longitude of the study site are 905 m (a.s.l.), 29°30.137', 79° 28.784', respectively. Annual average maximum temperature and minimum temperature were recorded 27.8°C and 14.8°C with mean annual rainfall 598 mm.

Biomass of *Dendrocalamus strictus*, *Bambusa vulgaris*, *B. multiplex*, *B. bambos* and *Phyllostachys nigra* was related to components of yield by linear regression model modified after Agarwal & Purwar (2010). Sampling was done during July 2011 by selecting five culms of various diameters and heights from each species. Total length, girth of culms at 1.0 m and 1.5 m, fresh and dry weight with branch and leaves of harvested culms was recorded. Culms were harvested and fresh weight with branch & leaves was recorded. For dry weight estimation shade drying was done until a constant weight achieved. On the basis of fresh and dry weight linear regression equations were developed for the estimation of above ground biomass (Singh et al. 2009). Biomass accumulation per clump was extrapolated to per ha basis by multiplying with 400 for *D. strictus* and *B. vulgaris*, 900 for *B. multiplex* and 100 for *B. bambos* assuming a spacing of 5 m x 5 m, 3 m x 3 m and 10 m x 10 m respectively (KAU 2002). For *P. nigra* extrapolation was done on the basis of number of culms per square meter. Carbon content estimation was done on the basis that 50 per cent of the total above ground biomass produced is carbon (Scurlock et al. 2000).

Results & Discussion

Above ground biomass of *D. strictus*, *B. vulgaris*, *B. multiplex*, *B. bambos* and *P. nigra* was related to its components of yields on the basis of regression equations (Table 1&2).

Linear regression equation indicated that above ground biomass produced depended on length of culms, girth to height at 1.0 m and 1.5 m by 96, 99, 100, 93 & 98 per cent on a fresh weight basis (Table 1) and 96, 99, 99, 97 & 93 per cent on a dry weight basis (Table 2) in *D. strictus*, *B. vulgaris*, *B. multiplex*, *B. bambos* and *P. nigra*, respectively.

Table 1: Linear relationship between fresh above ground biomass of bamboo species (y kg culm⁻¹) and yield components height (b_{1,m}), girth to height at 1m (b_{2, m}) and girth to height at 1.5 m (b_{3, m})

Bamboo species	Regression Equation
<i>Dendrocalamus strictus</i>	Y=(5.31)+(-1.79)b ₁ +(-607.73)b ₂ +(764.36)b ₃ R²=0.96 S.E ₁ =2.47, S.E ₂ = 935.44 S.E ₃ = 972.64
<i>Bambusa vulgaris</i>	Y=(-4.82)+(3.52)b ₁ +(56.66)b ₂ +(-155.46)b ₃ R²=0.99 S.E ₁ =0.28, S.E ₂ = 24.16 S.E ₃ = 30.00
<i>Bambusa multiplex</i>	Y=(-0.16)+(0.07)b ₁ +(4.41)b ₂ +(-2.54)b ₃ R²=1.00 S.E ₁ =15.75, S.E ₂ = 210.06 S.E ₃ = 1785.04
<i>Bambusa bambos</i>	Y=(2.95)+(0.63)b ₁ +(-210.01)b ₂ +(265.86)b ₃ R²=0.93 S.E ₁ =2.69, S.E ₂ = 214.75 S.E ₃ = 245.24
<i>Phyllostachys nigra</i>	Y=(-1.23)+(0.93)b ₁ +(-20.70)b ₂ +(13.29)b ₃ R²=0.98 S.E ₁ =0.54, S.E ₂ = 17.95 S.E ₃ = 11.45

Table 2: Linear relationship between dry above ground biomass of bamboo species (y kg culm⁻¹) and yield components height (b_{1,m}), girth to height at 1m (b_{2, m}) and girth to height at 1.5 m (b_{3, m})

Bamboo species	Regression Equation
<i>Dendrocalamus strictus</i>	Y=(5.99)+(-1.77)b ₁ +(-584.09)b ₂ +(712.13)b ₃ R²=0.96 S.E ₁ =1.82, S.E ₂ = 689.10 S.E ₃ = 716.50
<i>Bambusa vulgaris</i>	Y=(-2.85)+(2.20)b ₁ +(19.43)b ₂ +(-79.86)b ₃ R²=0.99 S.E ₁ =0.67, S.E ₂ = 58.44 S.E ₃ = 72.56
<i>Bambusa multiplex</i>	Y=(0.055)+(-0.035)b ₁ +(-0.20)b ₂ +(3.27)b ₃ R²=0.99 S.E ₁ =1.29, S.E ₂ = 17.17 S.E ₃ = 145.91
<i>Bambusa bambos</i>	Y=(5.17)+(-0.14)b ₁ +(-323.78)b ₂ +(363.98)b ₃ R²=0.97 S.E ₁ =1.32, S.E ₂ = 105.59 S.E ₃ = 120.58
<i>Phyllostachys nigra</i>	Y=(-1.34)+(0.88)b ₁ +(12.20)b ₂ +(-22.44)b ₃ R²=0.93 S.E ₁ =0.68, S.E ₂ = 22.45 S.E ₃ = 14.33

Multiple regression studies revealed that unit increase in length of culm decreased biomass on dry weight basis by 1.77, 0.035 & 0.14 per cent in *D. strictus*, *B. multiplex* and *B. bambos* whereas in *B. vulgaris*, and *P. nigra* biomass increased by 2.20 and 0.88 per cent respectively (Dhage et al. 2012). Similarly, girth at 1.0 m showed negative correlation with biomass (dry weight basis) in case of *D. strictus*, *B. multiplex* and *B. bambos* whereas *B. vulgaris*, and *P. nigra* showed positive correlation. In all the five species girth at 1.5 m was the major deciding independent variable for biomass estimation. Unit increase in girth at 1.5 m height (DBH) in *D. strictus*, *B. multiplex* and *B. bambos* increased biomass by 712.13, 3.27 & 363.98 per cent respectively. Riano et al (2002) has also reported a similar finding in *Guadua angustifolia*, 45 per cent of the whole fresh weight can be explained by the variation of DBH. Regression model has been developed for carbon stock estimation in *B. vulgaris*, *B. balcooa* and *B. cacharensis* with DBH as the independent variable (Nath & Das 2011). Net above ground biomass of five species after six years of plantation varied from 0.05 (*B. multiplex*) to 6.21 (*B. bambos*) kg culm⁻¹ on the basis of fresh weight and 0.02 to 4.31 kg culm⁻¹ on a dry weight basis in the mid-Himalayan region (Table 3). Dry weight was approximately 63.33, 35.72, 62.55, 36.68 and 4.58 per cent less than fresh weight in *D. strictus*, *B. vulgaris*, *B. multiplex*, *B. bambos* & *P. nigra*, respectively. Maximum estimated biomass was produced by *D. strictus* (106.49 t ha⁻¹ on a fresh weight basis) whereas on a dry weight basis maximum estimated biomass was recorded to *P. nigra* (89.76 t ha⁻¹). Among the five species minimum estimated biomass was in *B. bambos* (4.35 t ha⁻¹ on a fresh weight basis and 3.01 t ha⁻¹ on a dry weight basis) in the mid-Himalayan region.

Table 3 Carbon sequestration on the basis of estimated above-ground biomass in various bamboo species

Bamboo species	Biomass Kg culm ⁻¹		No. of culms per clump	Biomass Kg clump ⁻¹		No. of plants ha ⁻¹	Biomass (t ha ⁻¹)		Carbon sequestered (t ha ⁻¹)
	Fresh wt basis	Dry wt basis		Fresh wt basis	Dry wt basis		Fresh wt basis	Dry wt basis	
<i>Dendrocalamus strictus</i>	6.05	2.22	44	266.2	97.6	400	106.5	39.1	19.5
<i>Bambusa vulgaris</i>	2.53	1.62	16	40.4	26.0	400	16.2	10.4	5.2
<i>Bambusa multiplex</i>	0.05	0.02	261	14.0	5.3	900	12.6	4.7	2.4
<i>Bambusa bambos</i>	6.21	4.31	07	43.5	30.1	100	4.4	3.0	1.5
<i>Phyllostachys nigra</i>	0.41	0.39	23*	54.4	51.9	2,30,000	94.1	89.8	44.9

*Number of culms per sq mt

Culm dynamics, biomass, net primary production, soil microbial biomass and N-mineralization were estimated in a bamboo (*D. strictus*) plantation on mine spoil in the Singrauli coalfield in Madhya Pradesh, India. Above-ground net production was 17.0 to 24.7 t ha⁻¹ between 3 to 4, and 4 to 5 years from planting, respectively (Singh & Singh 1999). In the present study above ground biomass of *D. strictus* was estimated to be 39.1 t ha⁻¹ after six year of plantation. *B. bambos* showed maximum biomass (6.21 kg) on a per culm basis among all the five species but culm density was poor and as a result total biomass estimated per hectare was low. Above-ground biomass of *B. bambos* clumps averaged 2417 kg clump⁻¹ with an average accumulation of 241.7 Mg ha⁻¹ in Thrissur, Kerala, India

(Kumar et al. 2005). Carbon fixation and cycling were determined in a stand of *P. pubescens* in Japan. Total above-ground biomass produced was 137.9 t ha⁻¹ whereas biomass of rhizomes and fine roots was 44.6 t ha⁻¹ (Isagi et al. 1997). Above-ground biomass in *P. nigra* was estimated to be 94.1 t ha⁻¹ on a fresh weight basis in our study. For *Bambusa vulgaris* biomass was estimated 10.4 t ha⁻¹ on dry weight basis. Biomass estimation reports in *B. vulgaris* are very few, however Nath et al. (2009) have reported net above ground biomass 121.5 t ha⁻¹ contributed by three species out of which 17.9 per cent was *B. vulgaris*. Biomass in *B. multiplex* was estimated 12.6 t ha⁻¹ on a fresh weight basis and 900 plants ha⁻¹ with a spacing of 3 m x 3 m. *Bambusa multiplex* is mainly used in hedging, as a wind barrier or as an ornamental. Biomass estimation reports of this species could not be found.

Computation of carbon sequestration by all the five species was done on the basis of net above ground biomass produced in six years at ARS, Majhera (Table 3). Carbon sequestered by *D. strictus*, *B. vulgaris*, *B. multiplex*, *B. bambos* & *P. nigra* was 19.5, 5.2, 2.4, 1.5 & 44.9 t ha⁻¹ respectively.

Mostly sympodial bamboos are known to produce more biomass than monopodial bamboos (INBAR, 2009) but the comparative analysis of carbon sequestration between a monopodial bamboo *P. nigra* and clump forming other species revealed that in the Himalayan region at least one monopodial species has more potential to sequester carbon due to high density of culms. The high dry matter per cent in *P. nigra* furthers its potential as a good carbon sink at high altitudes.

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References

- Agarwal, A.; Purwar, J.P. 2009. Evaluation of above ground biomass produced by *Dendrocalamus asper* in North Western Himalayan Region of India. In: VIII World Bamboo Congress Proceedings, 4:91-96.
- Agarwal, A.; Purwar, J.P. 2010. Linear regression model for the estimation of above-ground biomass in various species of bamboo. In: 2010 INBAR Congress on Bamboo and Rattan Proceedings, 29.
- Dhage, V.D.; Suryawanshi, M.M.; Dhane, A.S.; Shelke, S.S. 2012. Seasonal incidence of two spotted mite (*Tetranychus urticae* Koch.) on gerbera under polyhouse condition, Journal of Agriculture Research and Technology, 37 (1), 82-86.
- INBAR, 2009. International Network for Bamboo and Rattan, Beijing, Home page. www.inbar.int.
- Isagi, Y.; Kawahara, T.; Kamo, K.; Ito, H. 1997. Net production and carbon cycling in a bamboo *Phyllostachys pubescens* stand, Plant Ecology, 130, 41-52.
- Kerala Agricultural University (KAU) 2002. Package of Practices Recommendations: Crops. 12th ed., Kerala Agricultural University, Trichur, 278p.
- Kumar, B.M.; Rajesh, G.; Sudheesh, K. 2005. Aboveground biomass production and nutrient uptake of thorny bamboo [*Bambusa bambos*(L.) Voss] in the homegardens of Thrissur, Kerala. Journal of Tropical Agriculture, 43 (1-2),51-56.
- Nath, A.J.; Das, A.K. 2011. Carbon storage and sequestration in bamboo –based smallholder homegardens of Barak Valley, Assam, Current Science, 100 (2), 229-233.
- Nath, A.J.; Das, G.; Das, A.K. 2009. Above ground standing biomass and carbon storage in village bamboos in North East India, Biomass and bioenergy, 33 (9), 1186-1196.
- Riano, N.M.; Londono, X; Lopez, Y.; Gomez, J.H. 2002. Plant growth and biomass distribution on

- Guadua angustifolia* Kunth in relation to ageing in the Valle del Cauca-Colombia, *Bamboo Science and Culture*, 16 (1), 43-51.
- Saxena, K. G.; Rao, K.S.; Sen, K.K.; Maikhuri, R.K.; Semwal, R. L. 2001. Integrated natural resource management: approaches and lessons from the Himalaya. *Conservation Ecology* 5 (2), 14. [online]
- Scurlock, J.M.O.; Dayton, D.C.; Hames, B. 2000. Bamboo: an overlooked biomass resource? *Biomass and Bioenergy*, 19(4), 229-244.
- Shanmughavel, P.; Francis, K. 2001. The dynamics of biomass and nutrients in bamboo (*Bambusa bambos*) plantations. *Journal of Bamboo and Rattan*, 1 (2), 157-170.
- Sharma, Y.M.L. 1987. Inventory and resources of bamboo, In: *Recent Research on bamboos*, A.N. Rao, G. Danarajan and C.B. Sastry (eds), Chinese Academy of Forestry and International Development Research Centre. pp.14-27.
- Singh, A.N.; Singh, J. S. 1999. Biomass, net primary production and impact of bamboo plantation on soil redevelopment in a dry tropical region, *Forest Ecology and Management*, 119 (1-3), 195-207.
- Singh, P.; Dubey, P.; Jha, K.K. 2004. Biomass production and carbon storage at harvest age in superior *Dendrocalamus strictus* plantation in dry deciduous forest region of India. In Singh, H.P.; Dadlani, N.K. Abstracts of the VII World Bamboo Congress, New Delhi, 27 February – 4th March, 2004. VIIth World Bamboo Congress, New Delhi, India, pp. 122.
- Singh, V.; Tewari, A.; Ram, J.; Singh, C. 2009. Aspect related changes in biomass stocks and carbon sequestration rates of *Shorea robusta* (Sal) forest of Central Himalaya. *Report and Opinion*, 1 (2), 56-60.
- Yen, Tian-Ming; Ji, Yi-Jia; Lee, Joou-Shian. 2010. Estimating biomass production and carbon storage for a fast-growing makino bamboo (*Phyllostachys makinoi*) plant based on the diameter distribution model. *Forest Ecology and Management*, 260 (3), 339-344.

Harvesting method optimizing shoot and culm production in *Dendrocalamus hamiltonii*

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Abstract

Dendrocalamus hamiltonii is a large, clump-forming bamboo with nutritious, palatable shoots and culms widely used for construction and weaving. The species is readily available and has great potential to contribute towards poverty alleviation efforts in southern Bhutan. In order to prevent unsustainable harvesting, for which precedence with other commercially utilized species exist nearby, silvicultural methods maximizing yield while ensuring sustainability need to be developed. We defined three harvesting treatments (selective cut, horseshoe cut, clearcut) based on farmers' goals for bamboo production and experimentally compared them with a no-intervention control treatment, implementing harvesting over two seasons. While the number of shoots produced did not differ between treatments, harvested output of shoots was highest with the horseshoe cut. Untreated clumps had higher total production of culms than harvested clumps. Harvested output of culms was with highest with horseshoe cut, not significantly different from clearcut. Accordingly, the horseshoe technique simultaneously maximized shoot and culm yield, but further follow-up is required to assess long-term sustainability of harvesting, in spite of an indicated increase in recruitment of new shoots. Even though the number of culms and the number of current-year culms seemed to influence the number of shoots regenerated, a much stronger relationship was detected between the number of culms harvested and the number of shoots regenerated, indicating compensatory growth mechanisms to guide shoot regeneration.

Keywords

Dendrocalamus hamiltonii; bamboo silviculture; clump management; shoot and culm production; Bhutan

Abbreviations

ANOVA – Analysis of Variance; ANCOVA – Analysis of Co-variance; MANOVA – Multivariate Analysis of Variance; MANCOVA – Multivariate Analysis of Co-variance; NTFP – Non-Timber Forest Product; PI – Productivity Index

Introduction

Globally, more than 1.6 billion people depend on forests for their livelihoods to a varying degree (Angelsen and Wunder 2003). People more strongly depend on forests in remote places with high degree of poverty (Sunderlin *et al.* 2008). Particularly in these areas, non-timber forest products frequently play a “safety-net” function for rural poor, thus providing opportunities for risk reduction. Bamboos as versatile and weakly perishable non-timber forest products are an example of such important sources of livelihood in many rural areas in which they occur (Lobovikov *et al.* 2011). Bhutan has identified commercialization of non-timber forest products (NTFPs) along with community forestry as key strategies for poverty alleviation through creation of employment and generation of income, especially for lower income groups (Gross National Happiness Commission 2008). This meets with the intention of local forest users to use forest products as an opportunity for employment and income generation, instead of relying on their subsistence farming (Samdrup 2011). The rate of return on investments with NTFPs is considerably higher, as compared to regular agricultural activities (Krishnankutty 2004; Pandit and Kumar 2010), explaining why bamboo-based local enterprises have successfully contributed to poverty alleviation elsewhere (Zhu 2003; Moktan *et al.* 2009). Accordingly, community-based management of bamboos within the legal framework of community forests also forms part of the ambitious plan to establish more than four hundred community forests in Bhutan by 2013 (Gross National Happiness Commission 2008). While administrative and marketing problems persist, the lack of appropriate methods for resource assessment and sustainable utilization remain key challenges to successful NTFP development (Wong *et al.* 2001; Social Forestry Division 2008).

Throughout monsoon Asia, bamboo culms are widely used for construction, fencing and handicrafts, while bamboo shoots are an important dietary supplement of high nutritional value (Stapleton *et al.* 1997; Bhatt *et al.* 2005a; Moktan *et al.* 2009) and have large economic potential in the region (Bhatt *et al.* 2003; Bhatt *et al.* 2004, 2005b). In Bhutan, presently both shoots and culms are mainly used for domestic purposes, while commercial markets for most species and products are weakly developed. The few successful NTFP-based cottage industries are frequently affected by declines in production, resulting from over-harvesting or improper management of the resource base due to lack of appropriate resource assessment and management guidelines (Prommegger *et al.* 2005; Moktan *et al.* 2009). *Dendrocalamus* spp. have been identified as one of the three bamboo species/genera for NTFP development in Bhutan, based on their abundance, easy propagation, multi-purpose use and economic potential (Social Forestry Division 2008). Suitable harvesting methods must maximize sustainable output of the target commodity. Bamboo shoot production mainly depends on the harvesting regime (thinning of culms), water and nutrient supply (Kleinhenz and Midmore 2001). As an activity generating immediate returns, the only reasonably applicable bamboo management intervention in the Bhutanese context is culm thinning, since irrigation, fertilizing and mulching are highly labor and cost intensive, for which farmers would have to divert resources away from agricultural production. At the same time, systematic clump management regimes are essential to prevent random harvesting resulting in decline of clump productivity (Virtucio 2006). Harvesting regimes relevant in our context have been developed for *D. strictus* in India (Tewari 1992), and *D. asper* (Decipulo *et al.* 2006) and *Bambusa blumeana* (Marquez 2006; Malab *et al.* 2009) in the Philippines, with the aim of maintaining balanced age distributions of young (1-3 year-old) culms.

In order to address the most important questions of sustainable production of *Dendrocalamus hamiltonii* Munro var. *edulis* Munro in southern central Bhutan, the objectives of the present study were to develop methods for suitable harvesting integrating local knowledge and focusing on the target commodities.

Methods

Species and study area

The distribution of *Dendrocalamus hamiltonii* Munro ranges from the central Himalayas to north-east India and includes the subtropical and warm-temperate broadleaf zones of Bhutan up to an altitude of 1800 m (Troup 1921; Seethalakshmi and Kumar 1998; Stapleton 2000). *D. hamiltonii* Munro var. *edulis* Munro is an especially palatable variety, common in central and eastern Bhutan (Stapleton *et al.* 1997). *D. hamiltonii* is a fairly large sympodial bamboo with pachymorph rhizomes and culms often growing up to 25 meters in height (Stapleton 2000). It commonly occurs in open forests, frequently after disturbances (Seethalakshmi and Kumar 1998) and is often cultivated. It has thin-walled culms which are very flexible making it suitable as all-purpose weaving material. Though thin walled, culms are also very popular as fencing material over other bamboo species because of their roughness and durability (Rai, pers. obs.). The foliage is harvested to feed cattle and horses and is grazed by mithun (Sundriyal and Sundriyal 2004). The shoots of *D. hamiltonii* are tasty and of high nutritional value (Bhatt *et al.* 2005a). The shoots start appearing at the beginning of June, are harvested between mid June and end of August and are consumed when fresh, or dried, shredded or pickled.

Tshanglajong village in Zhemgang district is located along the lower Mangduechu valley at an altitude ranging from 700 to 1000 m. The village is a relatively recent settlement, but the area has been cultivated previously, explaining the dominance of open *D. hamiltonii* forests (Rao and Saxena 1995) in the wider vicinity. A community forest focusing on management of *D. hamiltonii* was incorporated in Tshanglajong a year after the start of the present study.

Survey of local knowledge on bamboo ecology and use

In an action research framework, various social research methods were applied to gain insight into indigenous knowledge on bamboo ecology, harvesting methods, utilization, socio-economic significance and the people's goals for intended use. Specific methods applied included small group discussion and transect walks, and information was triangulated and compared in order to provide independent verification of the results obtained (Lawrence *et al.* 2008). Specific topics on which local knowledge was documented using participatory techniques included distribution, habitat characteristics, phenology, growth characteristics, age determination, morphology, yield, traditional harvesting techniques, utilization, income generation, employment, legal and regulatory constraints related to bamboos and their utilization. People's goals regarding bamboo utilization were derived in a participatory manner using the generated information.

Invitations to attend the small group discussion were sent to all households and as habitual in Bhutan, one representative per household most involved in the activity in question attended the discussion. Transect walks with key informants identified using the snowball sampling technique were conducted in the forest to verify the information obtained during the small group discussion.

Experimental comparison of harvesting methods

The research site is located half an hour walk west of the Tshanglajong village (27°06'27.54"N, 90°42'26.77"E) on an east-northeast facing slope at 870 m altitude with moderately moist site conditions. The experiment was established in spring 2009 with random selection of 16 bamboo clumps located in a relatively small area to mitigate unexplained variation in the data resulting from variations in environmental conditions. Selection criteria for bamboo clumps included homogenous site conditions, no damage or signs of harvesting and clearly defined borders. Treatments were

defined by a combination of harvesting prescriptions for shoots and culms (

Table) and applied randomly to previously measured clumps. The 16 bamboo clumps were evenly allocated to treatments and controls such that four clumps received each of the three harvesting treatments, and four clumps were reserved as controls. Shoots were monitored and harvested in July to August. In order to prevent borer damage, culms were monitored and harvested in November to December, as during the dry season, starch content of culms is very low, making them unattractive to insects (Dransfield and Widjaja 1995). Monitoring consisted of enumeration of harvestable shoots and culms. Shoots and culms of no value to the farmers (dead, broken, undersized, not of the right age) were excluded from enumeration. Harvesting of culms was restricted to culms older than two years, as besides their limited use, young culms have buds on the rhizome and should therefore be retained. Harvesting was carried out in seasons corresponding to roughly defined traditional harvesting seasons.

In order of harvesting intensity, treatments included untreated control, selective cutting, horseshoe cutting and clearcutting. No harvesting was carried out in the control treatment. Selective cutting was defined by low intensity removal of shoots and culms (25% each). As destruction of shoots near the perimeter of clumps may lead to clump congestion and ultimately to degradation (Troup 1921; Franklin 2008), we harvested culms starting in clump centers. The horse shoe method widely practiced in India and Nepal was applied, orienting the convex arch of the shoe facing uphill in order to prevent accumulation of debris in the arch (Figure 1). This way new shoots are mainly added on the outer arch of the horse shoe and therefore the clumps are expected to expand uphill (Bradshaw 1997). Both shoots and culms were harvested at 75% intensity. The clearcut treatment included the removal of 50% of new shoots and all culms older than two years (Table 1).

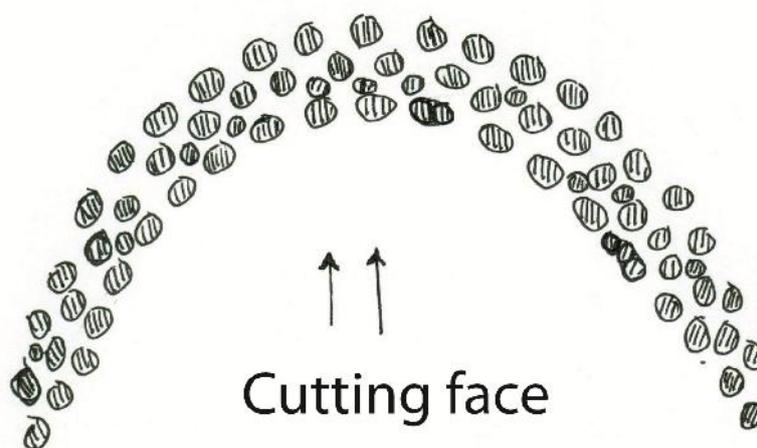


Figure 1: The horse shoe cutting method showing the cross section of live culms of a clump and the cutting direction.

For each clump, we measured initial clump diameter, enumerated initial number of present year and older live and of dead culms as well as the number of annually harvested shoots and culms. Harvesting and monitoring were carried out in summer and winter 2009 and 2010, as well as in summer 2011. Additionally, the collar diameter of bamboo shoots and their height was measured and the edible proportion of shoots was assessed in summer 2011. This data was not recorded for the control treatment.

Table 1: Harvesting regimes applied to clumps of *Dendrocalamus hamiltonii*

Treatment	Shoots harvested	Culms > 2 years harvested	Remarks
Control	0%	0%	no intervention
Selective cut	25%	25%	removal of dead culms and stumps, harvesting of shoots from inside out, harvesting of culms at base leaving two internodes intact
Horseshoe cut	75%	75%	removal of dead culms and stumps, convex arch facing upslope, harvesting of shoots and culms from inside of arch, harvesting of culms at base leaving two internodes intact
Clearcut	50%	100%	removal of dead culms and stumps harvesting of shoots from inside out, harvesting of culms at base leaving two internodes intact

Data Analysis

Qualitative information obtained during the small group discussion was triangulated against the information obtained during transect walks.

For bamboo clumps included in the experiment, the relationship between shoot recruitment and the number of culms per clump as well as the number of harvested culms per clump was initially tested using Pearson correlation coefficients. The difference in the ratio of the number of shoots to the number of culms per clump (Productivity Index) among treatments was tested using analysis of variance (ANOVA).

Shoot volume in 2011 was calculated using the conoid volume function with the parameters collar diameter and height. Edible shoot volume was calculated as the shoot volume multiplied by the edible proportion. Because volumes of shoots from the same clump are likely more correlated than of shoots from different clumps, differences in edible shoot volume among treatments were tested with a general linear mixed model (GLMM). This GLMM included a random effect for clumps with a compound symmetric error structure to account for the correlation among shoots in the same clump.

Since the recruitment of *Dendrocalamus* varies greatly between years (Taylor and Zisheng 1987; Decipulo *et al.* 2006; Marquez 2006), we pooled data over our two years of monitoring in order to evaluate shoot and culm production and harvesting. This resulted in dependent variables for combined shoot production 2010 and 2011, culm production 2010, combined shoot harvesting 2010 and 2011, and combined culm harvesting 2009 and 2010. At the time of analysis, the number of culms produced and harvested was not yet available for winter 2011 and therefore variables related to culms contained only one year's enumeration.

Since the simultaneous assessment of numbers of shoots and of culms represents a multivariate question, involving the interaction and trade-off between two response variables, a multivariate ANOVA (MANOVA) was deemed more appropriate than two separate ANOVA analyses (Scheiner 2001). Since the control treatment did not include harvesting by definition, data from these clumps were excluded from the analysis of shoots and culms harvested. As in the univariate analyses, the initial clump diameter and the initial number of culms in 2009 were included as covariates in analyses as proxies for initial clump size, resulting in a multivariate analysis of covariance (MANCOVA). The significance level of univariate ANCOVAs along with correlations between the dependent variables were used to test for significant differences among treatments and to calculate estimated treatment effects. Multivariate differences in response variables were evaluated via Wilks' λ , which is a multivariate F test of the ratio of the variance/covariance matrices of the errors versus the effects. As in a univariate ANOVA, appropriate interpretation of MANOVA results requires normality and homoscedasticity of residuals, which were evaluated using scatterplots. Where significant differences by treatment were indicated, a Scheffé test was performed on univariate estimated marginal means. However, because these means are not adjusted for the correlation between dependent variables in the

MANCOVA, we then performed multivariate comparisons among treatments via orthogonal contrasts. Analyses were conducted using SAS version 9.2 procedures PROC CORR, PROC GLM and PROC MIXED (SAS Institute Inc. 2002-2008).

Results

Survey of local knowledge and intentions

Of the six species of bamboos growing in and around Tshanglajong, *Dendrocalamus hamiltonii* (pakshing) was the only species used for a wide variety of purposes, such as fencing, weaving and construction. Farmers observed that *D. hamiltonii* grows best in valleys and depressions and in general under open forest canopy. They have encountered sporadic, but no mass flowering of *D. hamiltonii*, followed by monocarpic die-off of clumps. Farmers do not have particular methods of harvesting, nor do they apply local restrictions on collection time, but rather harvest shoots and culms whenever available or required. Stump height is determined by ease of cut and is usually higher towards clump centres and in highly congested clumps. Easily accessible culms of suitable quality usually near to clump edges are harvested on a preferential basis. Collection time for shoots, of which the top 30-40 cm are harvested, is determined by their emergence, generally in July and August. Parts of the population are occasionally engaged in weaving of mats and baskets for domestic, as well as for commercial purpose, but in spite of its abundance, only two farmers are intensively involved in bamboo harvesting and processing. The use of culms for different purposes is determined by their physical qualities, which is a function of their age, determined by external signs confidently up to three years. Young culms are used for weaving, while older ones are used for construction purposes. Products are either sold off farm, in Tingtibi, the nearest town at 8 km distance, or Zhemgang, the district headquarters located 42 km away. Bamboo culms are presently not sold. People are interested in harvesting both shoots and culms, but do not have much time and resources to devote towards bamboo activities due to unreliable markets.

Bamboo clump properties prior to application of treatments

Prior to application of treatments in 2009, mean clump diameter was 308 cm, and on average a clump had 21 culms with a median culm diameter of 8.56 cm. About 15% of culms were dead, while 30% were current-year culms (Table 2).

Table 2: Bamboo clump characteristics prior to application of treatments in 2009

Parameter	Mean	Standard Error
Clump diameter [cm]	308.44	74.68
Total number of culms	20.75	8.16
Median culm diameter [cm]	8.56	0.89
Proportion of current-year culms	0.298	0.121
Proportion of dead culms	0.151	0.105

Effects of clump size and harvesting intensity on regeneration of shoots

The correlation between the number of culms per clump in 2009 prior to harvesting and the number of shoots regenerated per clump in 2010 was relatively strong, but marginally not significant (data not presented; $r=0.447$, $p=0.083$). Similarly, the correlation between the number of current-year culms in 2009 prior to harvesting and the number of shoots in 2010 was marginally not significant (data not presented; $r=0.470$, $p=0.066$). On the other hand, the correlation between the number of culms harvested per clump in 2009 and the number of shoots regenerated in 2010 was stronger and significant (Figure 2; $r=0.703$, $p\leq 0.01$). There was no relationship between the number of culms per clump in 2009 prior to harvesting and the number of culms harvested per clump in 2009 (data not presented; $r=0.307$, $p>0.05$).

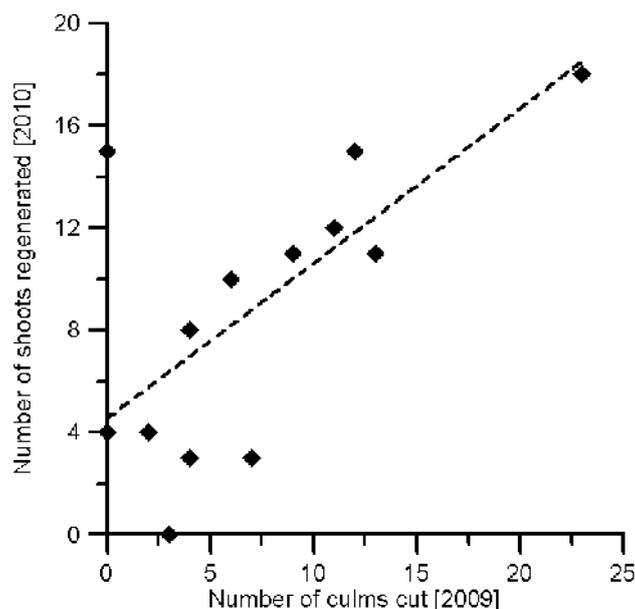


Figure 2: Relationship between number of culms harvested per clump and number of shoots emerged next summer

Productivity Index

Although selective cutting and clearcutting had much higher shoot/culm ratios than the control or horse shoe cut treatments, the ratio of shoots to culms per clump in 2010 was not significantly different between treatments (Table 3; ANOVA results not shown, $p > 0.05$). Selective cutting also produced the greatest edible shoot volume, but this difference was not significant (Table 3; GLMM results not shown, $p > 0.05$).

Table 3: Ratio of shoots to culms (productivity index) in 2010 and edible shoot volumes by treatment (means \pm SE resulting from ANOVA/GLMM)

Treatment	Shoots/culm	Edible shoot volume [cm ³]
Control	0.378 (± 1.819)	Not measured
Selective cut	4.833 (± 1.819)	693.60 (± 221.05)
Horseshoe cut	1.681 (± 1.819)	569.85 (± 178.21)
Clearcut	4.396 (± 1.819)	525.02 (± 187.85)

Production of shoots and culms under experimental treatments

The multivariate test of differences among treatments using Wilks' λ criteria was statistically significant and the model explained a large proportion of the variation of the response variables number of shoots per clump and number of culms per clump (MANCOVA; $\lambda = 0.138$; $p \leq 0.01$). The number of culms before application of treatments was a significant covariate in a multivariate context ($\lambda = 0.231$, $p \leq 0.001$) and was significant in both separate univariate ANCOVAs (Table 4; $p \leq 0.05$). The number of shoots produced did not differ between treatments in a univariate context, and orthogonal contrasts did not indicate significant differences when adjusting for correlations between the two dependent variables (Figure 3; contrasts, $p > 0.05$). Contrasts for the number of culms produced, on the other hand, indicated that there were significantly higher numbers produced in the control treatment as compared to other treatments, which did in turn not differ from each other (Figure 3; contrasts, $p \leq 0.01$).

Table 4: Type III test of fixed effects of *Dendrocalamus hamiltonii* (a) total number of shoots per clump, (b) with total number culms per clump, (c) shoots harvested per clump, and (d) culms harvested per clump as dependent variables

Response variable	Source	DF	Type III SS	Mean Square	F Value	Pr > F
(a) Shoots per clump	Treatment	3	153.80	51.27	1.24	0.3410
	Number of culms 2009	1	327.49	327.50	7.94	0.0167
(b) Culms per clump	Treatment	3	812.42	270.81	9.27	0.0024
	Number of culms 2009	1	165.00	165.00	5.65	0.0367
(c) Shoots harvested per clump	Treatment	2	171.40	85.70	9.59	0.0075
	Number of culms 2009	1	186.78	186.78	20.91	0.0018
(d) Culms harvested per clump	Treatment	2	304.63	152.31	23.41	0.0005
	Number of culms 2009	1	247.44	247.44	38.03	0.0003

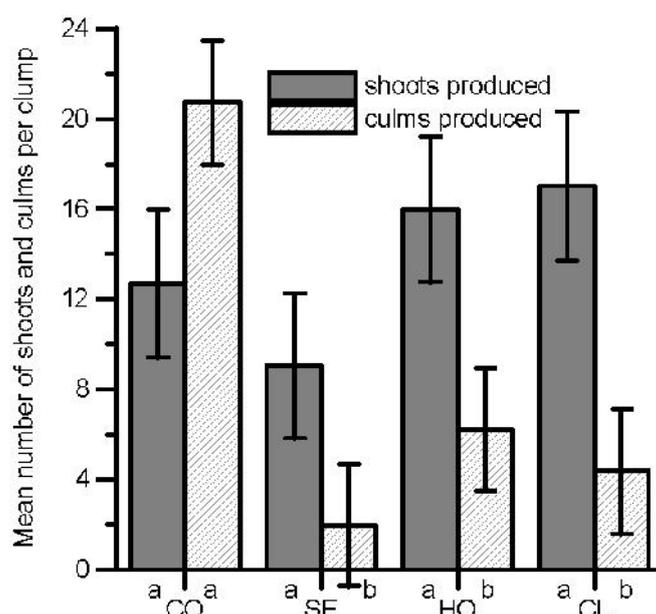


Figure 3: Total number of shoots and culms produced per *Dendrocalamus hamiltonii* clump under different harvesting regimes (estimated marginal means \pm SE resulting from MANCOVA; CO-control, SE-selective cut, HO-horseshoe cut, CL-clearcut)

Harvesting of shoots and culms under experimental treatments

The multivariate test of differences between groups using the Wilks' λ criteria was statistically significant and the model explained a large proportion of the variation of the response variables (MANCOVA; $\lambda=0.128$, $p \leq 0.01$). The number of culms before application of treatments was a significant covariate in the multivariate model ($\lambda=0.173$; $p \leq 0.01$) and was significant in both separate univariate ANCOVAs (Table 4; $p \leq 0.01$). The number of shoots harvested was significantly higher with horseshoe cut as compared to clearcut, while selective cut did not differ from either treatment (Figure 4; contrasts, $p \leq 0.05$). The number of culms harvested was significantly higher with horseshoe cut, as compared to the other two methods (Figure 4; contrasts, $p \leq 0.001$).

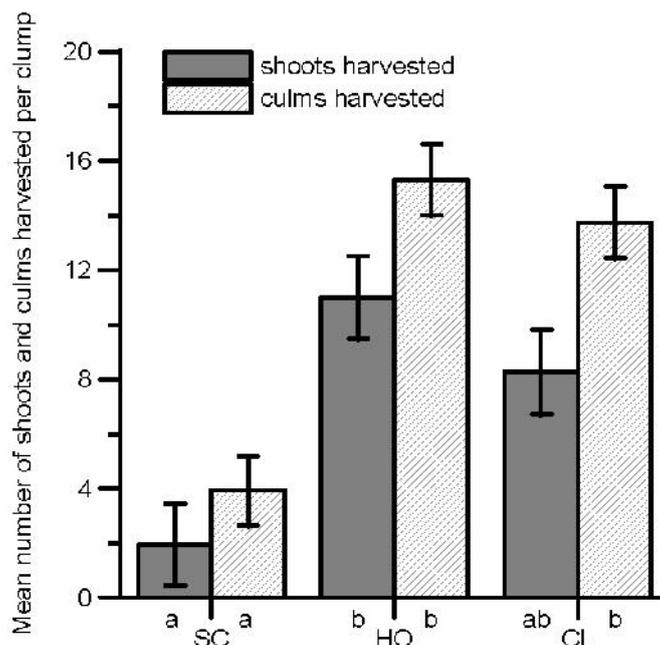


Figure 4: Total number of shoots and culms harvested per *Dendrocalamus hamiltonii* clump under different harvesting regimes (estimated marginal means \pm SE resulting from MANCOVA; SC-selective cut, HO-horseshoe cut, CL-clearcut)

Discussion

Experimental design based on farmers' objectives

Results of the social survey showed that farmers are interested in low input clump management practices and simultaneously maximizing harvestable number of shoots and culms, which has also been confirmed by other studies (Trinh Thang *et al.* 2011). Due to financial and labor implications, we thus had to rule out irrigation and fertilization as possible treatments in the trial. Lack of markets has also been identified independently as the reason for low interest of farmers to devote more resources towards bamboo management (Trinh Thang *et al.* 2011). In order to provide a benchmark of comparison between harvesting treatments and unmanaged clumps, the control treatment defined by no harvesting was included. Cleaning of dead culms and stumps was included in all harvesting treatments, in order to reduce clump congestion and to ease harvesting. The selective cut treatment was designed as a low-input thinning without spatial regulation of culms to be removed. In the course of harvesting, selective cut proved rather problematic to implement, as cutting of low stumps for culms marked for harvesting was difficult and frequently dangerous in clump centres, despite reduced culm densities. The horseshoe cut method (Tewari 1992; Bradshaw 1997) was included as a more labor-intensive alternative, requiring skills in proper spatial arrangement of culms to be removed. After the initially greater labor investment, the horseshoe method proved easier to administer, as culms and shoots were readily accessible for harvesting from clump edges. We were interested to see whether shoots located closer to the edge of clumps were less likely to survive (Franklin 2008), in which case the horseshoe method would have provided low yields of shoots and culms. Bamboo shoots are heavily browsed by wild animals and destroyed by insects (Taylor and Zisheng 1987), also observed with *D. hamiltonii* in our study site (Trinh Thang and Dorji 2010) and therefore we expected that this would lower shoot yields with treatments maximizing edge effects (horseshoe cut). The clearcut method was an alternative, which can be most easily and safely implemented in unmanaged, congested clumps of *D. hamiltonii* with most culms interlocking and under tension, resulting in dangerous

situations during harvesting. Maintenance of a balanced age distribution with removal of older clumps is generally deemed beneficial for clump vigor (Malab *et al.* 2006; Nath and Das 2011), but our study design did not allow for testing the effects of different age distributions on productivity. We applied harvesting treatments annually, resembling present local practices. Investigation on the management of *D. strictus*, which is a close relative of *D. hamiltonii*, revealed that sustainable harvesting depends on felling intensity, cutting methods, and felling cycle. Recommendations include a three- to four-year felling cycle with retention of new culms along with a certain number of old culms (Tewari 1992). Our study design and duration did not allow drawing any conclusions on cutting cycles, which needs to be investigated in the future.

Regeneration of bamboo clumps

While establishing a nearby study on intensive management of *D. hamiltonii* clumps for bamboo shoot production, Trinh and Dorji (2010) found comparable numbers of culms per clump and proportions of current-year old culms in untreated clumps, as reported in the present study. Our results were in contrast with considerably lower annual recruitment rates reported for other clump-forming bamboos (13.7% with *Fargesia scabrida*, 8.2% for *F. spatatea* (Taylor and Zisheng 1987), 3.2-22.2% for *Gigantochloa scortechinii* (Azmy 1999), and 11-27% for *Bambusa blumeana* (Malab *et al.* 2006)), but within the range reported for *D. strictus* of 12-83% (Lepcha *et al.* 2008).

Our recruitment rate of new shoots per culm (Productivity Index) of 38% per year with untreated clumps was higher than culm recruitment reported for *D. strictus* (30%: Singh and Singh (1999); and 11.4%: Tripathi and Singh (1996)) and comparable to that of *Gigantochloa scortechinii* (8.2-41.7% (Azmy 1999)). Assuming a shoot survival rate of 0.7 (Tripathi and Singh 1996), our results correspond well with those found for *D. strictus* and also with the proportion of current-year culms we found at the time of trial establishment. The effect of harvesting treatments did not lead to significantly increased Productivity Indices, even though mean PIs for horseshoe and clearcut treatments were a multiple of the mean PI for the control treatment. Increased PI as a result of clump cleaning has been reported for *Bambusa blumeana* (Malab *et al.* 2006). Similarly, culm harvesting led to increased proportions of present-year culms with *D. strictus* (Lepcha *et al.* 2008).

Our results do not fully support the findings of other studies on harvesting large, clump forming bamboos, according to which the number of culms (Vázquez-López *et al.* 2004) and especially the number of current-year culms (Malab *et al.* 2006; Hogarth and Franklin 2009) is closely correlated with shoot production. Even though we found indication for the above relationships to possibly hold true for *D. hamiltonii*, the number of culms harvested more closely influenced the number of shoots recruited in the following season. This relationship can be explained by the compensatory growth mechanism (McNaughton 1983), according to which bamboos compensate harvesting losses with increased recruitment of new shoots (Franklin 2008). The correlation between the number of mature culms and newly recruited shoots may rather be an effect of clump size, according to which larger clumps, indicated by greater number of mature culms, potentially produce greater number of new shoots.

Differences in edible shoot volume between control and thinned clumps could not be assessed, as no measurements were made for the control treatment. Culm thinnings without fertilization resulted in non-significant increases in shoot weight in *D. asper* and *B. blumeana* (Decipulo *et al.* 2006; Marquez 2006).

Shoot and culm production and harvesting in different treatments

In spite of continuous removal of shoots in the course of harvesting, we did not find significant differences in total shoot production between treatments, due to increased recruitment of shoots with harvesting (Vázquez-López *et al.* 2004; Decipulo *et al.* 2006). We did not find evidence for clump congestion restricting shoot production (Decipulo *et al.* 2006; Malab *et al.* 2006), as shoot production appeared rather constant irrespective of removal. A slight trend in our data suggested however, that selective cutting, defined by lower harvesting intensity of old shoots, may result in weaker recruitment of new shoots, while treatments removing a larger proportion of old culms indicated increased shoot production. This non-significant trend may support the theory that younger culms are more likely to produce shoots (Kleinhenz and Midmore 2001; Malab *et al.* 2006). Culm production was significantly higher in the control treatment, as no removal of culms took place.

The high harvested output in shoots and culms with the horseshoe cut was likely a result of increased edge length of clumps and decreased distance of culms from the clump edges. Horseshoe cutting avoids congestion of clumps, and leads to improved clump vigor, manifested through increased shoot production (Malab *et al.* 2006; Franklin 2008). The comparatively high harvest output in the clearcut treatment is likely a result of compensatory growth response to virtually complete removal of photosynthesizing biomass (McNaughton 1983). Our design did not allow evaluation of a possible trade-off between shoot and culm production, as observed with *D. asper* (Decipulo *et al.* 2006).

Conclusions

Before markets are readily available for bamboo products, farmers have low willingness to invest in management of *Dendrocalamus hamiltonii*. The notable exceptions are silvicultural interventions, resulting in harvest of shoots and culms, both for domestic consumption and for limited sale. Tested silvicultural methods, especially the horseshoe cut method, lead to high-level yield of shoots and culms, not jeopardizing clump productivity in the first two years after application of treatments. The horseshoe cut method was easy to administer after initially greater investment of labor and maximized safety of work while harvesting. Longer follow-up of the trial is necessary to be able to draw definitive conclusions on the sustainability and productivity of harvesting methods.

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References

- Angelsen, A.; Wunder, S. 2003. Exploring the forest—poverty link: key concepts, issues and research implications. CIFOR Occasional Paper 40. CIFOR, Bogor, 58 pp.
- Azmy, M. 1999. Regeneration of natural stand bamboos of *Gigantochloa scortechinii*. *Journal of Tropical Forest Science*, 11, 639-650.
- Bhatt, B.P.; Singh, K.; Singh, A. 2005a. Nutritional values of some commercial edible bamboo species of the North Eastern Himalayan region, India. *Journal of Bamboo and Rattan*, 4, 111-124.

- Bhatt, B.P.; Singha, L.B.; Sachan, M.S.; Singh, K. 2004. Commercial edible bamboo species of the North-Eastern Himalayan Region, India. Part I: Young shoot sales. *Journal of Bamboo and Rattan*, 3, 337-364.
- Bhatt, B.P.; Singha, L.B.; Sachan, M.S.; Singh, K. 2005b. Commercial edible bamboo species of the North-Eastern Himalayan region, India. Part II: Fermented, roasted and boiled bamboo shoots sales. *Journal of Bamboo and Rattan*, 4, 13-31.
- Bhatt, B.P.; Singha, L.B.; Singh, K.; Sachan, M.S. 2003. Commercial edible bamboo species and their market potentiality in three Indian tribal states of the North Eastern Himalayan Region. *Journal of Bamboo and Rattan*, 2, 111-133.
- Bradshaw, R.I. 1997. *Bamboo in Nepal: a management guide*. 12 pp.
- Decipulo, M.S.; Ockerby, S.E.; Midmore, D.J. 1999. Managing clumps of *Dendrocalamus asper* in Bukidnon, the Philippines. In Midmore, D.J. ed., *Silvicultural management of bamboo in the Philippines and Australia for shoots and timber*. Los Baños, 22-23 November 2006. pp. 36-45. ACIAR, Canberra, Australia.
- Dransfield, S.; Widjaja, E.A. ed., 1995. *Plant resources of South-east Asia No.7 Bamboos*. Backhuys Publishers, Leiden.
- Franklin, D.C. 2008. Fate of Culm Shoots in Wild Stands of a Tropical Clumping Bamboo. *Journal of Sustainable Forestry*, 26, 97-111.
- Gross National Happiness Commission 2008. *Tenth Five Year Plan (2008-2013)*. Royal Government of Bhutan, Thimphu, 170 pp.
- Hogarth, N.J.; Franklin, D.C. 2009. Observations on the clonal parentage of culms in wild stands of a clumping bamboo from northern Australia. *Journal of Tropical Forest Science*, 21, 139-146.
- Kleinhenz, V.; Midmore, D.J. 2001. Aspects of bamboo agronomy. *Advances in Agronomy*, 74, 99-145.
- Krishnankutty, C.N. 2004. Benefit-cost analysis of bamboo in comparison with other crops in mixed cropping home gardens in Kerala State, India. *Journal of Bamboo and Rattan*, 3, 99-106.
- Lawrence, A.; Kinhal, G.; Luintel, H.; Molteno, S.; Gillett, S. 2008. *Participatory science for sustainable wild harvests - a methods handbook*. University of Oxford, Environmental Change Institute Oxford, 218 pp.
- Lepcha, S.T.S.; Bisht, N.S.; Singh, C.J.; Dhiman, V. 2008. Impact of cultural operations on the production of new culms in *Dendrocalamus strictus* (Roxb.) nees in Uttarkhand. *Indian Forester*, 134, 859-865.
- Lobovikov, M.; Schoene, D.; Yping, L. 2011. Bamboo in climate change and rural livelihoods. *Mitigation and Adaptation Strategies for Global Change*, 1-16.
- Malab, S.C.; Batin, C.B.; Malab, B.S.; Alipon, M.A.; Midmore, D.J. 1999. Improving productivity of a previously unmanaged *Bambusa blumeana* plantation for culms and shoots in Ilocos Norte, the Philippines. In Midmore, D.J. ed., *Silvicultural management of bamboo in the Philippines and Australia for shoots and timber*. Los Baños, 22-23 November 2006. pp. 24-53. ACIAR, Canberra, Australia.
- Malab, S.C.; Batin, C.B.; Malab, B.S.; Alipon, M.A.; Midmore, D.J. Improving productivity of a previously unmanaged *Bambusa blumeana* plantation for culms and shoots in Ilocos Norte, the Philippines. In Midmore, D.J. ed., *Silvicultural management of bamboo in the Philippines and Australia for shoots and timber*. Los Baños, 24-53. ACIAR,
- Marquez, C.B. 2009. Improving and maintaining productivity of *Bambusa blumeana* for quality shoots and timber Iloilo and Capiz, the Philippines. In Midmore, D.J. ed., *Silvicultural management of bamboo in the Philippines and Australia for shoots and timber*. Los Baños, 22-23 November, 2006. pp. 46-60. ACIAR, Canberra, Australia.
- McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos*, 40, 329-336.

- Moktan, M.R.; Norbu, L.; Dukpa, K.; Rai, T.B.; Dorji, R.; Dhendup, K.; Gyeltshen, N. 2009. Bamboo and cane vulnerability and income generation in the rural household subsistence of Bjoka, Zhemgang, Bhutan. *Mountain Research and Development*, 29, 230-240.
- Nath, A.J.; Das, A.K. 2011. Population status and regeneration of a tropical clumping bamboo *Schizostachyum dullooa* under two management regimes. *Journal of Forestry Research*, 22, 43-46.
- Pandit, B.H.; Kumar, C. 2010. Factors influencing the integration of non-timber forest products into field crop cultivation: A case study from eastern nepal. *Journal of Sustainable Forestry*, 29, 671-695.
- Prommegger, W.; Budur, K.; Dorji; Chhetri, P.B. 2005. Lemon grass distillation in eastern Bhutan - a scenario analysis. RNR-RC Jakar, Bumthang, Bhutan, 35 pp.
- Rao, K.S.; Saxena, K.S. 1995. Effect of land use on *Dendrocalamus hamiltonii* regeneration during early secondary successional stages in northeast India. *Journal of Tropical Forest Science*, 7, 347-354.
- Samdrup, T. 2011. Improving the contribution of community forestry to poverty reduction in Bhutan. Department of Economic and Social Sciences. BOKU, Vienna, 64 pp.
- SAS Institute Inc. 2002-2008. SAS 9.2. SAS Institute Inc., Cary, NC, USA.
- Scheiner, S.M. 2001. MANOVA. In Scheiner, S.M., Gurevitch, J. ed., *Design and Analysis of Ecological Experiments*. Oxford University Press, New York, pp. 99-115.
- Seethalakshmi, K.K.; Kumar, M.S.M. 1998. *Bamboos of India*. Kerala Forest Research Institute, Peechi, 342 pp.
- Singh, A.N.; Singh, J.S. 1999. Biomass, net primary production and impact of bamboo plantation on soil redevelopment in a dry tropical region. *Forest Ecology and Management*, 119, 195-207.
- Social Forestry Division, D.o.F. 2008. National strategy for the development of non-wood forest products 2008-2018. Ministry of Agriculture, Thimphu, Bhutan, 46 pp.
- Stapleton, C.M.A. 2000. Bambusae. In Noltie, H.J. ed., *The grasses of Bhutan, Flora of Bhutan*. Royal Botanic Garden, Edinburgh, pp. 482-515.
- Stapleton, C.M.A.; Barrow, S.; Pradhan, R. 1997. *Bamboo and Cane Study of Zhemgang Dzongkhag*. Royal Government of Bhutan, Ministry of Agriculture, Thimphu, Bhutan, 28 pp.
- Sunderlin, W.D.; Dewi, S.; Puntodewo, A.; Müller, D.; Angelsen, A.; Epprecht, M. 2008. Why forests are important for global poverty alleviation: a spatial explanation. *Ecology and Society*, 13, 24.
- Sundriyal, M.; Sundriyal, R.C. 2004. Wild edible plants of the Sikkim Himalaya: Marketing, value addition and implications for management. *Economic Botany*, 58, 300-315.
- Taylor, A.H.; Zisheng, Q. 1987. Culm dynamics and dry matter production of bamboos in the Wolong and Tangjiahe Giant Panda Reserves, Sichuan, China. *Journal of Applied Ecology*, 24, 419-433.
- Tewari, D.N. 1992. *Silviculture and management of bamboos in India*. In Tewari, D.N. ed., *A monograph on bamboo*. International Book Distributors, Dehra Dun, pp. 169-186.
- Trinh Thang, L.; Dorji, T. 2010. Bamboo for shoot production: identifying the best management options using an action learning approach. Thimphu, 20 pp.
- Trinh Thang, L.; Samten, W.; Dendup, T. 2011. Report on evaluation of action learning research site, bamboo management training and bamboo shoot enterprise development feasibility study in Zhemgang. Social Forestry Division, Department of Forests, Thimphu, Bhutan, 36 pp.
- Tripathi, S.; Singh, K. 1996. Culm recruitment, dry matter dynamics and carbon flux in recently harvested and mature bamboo savannas in the Indian dry tropics. *Ecological Research*, 11, 149-164.
- Troup, R.S. 1921. *The silviculture of Indian trees III*. Oxford University Press, Oxford, U.K., 1195 pp.

- Vázquez-López, J.M.; Vibrans, H.; García-Moya, E.; Valdez-Hernández, J.I.; Romero-Manzanares, A.; Cuevas-Guzmán, R. 2004. Effects of harvesting on the structure of a neotropical woody bamboo (*Otatea: Guaduinae*) populations. *Interciencia*, 29, 207-211.
- Virtucio, F.D. 2009. General overview of bamboo in the Philippines. In Midmore, D.J. ed., *Silvicultural management of bamboo in the Philippines and Australia for shoots and timber*. Los Baños, 22-23 November 2006. pp. 18-23. ACIAR, Canberra, Australia.
- Wong, J.L.G.; Thornber, K.; Baker, N. 2001. Resource assessment of non-wood forest products - experience and biometric principles. FAO NWFP Series 13, Rome, 110 pp.
- Zhu, Z. 2003. The industrialization and market orientation of bamboo shoot production in Lin'an County: A case study. *Journal of Bamboo and Rattan*, 2, 441-452.

Macroproliferation Technology For Raising Large Scale Plantations Of Sympodial Bamboos.

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Abstract

Mass propagation of bamboos for raising industrial and commercial plantations was *an ever existing major enigma* world over, till the later years of 20th century. A latest low cost *macroproliferation technology* was developed in the last decade of the 20th century in 1991, at Forest Research Institute under Indian Council of Forestry Research and Education, Dehra Dun for mass propagation of sympodial bamboos for raising larger bamboo plantations. Thus, it solves the ever existing major enigma pertaining to non-availability of field planting stocks in massive numbers, for raising plantations. Macroproliferation technology has been found to be highly useful for sustained production of field plantable bamboo saplings in massive numbers rapidly, perpetually and plentifully for any desired number of years depending upon the targets and the facilities available. The dependence on seed production in nature is eliminated to the great extent.

The recently developed multiple intake macroproliferation technology has opened up many new avenues for research activities in the field of bamboo research. Ever since this technology was developed in 1991, great interest has been evoked amongst the bamboo scientists, researchers and growers to further explore the full potential of this technology. Seedlings raised by seed sowing; limited number of saplings available from the conventional clonal propagation methods viz. off-set, rhizome divisions, branch cuttings or culm cuttings etc. of bamboos can be successfully used for production of massive field planting stocks for raising larger plantations. It is not only capable of enhancing the multiplication rate of tissue culture bamboo plants massively but also reduces of the cost of production of field plantable saplings remarkably. Macroproliferation technology has been found to be highly efficient and dependable for production of field plantable saplings in massive numbers.

Keywords

Macroproliferation Technology; Propagules Production; Bamboo Plantations.

Introduction

Bamboo is an ideal environmental as well as economic investment which can be utilized in a variety of ways. Environmentally, the above ground parts of bamboo plant check pollution and the underground parts rhizome and roots check soil erosion whereas the emerging shoots are eaten. Bamboo is a wonderful sink of carbon dioxide with carbon sequestration rate as high as 47 per cent amounting to 12 - 17 tonnes of carbon dioxide per hectare per annum. It is a miraculous 'oxygen factory' as it generates 35 per cent more oxygen than other timber species and also its biomass yield is 2-6 times more than other timber species. Bamboo is considered as the fastest growing plant on degraded lands but also growing rapidly and covering ground quickly to prevent soil run-off and yield biomass 2-6 times more than the other timber species. Bamboo is gaining attention as an alternative forest crop with multiple uses and benefits (Bhatia 2003). Sands (2009) is of opinion that bamboo plantations will soon become possibly the greatest natural carbon sink. Each acre made up of bamboo sequesters up to 40 tons of carbon dioxide. The plant 'eats' atmospheric carbon dioxide and through the process of photosynthesis turns it into sugars. The bamboo plant transforms these sugars into the compounds that make up bamboo fiber. The carbon from atmosphere is thus locked up in the bamboo fiber itself. Bamboo is only effective for long term carbon sequestration if the bamboo culms are regularly harvested and that harvest turned into durable goods or biochar. Left un-harvested bamboo culms levels off the sequestration rate. By harvesting the 20% of the biomass of the plant each year as 3+ year old mature bamboo culms, the high rate of carbon sequestration are maintained for the 50-75 year life of the bamboo plant. Unlike most trees, bamboo plants are not killed when bamboo culms are harvested. Each year the rhizome is expanding, sequestering additional carbon for the life of the bamboo plant. Additionally, India has promising opportunities for climate change mitigation due to presence of large bamboo resource and has great potential for its cultivation and utilization (Dube 2008). Climate change is a serious reality world over. All the countries are going to be adversely affected by climate change. Bamboo being the fastest growing plant, bamboo plantations can be one of the important components to mitigate the impact of the climate change in shorter time.

In the olden days, the natural crop of bamboo was abundant enough to meet the requirements and bamboo was not considered as a resource but rather a weed. The old working plans in some of the states of India prescribed eradication of bamboo as a weed of teak plantations. Bamboo began to be considered as a resource of value only with the establishment of paper mills, using bamboo as raw material. These industrial uses suddenly enhanced the demand for bamboo by an order of magnitude over the traditional uses (Adarsh Kumar 1993). Due to over exploitation of bamboo forests for industrial and commercial purposes, the bamboo stock is gradually decreasing (Gadgil and Prasad 1978). Thus there is bound to be a situation of acute scarcity in near future if remedial measures are not taken soon.

Tewari (1988) called bamboo as 'poverty alleviator' as bamboo plays an important role in the rural economics of the developing countries. Thousands of rural people are engaged in the traditional crafts of making mats, baskets etc. to earn their livelihood. Additionally, bamboo is very important raw material for several small and large scale industries besides it is also used as construction material. From the raw material known as the "poor man's timber", bamboo currently has been elevated to the status of 'The timber of the 21st century'.

Bamboo is the natural vehicle for development. Indian subcontinent needs another green revolution – *the "Bamboo Based Green Revolution"* for sustainable development assisting poverty alleviation, employment generation and prosperity accumulation. Kamesh Salam (2009) has expressed that trees

are crucial for the global environment as they absorb carbon dioxide. They are important instruments to minimize global warming and related problems. It is for this reason that it has become important to find an alternative wood. Here bamboo has the advantage of growing fast and being a perfect material for a wide application of industrial products. By using fast growing alternative and renewable resources such as bamboo, we can reduce the pressure on tree forests, safe guarding the natural environment and the needs of future generations. Bamboo presents a viable alternative to check deforestation. The country suffers from deforestation to the extent of 1.5 million hectares annually because of timber and fuel wood requirements. Due to shrinkage in extent alarming decrease in the density, the supply of industrial wood from forests have been greatly reduced and there is an urgent need to look for alternative source to meet the increasing demands of our growing society. In this search, bamboo has emerged as a very ideal naturally renewable alternative source. Ecologically, bamboo plants are also valuable for windbreaks and soil conservation because of their heavy foliage and extensive rhizome-root system for ecological protection to river banks, lake shores, hillsides and homesteads besides these ensure returns and productivity. Bamboo has also been recommended as a target species for plantations in order to progress towards a greener world for pollution free environment besides for economic prosperity. Hence, the bamboo plantations not only decrease the quantity of carbon dioxide in the environment but will also be of great advantageous in fighting the war against global warming, world over.

The potential production of bamboo from all sources is reported to be near about 4.5 million tons per annum (Tewari 1992). With the trend of decrease in production and rise in population, the gap between supply and demand is going to be larger. This position clearly elucidates the need for increasing bamboo production. Adequate attention on raising bamboo plantation under various programs has not been given so far. Now we need to involve farmers and villagers in bamboo production. Besides protecting natural vegetation of bamboo, the activity has to be brought outside the conventional forestry on lands other than forest lands. It is the time that cultivation of bamboo is intensified through small and marginal farmers (Kamesh Salam 2002). The importance of bamboo in mountain ecosystem and mountain societies is that bamboo has the advantage of short rotation relative to other tree species, effective regeneration ability and good properties for a variety of uses. The properties of bamboo that are similar, or even superior, to those of wood, make it a suitable substitute for wood. If the bamboo resources of the tropical and subtropical areas are utilized appropriately for various uses, the potential for saving wood is remarkable (Wang et al. 2005).

Intensive bamboo propagation is necessary not only to increase biomass and species conservation but also to cultivate the economically important species for financial gains and to supply bamboo to meet the market demands (Rao 1992). But increasing demand and over exploitation is continuously depleting the bamboo production in most of the Asian countries. A time has come to take this matter seriously to device necessary measures for the management of bamboo areas intensively so that they are again brought back to rejuvenation and productivity. As a highly renewable and versatile resource, bamboo receives more attention from many sectors of modern civilization. In the post industrial world the outstanding productivity of this plant and versatility of the material will ensure the global importance of bamboo (Hanke 1990). As a highly renewable and versatile resource, bamboo receives more attention from many sectors of modern civilization. In other words, "Bamboo fever is wide spread throughout the world indeed" (Hsiung 1991). No doubt, this amazing tree grass has played a significant role in the life and activities of man and perhaps no other growing plant on earth has so many and as varied uses as bamboo. Yet, we know very little about several aspects of this fascinating plant and these are receiving high priorities in the research activities of bamboo specialists.

National Bamboo Mission (India)

Based on India's rich biodiversity and rich culture of bamboo utilization with greater potentials has triggered programs nationwide for economic and industrial development through the use of bamboo in a most environmental friendly way. Large targets for plantations across the country have been fixed. The emphasis of the National Bamboo Mission (India) is on an area based regionally differentiated strategy, for both forest and non-forest areas. A number of activities are proposed to be taken up for increasing production and productivity of bamboo through appropriate varieties with potential for yield, plantation development and dissemination of technologies through a seamless blend of traditional wisdom and scientific knowledge, along with the convergence and synergy amongst stakeholders. Besides ensuring proper post-harvest storage and treatment facilities, marketing and export to assure appropriate returns to growers/producers. Also to use bamboo development as an instrument of poverty alleviation and employment generation for skilled and unskilled persons, especially unemployed youth particularly in the rural sector for eco-rehabilitation purposes.

Mass Propagation of Bamboos – An Ever Existing Major Enigma

Mass propagation of bamboos for raising industrial and commercial plantations was an ever existing major enigma, world over till the later years of 20th century. Propagation of bamboo by seed is the best method but the main hurdle in raising bamboo plantation is the rare seed production in nature due to very long seeding intervals. The requirement of 'bamboo timber' for multiple uses by the industries and the common man will definitely increase in far greater dimensions. For this purpose larger bamboo plantations, intensive plantings of bamboo around villages, farmer's land, wastelands and other non-bamboo areas etc. need to be carried out. In order to meet the future requirements, more and more bamboo needs to be grown. The traditional/conventional methods of vegetative propagation of bamboos appear to be inadequate to meet the desired level of demand for field plantable saplings in massive numbers for raising large plantations. McClure (1966) highlighted certain problems *viz.* meager development of roots; decay of rhizomes and slowness of rhizome buds to break dormancy in propagation of clump forming bamboos through rhizomes. Banik (1980) stated that "a truly successful method for vegetative propagation has not yet been found. Successful propagules must develop roots and rhizome if they are to survive after being planted.

Prof. Liese (1985) stated that vegetative propagations by cuttings from culm, branch or rhizome is commonly practiced. So far several methods are applied, but for practical purposes especially for establishing larger plantations, the degree of failure is still rather high. Prof. Liese (1991) further added that in spite of intensive efforts made at various institutions, universally applicable method for vegetative propagation of bamboo is not yet available. Bamboo seems to be a difficult species to multiply, nobody seems to understand the bamboo just enough to propagate it in massive numbers (Anon. 1990). Sharma (1990) stated that Pathak (1899) was perhaps the first to attempt the propagation of the common 'male bamboo' (*Dendrocalamus strictus*) by cuttings. Since then several papers have appeared dealing with the vegetative propagation of bamboos. None of these earlier attempts have standardized the technique of bamboo propagation by vegetative methods. Rao and Rao (1990) are of opinion that the vegetative methods of bamboo propagation *viz.* off-set planting, rooting of culm and branch cuttings are of limited value for the large scale propagation of clump forming sympodials. The propagules, thus produced are bulky heavy, difficult to handle and transport. Thus to generate field planting stocks of bamboos on mass scale for raising industrial and commercial plantations is definitely an uphill task. Othman and Nor (1993) stated that the main problem faced in establishing large-scale bamboo plantations is the non-availability of planting material. None of the conventional methods of propagation is universal and effective for all the species of bamboos. Each carries its own inherent risks (Anon. 1994).

Srinivasan (Anon. 1994) mentioned that the key to the success will be cost effective and simple plant propagation techniques. Nautiyal et. al (2008) are of opinion that the traditional /conventional rhizome-off-set methods of propagation can be used only for production of few propagules. These are very expensive methods being labour intensive for excavation and transport. The availability of off-sets / rhizomes per clump is limited as only young (1-2 years old) culms can be used as propagules. The field survival percentage remains between 5-50 per cent. Both whole culm cutting and ground layering methods need sufficient space near the clump, which may not always be available. The culm cutting method of propagation is comparatively well studied but the survival percentage is quite low and the method is also expensive. Bamboos can be multiplied vegetatively with ease but large plantations are difficult to raise because lack of regular and plentiful supply of seed / propagation material is a serious constraint for mass propagation of bamboos and simultaneously the resulting clumps are liable to flower with the parent clump and therefore, the life could be short and uncertain (Nawa Bahar and Singh 2008). Similarly, Manoj Chandran (2008) has also cautioned that propagation from rhizome, offset and culm cuttings of unknown age is also unreliable as the saplings developed and planted in the field for raising bamboo plantations, may flower, dry and die before their economic use starts or much before utilization of full potential of bamboo culm production of the plantation. Sastri (2008) is of opinion that supply of planting material for large scale commercial plantations of the desired species could be a limiting factor due to lack of seed and/or other propagules. Troup (1921) described the seed germination and seedling growth pattern followed by the 'seedling division' as one of the method of propagation of *Dendrocalamus strictus*, besides describing other methods viz. off-set planting, culm cuttings and branch cuttings etc. for propagation of bamboo. "The 'seedling division' consisted in dividing up the mass of rhizome and transplanting the culms (tillers) in small clump (bunches) of two or three with rhizomes attached; transplanting is best carried out immediately before the growing season commences". Whereas, Banik (1987) described the growth pattern of *Bambusa tulda* from seed to seedling and observed that a bamboo seedling attains 4 -5 culms stage at the age of nine months. Seedlings at this stage are ready for multiplication and may be separated into three units in such a way that each piece has roots, old and young rhizome with buds and shoots. Every year the seedling gets multiplied three times of the initial stock. Out of this, two-thirds of the seedlings may be planted in the field. The rest can again be multiplied after nine months and the process can be repeated every year. However, Banik (1987) further continued, "detailed scientific study is essential on such a macroproliferation of bamboo seedlings to develop a new dependable technique for bamboo propagation at least for a few years".

Mass Propagation of Bamboos - An Ever Existing Major Enigma solved through the development of Macroproliferation Technology

Adarsh Kumar (1991, 1992, 1993) had developed a new low cost universal technology, named by him as *macroproliferation technology*, for mass propagation of economically important sympodial bamboos viz. *Bambusa bambos*, *B. tulda*, *Dendrocalamus hamiltonii* and *D. strictus* in the beginning of the last decade of the 20th century. It has now become possible to produce bamboo field planting stocks vegetatively for raising larger industrial and commercial plantations perpetually and plentifully, depending upon the targets and the facilities available, for any desired number of years without dependence on seed production in nature. Hence, it has solved the ever existing major enigma pertaining to non-availability of field planting stocks in massive numbers, for raising high yielding industrial and commercial plantations. The technology developed is simple, easy, cost effective and involved the use of locally available materials. The field plantable saplings thus produced can be handled easily as these remain small in size. This macroproliferation technology has been described as a major breakthrough (Anon. 1992), thus, it can solve the existing difficulties of vegetative

propagation (Liese 1992), and it is one of the pioneer achievements in the field of bamboo research, picked up by the media (Rawat et al. 2009a).

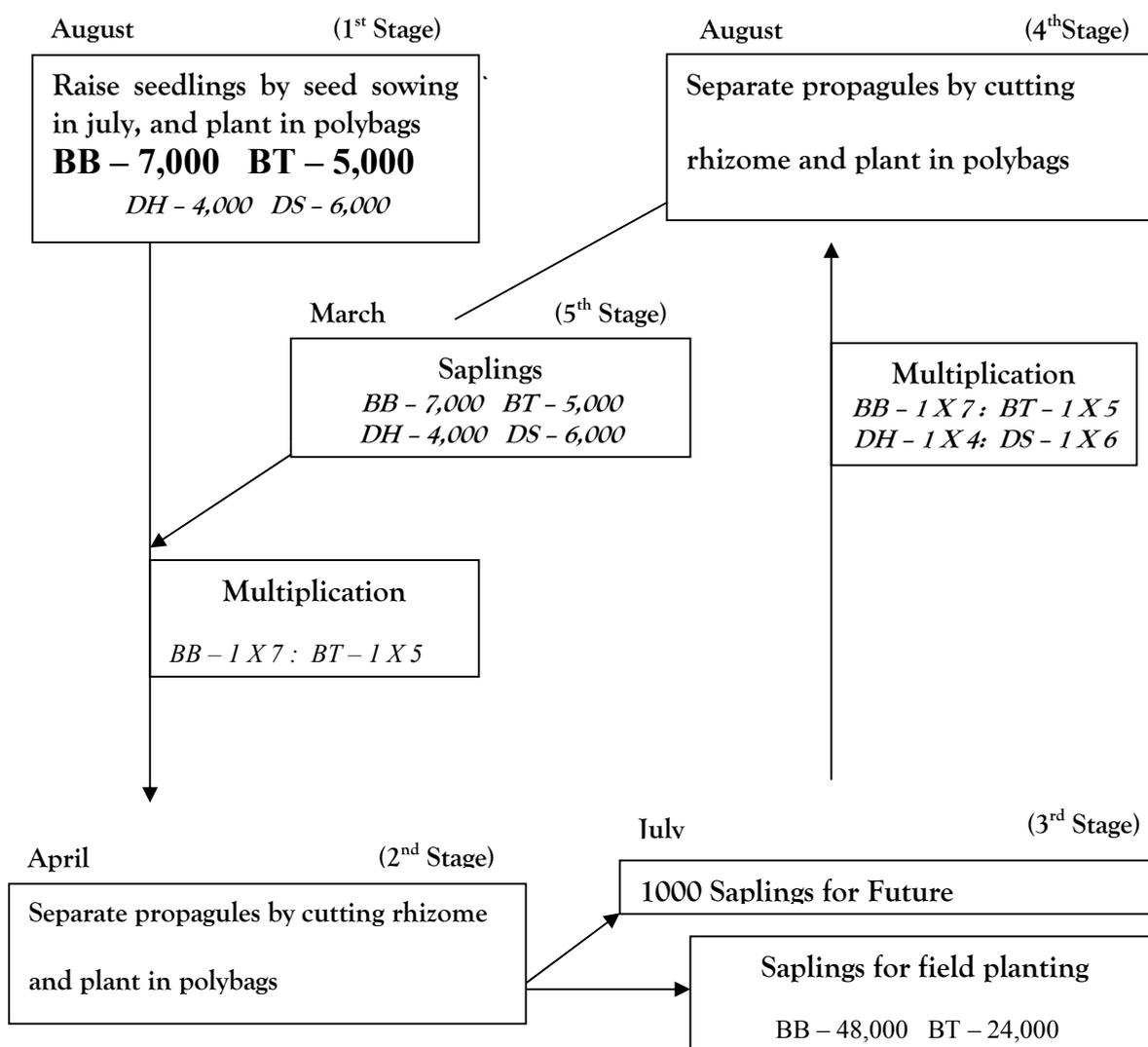
According to this technology (Plan-1) bamboo seeds are sown in July in germination boxes or in the nursery beds. After one month, in August, the young seedlings of *Bambusa bambos*=7,000; *B. tulda*=5,000; *Dendrocalamus hamiltonii*=4,000 and *D.strictus*=6,000 are pricked out and planted in polybags (Stage-1). From August to March bamboo seedlings are maintained by regular watering, weeding and soil working. The tillers ranging from 3-10 in numbers developed in each polybag of *B. tulda*, *D. hamiltonii* and *D .strictus* whereas in *B. bambos* it ranged between 6 and 14. The averages being *B. bambos* : 7.0, *B. tulda* :5.0, *D. hamiltonii* : 4.0 and *D. strictus*: 6.0. In the first week of April (Stage-2), the bamboo saplings are carefully removed from the polybags. Each proliferated tiller alongwith some rhizome and some roots is separated by cutting the rhizome. These act as propagules. (*Bambusa bambos* = 49,000; *B. tulda*=25,000;) *Dendrocalamus hamiltonii*=16,000 and *D. strictus*=36,000). Each propagule is planted in fresh polybag, as was done earlier in August. From April to June the propagules grow into saplings which are maintained by regular watering, weeding and soil working. In the first week of July (Stage-3), field plantable bamboo saplings are available in large numbers i.e. *B. bambos* = 49,000; *B. tulda* = 25,000; *D .hamiltonii* = 16,000 and *D. strictus* = 36,000. Out of these, 1000, or in multiples, saplings of each species are retained in the nursery for future propagation work. In August (Stage-4), propagules are prepared by separating the tillers by cutting the rhizome of 1000 saplings (retained in the nursery in July for future multiplication) and planted in poly bags. These were allowed to grow till March (Stage-5) next year by regular watering weeding and soil working, to develop into saplings. In April (Stage-2) fresh propagules were prepared by cutting the rhizomes of each of these saplings. From April to June these propagules grow into saplings. In the first week of July (Stage-3), field plantable bamboo saplings are available in large numbers i.e. *B. bambos* = 49,000; *B. tulda* = 25,000; *D .hamiltonii* = 16,000 and *D. strictus* = 36,000. Out of these, 1000 or in multiples saplings of each species are retained in the nursery for future propagation work. The whole cycle according to Plan-1 is repeatable for several years as per the requirements of field plantable saplings.

With the gradual increase in physiological age of the rhizome system but having only four months old (April to June) aerial growth, the resultant field plantable saplings possess well developed roots and rhizome system thus become more and more vigorous and shows almost 95-100 per cent survival in the field plantings. The field plantable sapling stocks having four months aerial growth, remain small in size which is additional advantage as these are easy to handle and transport.

Prof. Liese (1992) described this technology as ‘most remarkable’ and further added that ‘it can solve the existing difficulties’ of mass propagation of bamboos. Tewari (1992) stated that this macroproliferation technology of vegetative propagation is potentially universally applicable and can be used for mass production of field plantable saplings of sympodial bamboos. Gupta (1992) expressed that the technology on multiplication of bamboo seedlings through macroproliferation developed by Adarsh Kumar (1991,1992) has great potential in solving the problem regarding bamboo propagation on mass scale which is a great achievement in the field of bamboo research. Joshi and Dhiman (1994) are of opinion that singling of macroproliferated shoots appear to be potential method of multiplying bamboo particularly with short seed supply.

Plan-1. Plan for perpetual mass production of sympodial bamboos vegetatively through macroproliferation technology (Adarsh Kumar 1991, 1992, 1993). *BB*=*Bambusa bambos*; *BT*=*Bambusa tulda*; *DH*=*Dendrocalamus hamiltonii*; *DS*=*Dendrocalamus strictus*)

In the first year, field plantable saplings in massive numbers may be available through stages 1-2-3. From second year onwards the same numbers of field plantable saplings may be available from stages 3-4-5-2 and so on



Banik (1994) stated, "Recently in India a detailed plan has been developed by Adarsh Kumar (1991, 1992) for continuous production of field plantable saplings in massive numbers every year for any desired number of years". Dransfield and Widjaja (1995) reported that vegetative propagation system called 'macroproliferation of seedlings' has been successfully developed in India for large scale propagule production. This method ensures a continuous supply of propagules. John et.al (1995) emphasized that large scale cultivation is the only way to prevent further depletion, and to ensure a constant and sustained supply of raw material for growing industrial uses. Adarsh Kumar (1996) stated that massive planting stocks were produced every year for a number of years using recently developed macroproliferation technology. The dependence on bamboo seed production in nature is eliminated

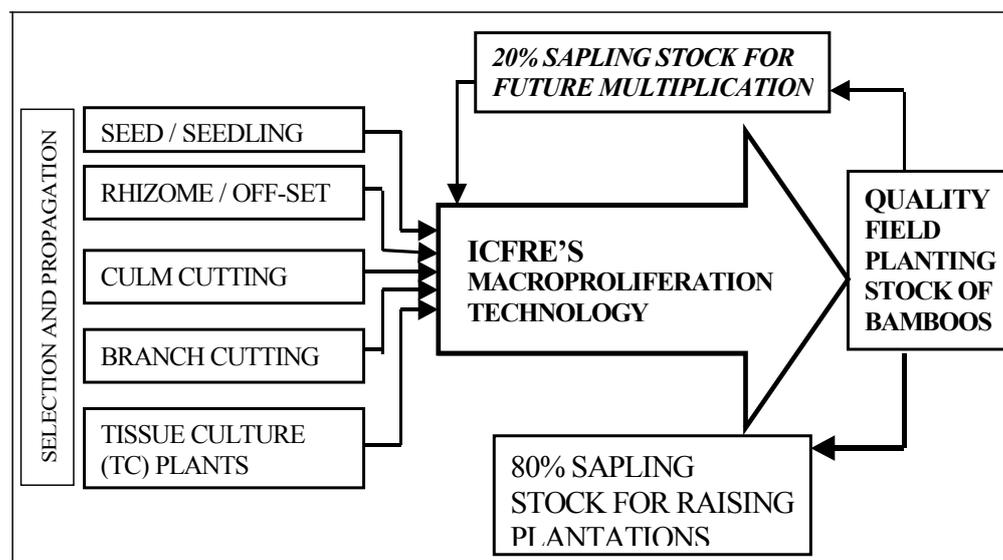
from second year onwards for perpetual production of bamboo field planting stocks.

Katwal (2002) stated that macroproliferation technology will help farmers to carry out macroproliferation of selected bamboo germplasm quickly and economically without using any sophisticated facility. Jagdish Kishwan et.al (2005) considered macroproliferation technique as the current technique of mass multiplication of bamboos. Recently, Nautiyal *et al.* (2008) reported the mass propagation of *Arundinaria falcate*, *Bambusa nutans*, *B. vulgaris*, *B. multiplex*, *Dendrocalamus asper* and *D. membranaceous* through macroproliferation technology. Prasad (2008) produced large planting stocks of bamboos by the use of macroproliferation technique. Nath et. al (2008) stated that the bamboo plantation efforts constrained due to the unavailability of quality planting stock may be overcome through the research and developmental activities of Indian Council of Forestry Research and Education (ICFRE), Dehra Dun (India) by the use of new macroproliferation. technology developed for mass propagation of sympodial bamboos.

The recently developed, low cost macroproliferation technology has opened up many more new avenues for research activities in the field of bamboo research. Rawat et al. (2009 a & b) stated that ever since macroproliferation technology was developed by Adarsh Kumar (1991,1992,1993), great interest is being continuously shown by the bamboo researchers and bamboo growers to further explore the full potential of the macroproliferation technology. It is highly flexible, as mass propagation of bamboos can be under taken from seed (Adarsh Kumar 1991, 1992,1993; Adarsh Kumar et al. 1994); off-set and branch cuttings (Koshi and Gopakumar, 2005); Rain Forest Research Institute, Jorhat (India), has developed a protocol to induce juvenility and generate saplings from the mature culms, followed by mass multiplication through macroproliferation technology (Katwal 2002); culm cuttings (Dubey et al. 2008); and tissue culture (TC) plants (Preetha et al. 1993; Arya and Arya, 1999). The saplings available from selected superior mother clumps by using conventional clonal methods of propagation of bamboos viz. off-sets, culm and branch cuttings etc. and tissue culture (TC) plants besides seedlings raised by seed sowing, can be used for the macroproliferation technology (Plan-2) for production of massive field planting stocks of bamboos for raising industrial and commercial plantations during the prolonged vegetative phase of the bamboo clumps.

Macroproliferation technology plays major role in production of massive quantities of field planting stocks of commercially important prioritized sympodial bamboos identified by National Bamboo Mission (India) in order to increase the coverage of area under bamboo in potential forest and non forest areas with suitable species to enhance yields. Large targets for plantations across the country have been fixed. The National Bamboo Mission (India) is continuing to cover over 1.76 lakh hectare area through bamboo growth. This requires, over 70 million field plantable saplings to raise bamboo plantations. In short it can be said, every action counts, every person counts, bamboo plantations could emerge as 'Green Gold Mines' in near future.

Plan-2. Multiple approaches to macroproliferation technology for massive production of quality planting stock of bamboo saplings through clonal and tissue culture propagation. (ICFRE=Indian Council of Forestry Research and Education, Dehra Dun, India)

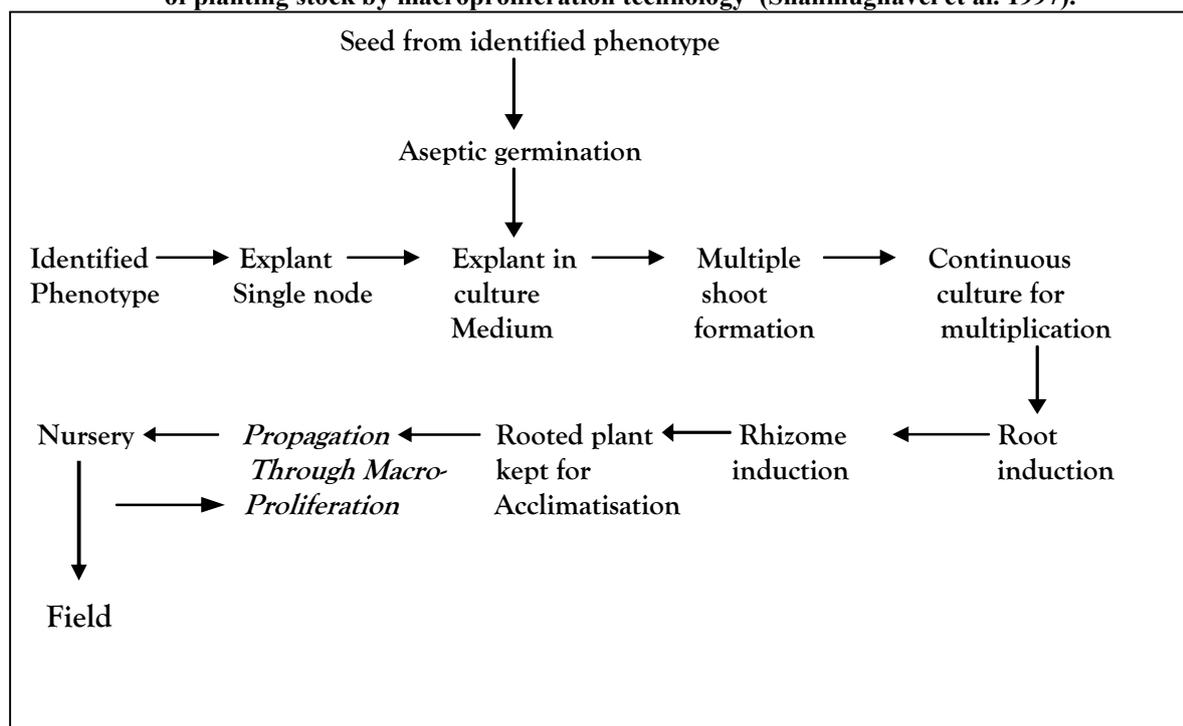


Enhancement of Planting Stocks of Tissue Culture Plants

This macroproliferation technology of mass propagation of bamboos has also been found highly advantageous by the tissue culture specialists to multiply *Dendrocalamus asper* plants which were earlier developed through tissue culture. Arya and Arya (1999) reported that the macroproliferation technology enhanced the multiplication rate of tissue culture raised bamboo plants and ensured a very high rate (95 per cent and above) of establishment and survival in the field in a short interval of time. These plants were multiplied twice a year for two years as per the technology. Preetha et al. (1993) used macroproliferation technology to enhance the quantity of *Dendrocalamus strictus* and *Bambusa bambos* rooted plants which were earlier developed through tissue culture and hardened in the shade house for 20-30 days. The field planting stock was increased 4-5 times by adopting this technology. Shanmughavel et al. (1997) suggested that in order to increase the tissue culture plants before transfer to the field, macroproliferation should be practiced (Plan-3). By this technology a large number of identified planting stock of *Bambusa bambos*, *B. polymorpha*, *Dendrocalamus asper*, *D. giganteus*, *D. strictus* and *Phyllostachys edulis* can be made available.

The macroproliferation technology not only enhances the multiplication rate of tissue culture (TC) bamboo planting stocks massively but also results in the reduction of the cost of production of field plantable saplings (of TC plants) remarkably. The operational guidelines framed by Government of India (Anon. 2007) for production of quality planting material of bamboos through tissue culture, suggested the mass multiplication of tissue culture (TC) plants through the macroproliferation technology for the enhancement of the planting stocks.

Plan-3. Multiplication of bamboos by tissue culture and further enhancement in production of planting stock by macroproliferation technology (Shanmughavel et al. 1997).



Economics of Planting Stock Production

Nautiyal et al. (2008) found that unlimited planting stock may be produced at the lowest cost i.e. @ Indian Rs. 2.50 (=US\$ 0.05) per sapling through macroproliferation technology. They have worked out the comparative economics of bamboo planting stock production by different methods of vegetative propagation as under (Table-2):

Table-2. Comparative costings regarding production of bamboo planting stocks by different methods of vegetative propagation (Nautiyal et al. 2008).

Sl No.	Method	Cost per plant (Rs.)	Remarks
1.	Off-set planting	50.00	Labour intensive, heavy planting stock
2.	Rhizome planting	30.00	Labour intensive, heavy planting stock
3.	Whole culm cutting	15.00	Labour intensive
4.	Layering	05.00	Labour intensive
5.	Culm cutting	08.00	Limited planting stock may be produced
6.	Branch cutting	03.00	Unlimited planting stock may be produced
7.	Macroproliferation Technology	02.50	Unlimited planting stock may be produced

Safe Transportation of Field Plantable Saplings of Bamboos

The plantation activities also involve safe transportation of bamboo planting stocks from nurseries to planting sites. According to Adarsh Kumar and Jain (2009) their safe transportation from nursery to field planting site is as vital as production of field plantable bamboo saplings in the nurseries. It is

absolutely essential that the saplings reach the destination in undamaged and uninjured condition. This is one of the most practical and applied aspect of the plantation technology. Despite constantly improving afforestation technologies, many organizations often face decline in early survival of planted material for raising plantations. Many a times these failures cannot be attributed to insects, diseases or adverse weather. The most common reasons for these failures may be due to breakdowns in what can be thought of as the “afforestation system”. At various points between nursery bed and the field planting site, seedlings/saplings get “critically wounded” by events that are considered to be insignificant. Combination of these “insignificant events” add up to poor seedling/ sapling survival or complete plantation failures. All the investment in tree breeding/selection, nursery culture and careful handling can be lost if transportation of seedlings/saplings for raising plantations is not given due attention. The bamboo seedlings / saplings are live, tender and delicate plants which need proper protection against high ambient temperature, gusty wind and mechanical vibrations due to speed of the truck (carrying field planting stocks) during transit. The trucks need to have double layered tarpaulin roofing to protect the planting stocks from heat of the sun; proper ventilation for the respiration by the planting stocks and also to prevent the accumulation of carbon dioxide besides protecting the saplings from the gusty wind. The mechanical vibrations due to speed of the truck (carrying field planting stocks) during transit may be checked to the great extent at a speed not exceeding 40-50 kilometers per hour at any time during transit. Further, the truck driver needs to be made aware of the possible damage to the saplings due to the vibrations caused by the high speed of the truck. Beside these, proper care for the planting stock is also utmost requirement to maintain sufficient moisture for the plants to maintain uptake of nutrients, during transit.

Earlier, Adarsh Kumar and Mohinder Pal (1993) stated that the conventional methods are still useful for raising planting stocks on very small scale, but for large scale propagation of bamboos, macroproliferation method has many benefits and can be directly used in the forest nurseries. Recently, Banik (2008) has also expressed that macroproliferation method has subsequently been used to multiply plants generated by culm cuttings, branch cuttings and micropropagated tissue cultured (TC) plants.

References

- Adarsh Kumar 1991. Mass production of field planting stock of *Dendrocalamus strictus* through macroproliferation - A Technology. *Indian Forester* , 117(12), 146-152.
- Adarsh Kumar 1992. A New technology for mass production of field planting stock of *Bambusa arundinacea* through macroproliferation. *Proceedings International Symposium on Industrial use of Bamboo*. 7-11 December 1992, Chinese Academy of Forestry, Beijing, China: 56-60.
- Adarsh Kumar 1993. New low cost technology for mass propagation of bamboos. *ICFRE Publication No. 29*, Indian Council of Forestry Research & Education, Dehra Dun, India.
- Adarsh Kumar 1996. Macroproliferation – A new technology for mass production of field planting stocks of sympodial bamboos for raising massive bamboo plantations. National Seminar on Bamboo, 28th-29th November 1996, Aranya Bhawan, Bangalore, India.
- Adarsh Kumar; Jain, S.S. 2009. Safe transportation of field planting stocks of sympodial bamboos with special reference to saplings. *Indian Forester*, 135 (5), 595-599.
- Adarsh Kumar; Mohinder Pal 1993. Recent developments in bamboo propagation for rapid mass production of field planting stock. *Proc. National Seminar on Forest Produce*, 15-16 October 1993. Institute of Forest Genetics and Tree Breeding, Coimbatore, India.
- Adarsh Kumar; Mohinder Pal; Shiv Kumar 1994. Mass production of field planting stock of *Dendrocalamus hamiltonii* vegetatively through macroproliferation. *In: Bamboo in Asia and the*

- Pacific. *Proceedings of the IVth International Bamboo Workshop held in Chiangmai, Thailand, November 27-30, 1991.* Technical Document GCP/RAS/134/ASB Forspa Publication No. 6. IDRC- FAO: 123-127.
- Anon. 1990. Some other notes on the Bamboo. *Canopy International* 16 (1): 10.
- Anon. 1992. Annual Report 1991-92. *Indian Council of Forestry Research & Education*, Dehra Dun, India.
- Anon. 1994. Report: Propagation and Supply of Propagating Materials. *In: Constraints to Production of Bamboo and Rattan- INBAR Report No. 5 :Report of INBAR Research Consultation , 9-13 May 1994, Bangalore, India: INBAR, New Delhi, India: 2-23.*
- Anon. 2007. Operational guidelines for large scale production and demonstration of quality planting material of bamboo. Department of Biotechnology, Ministry of Science & Technology, Government of India, New Delhi, India.
- Arya, S.; Arya, I.D. 1999. Micro and macro-propagation of edible bamboo (*Dendrocalamus asper*). *Proc. International Workshop on Forestry Research in conservation of Natural Forests (Eds. Negi, R. S. et al.) UNDP-ICFRE, Dehra Dun, India, 209-213.*
- Banik, R.L. 1980. Propagation of bamboos by clonal methods and by seed. *In: Bamboo Research in Asia (Eds. Lessard, G. and Chouinard, A.), Proc. Bamboo Workshop held in Singapore 28-30 May 1980, 139-150.*
- Banik, R.L. 1987. Techniques of bamboo propagation with reference to prerooted and prerhizomed branch cuttings and tissue culture. *In: Recent Research on Bamboos (Eds. Rao, A. R., Dhanrajan, G., Sastry, C. B.) CAF, China and IDRC, Canada. Proc. Intl. Bamboo Workshop, October 6-14, 1985, Hongzou, China: 160-169.*
- Banik, R.L. 1994. Review of conventional propagation research on bamboos and future strategies. *In: Constraints to Production of Bamboo and Rattan- INBAR Report No. 5, Report of INBAR Research Consultation , 9-13 May 1994, Bangalore, India: INBAR, New Delhi, India: 115-142.*
- Banik, R.L. 2008. Issues in production of bamboo planting materials- Lessons and strategies. *Indian Forester* 134(3): 291-304.
- Bhatia, V. 2003. Bamboo shoots as an option for Agroforestry. *Proceedings of National Seminar on Forest Resource Management, 15-17 November 2003, Department of Forestry, C.S. Azad University of Agriculture, Kanpur, India: 93-104.*
- Dransfield, S.; Widjaja, E. A. 1995. Plant Resources of South-East Asia, No.-7: Bamboo. Backhus Publishers, Leiden , 189 pp.
- Dubey, R. M.; Das, P. S.; Reeta Choudhury 2008. An investigation into macroproliferation of some selected bamboo species of Assam. *Indian Forester*, 134 (3): 367-378.
- Gadgil, M.; Prasad, S.N. 1978. Vanishing bamboo stocks. *Commerce* 136 (3497): 1000-1004.
- Gupta, B.N. 1992. Personal communication
- Hanke, D.E. 1990. Seeding the bamboo revolution. *Nature*, 344 (6264) : 291-292.
- Hsiung, W. 1991. Prospects of bamboo development in the world. *Jour. Amer. Bamboo Soc.* 8 (1&2) : 168-177.
- Jagdish Kishwan; Sharma, K.K.; Ratho, S.K. 2005. Bamboo based agro-forestry models. *In: Agroforestry Manual for Asia Pacific Region. Government of India, Ministry of Environment and Forests, New Delhi and United Nations Convention to combat desertification, Bonn, Germany.*
- John, C. K.; Nadgauda, R. S.; Mascarenhas, A. F. 1995. Bamboo – some newer prospectives. *Current Science* 68(9): 885-895.
- Joshi, N. K.; Dhiman, R. C. 1994. Vegetative propagation in operational forestry –problems and prospects. *In: Indian Forestry New Trends (Ed. Joshi, N. K.), Indian Council of Forestry Research & Education, Dehra Dun.*

- Kamesh Salam 2002. Keynote address – Expert consultation on sustainable utilization of bamboo resources subsequent to gregarious flowering in the northeast. Rain Forest Research Institute, Jorhat, 24th April 2002.
- Kamesh Salam 2009. Foreword. *Proc. VIII World Bamboo Congress*, Bangkok, Thailand.
- Katwal, R.P.S. 2002. Inaugural Address – Expert consultation on strategies for sustainable utilization of bamboo resources subsequent to gregarious flowering in the North-East. 24-25th April 2002 (CBTC, Guwahati and UNIDO), Rain Forest Research Institute, Jorhat, India.
- Koshi, K.C.; Gopakumar, B. 2005. An improvised vegetative propagation technique for self incompatible bamboos. *Current Science* 89 (9): 1474-1476.
- Liese, W. 1985. Bamboo – biology, silvics, properties, utilization. Schriftenreihe der Deutsche Gesellschaft – für – Technische, Zusammenarbeit No. 180, Eschborn, Germany, 132pp.
- Liese, W. 1991. Progress in bamboo research. *Jour. Amer. Bamboo Soc.* 8 (1&2): 151-167.
- Liese, W. 1992. Personal communication.
- Manoj Chandran 2008. Development of rhizome banks for bamboo multiplication. *Indian Forester* 134 (3): 445-447.
- McClure, F.A. 1966. The Bamboos: A fresh perspective. Harvard University Press, Cambridge, Massachusetts, U.S.A.
- Nath, V.; Pal, R.S.; Banerjee, S.K. 2008. BAMBOO: Its distribution, production, Habitat and agroforestry potential. *Indian Forester*, 134(3): 387-396.
- Nautiyal, S.; Negi, S.S.; Biswas, Sas; Rathore, R. K. 2008. Farmer's friendly cost effective propagation techniques of bamboo. *Proc. International Conference on Improvement of Bamboo Productivity and Marketing for Sustainable Livelihood*. 15-17 April 2008, New Delhi, India : 253-271.
- Nawa Bahar; Singh, V.R.R. 2008. Storability of bamboo seeds: a brief review. *Indian Forester* 134 (3): 441-444.
- Othman, A.R.; Nor, H.M. 1993. Vegetative propagation by branch and culm cuttings of selected bamboos of Malaysia. *BIC-India Bulletin*, 3(2):24-28.
- Pathak, S.L. 1899. Propagation of the male bamboo by cutting in the Pinjaur- Patiala forest nursery. *Indian Forester*, 25: 307-308.
- Prasad, P. N. 2008. Propagation of bamboos in Manipur. *Indian Forester* 134 (3): 325-332.
- Preetha, R.; Yasodha, R.; Madhavi, S.; Sumathi, R.; Stanley, J.; Gurumurthi, K. 1993. Tissue culture of forest plants. *Proc. Workshop on production of genetically improved planting materials for afforestation programmes*, 18-25 June 1993, Coimbatore, India, 76-97.
- Rao, A. N. 1992. Further research needed to improve bamboo in South and South-east Asia. In: Bamboo and its use. *International . Symposium on. Industrial Use of Bamboo*, Beijing, China, 7-11 December 1992: 3-9.
- Rao, I.V.R.; Rao, I.U. 1990. Tissue culture approaches to the mass-propagation and genetic Improvement of bamboos. In: Bamboos Current Research (eds. Rao, I.V.R., Gnanaharan, R., Sastry, C.B.) *Proc. International Bamboo Workshop* 14-18 November 1988 held at Cochin, India; The Kerala Forest Research Institute, India and International Development Research Centre, Canada: 151-158.
- Rawat, G.S.; Adarsh Kumar; Jain, S.S. 2009(a). Multiple approaches to ICFRE's macroproliferation Technology for massive production of Bamboo field planting stocks for raising larger plantations *In:Advances in Bamboo Plantation Management and Utilization*.(Eds. Arya I.D;Sarita Arya;Rathore T.S and Tarun Kant); *Proc. National Seminar on Bamboo - "Plantation, Management and its Utilization"* 17th to 19th March 2009, Arid Forest Research Institute, Jodhpur, India:191-201.

- Rawat, G.S.; Adarsh Kumar; Jain, S.S. 2009(b). Production of quality planting material of sympodial bamboos for raising plantations in India. *Proc. VIII World Bamboo Congress*, 16-18 September 2009, Bangkok, Thailand Vol. 6: 89-106.
- Sands, D.E. 2009. Bamboo and climate change: The Imperative. *Proc. VIII World Bamboo Congress*, Bangkok, Thailand 16-19 September 2009; Vol. 1: 14-16.
- Sastry, C. B. 2008. A 2020 vision for bamboo in India- Opportunities and Challenges. . *Proc. International Conference on Improvement of Bamboo Productivity and Marketing for Sustainable Livelihood*. 15-17 April 2008, New Delhi: 6-15.
- Shanmughavel, P.; Francis, K.; George, M. 1997. Plantation Bamboo- Establishment and management : By tissue culture and macroproliferation. International Book Distributors, DehraDun. 39-45.
- Sharma, M.L. 1990. Vegetative propagation of bamboos. Successes and Failures. *Proc. National Seminar on Bamboo*, 19-20 December 1990, Aranya Bhawan, Bangalore, Karnataka Forest Department and The Bamboo Society of India, Baglore, India.
- Tewari, D. N. 1992. A Monograph on Bamboo. International Book Distributors, Dehra Dun, India.
- Tewari, D. N. 1988. Bamboo as poverty alleviator. *Indian Forester*, 114(10): 610-612.
- Troupe, R. S. 1921. The Silviculture of Indian Trees, Vol.3. Clarendon Press, Oxford.
- Wang, K.L; Hong, L.T; Ramanatha Rao V. 2005. Bamboo Resources and Traditional Culture in Xishuangbanna, Yunnan, Southwest China, IPGRI-APO, Serdang. Maylasia. 113p.

Propagation of a montane bamboo-*Thamnocalamus falconeri* Hook. f. ex Munro. through farmer-friendly technologies to enhance the rural economy in Garhwal Himalayas

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Abstract

The study was carried out in *Thamnocalamus falconeri* with the objective of developing vegetative propagation techniques, viz. offsets, clump division, rhizome cuttings, air layering, ground layering, macro-proliferation and culm cuttings, which have not been reported hitherto in this species. *Thamnocalamus falconeri* Hook. f. ex Munro. (local name, Dev Ringal) is an important hill bamboo associated with lives of many people residing in the Himalayas. The species is dwindling fast in natural forests and deserves special attention. Flowering and seed production in Dev Ringal occurs only every 28-33 years, hence seed is not usually available. Alternative techniques are required to build up sufficient stock for planting on a regular basis. Of all the techniques investigated, macro-proliferation of seedlings yielded maximum planting stock, with survival rate of 98-100%. With 100 seedlings in the beginning, 560 propagules are obtainable in 8 months and 3136 propagules after two multiplications through this technique. In comparison, starting with 100 culm cuttings, 1904 saplings were produced after two multiplications. The two techniques, viz. macro-proliferation of seedlings and culm cutting propagation are discussed in detail. The techniques presented are easy, cost effective, farmer-friendly, sustainable and do not involve any special technicalities.

Keywords

Thamnocalamus falconeri, vegetative propagation, macro-proliferation, layering, culm cutting, offset planting

Introduction

Bamboos are a group of tall arborescent grasses associated with the lives of millions of people in Asia, Africa and Latin America. India, China and Myanmar have 19.8 million hectares of bamboo reserves which is 80% of the World's bamboo forests, out of which India's share is 45% with nearly 130 species (Sharma 1980). India has the second largest bamboo resources after China. Presently bamboo forests in India occupy ten million hectares, of which 28% lie in north-eastern states. Realizing the economic importance of bamboo, India has announced National Bamboo Mission Board for promoting bamboo based industries. National Mission on bamboo Technology and Trade development has launched several initiatives to place bamboo as a key species for Research and Development. Among non-timber forest products (NTFPs), bamboo occupy a special place in socio-economic lives of rural India. Garhwal Himalayas are represented with four hill bamboo species (locally known as Ringal). These are classified as - *Sinarundinaria anceps* ((Mittf.) Chao & Renv. also known as *Arundinaria jaunsarensis* (Gamble) or *Yushania jaunsarensis* (Gamble); *Arundinaria falcata* (Nees ex. Munro) also known as *Sinarundinaria falcata* (Nees) Chao & Renv., or *Drepanostachyum falcatum* (Nees) Keng.; *Thamnocalamus spathiflorus* (Trin.) Munro also named as *Arundinaria spathiflora* (Trin.) and *Thamnocalamus falconeri* Hook f. ex Munro., often being known as *Himalayacalamus falconeri* (Munro) Keng f. or *Drepanostachyum falconeri* (Hook f. ex Munro). All such genera of hill 'Ringal' bamboos share 3 stamens and relatively small stature, producing culms of less than 3cm in diameter in the western Himalayas, with small branches, often in large numbers. They occupy a special place in the lives of communities residing in the hills, providing livelihood support to a large number of traditional artisans and rural communities. Many cultural traditions of rural communities are intimately connected with bamboo resources. The culms of *A. falcata* and *T. falconeri* are put to day to day use by the local people for making baskets including kiltas, kandis, mats, hookah pipes, stakes for cash crops fuelwood, fodder, roofing, thatching, flooring of houses, fencing of houses and gardens, walking sticks, brooms, weapons and tools. The small brittle culms with swollen nodes of *T. spathiflorus* make it unsuitable for weaving, however, it is extremely important for wildlife , providing food for Red pandas and bears and shelter for birds like pheasants.; fresh sprouts of *T. falconeri* are cooked and used as vegetable (Stapleton 1994) and *A. falcata* apparently also has medicinal value (Kapoor 1991). Many people earn their livelihood by making baskets and other handicrafts from these resources.

Apart from their economic utility, they play an important ecological role in soil conservation checking soil erosion (Naithani 1993). *T.spathiflorus* forms the favoured habitat of several red-listed wild animal species viz., Musk Deer and the Pheasant species such as Monal and Western Tragopan (Goraya *et al.* 2003).

Unfortunately these natural resources of hill bamboos are being depleted at an alarming rate due to degradation of natural habitat, forest fires, unscientific and illegal harvesting for day to day use by the rural populace along with insufficient plantation. The problem is further compounded by gregarious flowering resulting in death of entire clumps following seeding, with the result that many areas in Garhwal Himalayas where bamboo grew in dense thickets a decade ago now lie barren. The resultant regeneration takes time to establish and has to face intense grazing pressure, forest fires, upcoming weeds as the parent stock completely dies off after flowering. This calls for large replanting efforts which require enormous quantities of planting material. Propagation through seeds is an easy approach but seeds of Ringal bamboos are not easily available due to long flowering and seeding interval.

Considering the limitation in seed supply due to long flowering interval (28-30 years) in *Arundinaria falcata* (Troup 1921; Campbell 1988), 60 years in *Thamnocalamus spathiflorus* (Trin) Munro by Campbell (1988) and 16-17 years by Anon (1981), 15 years interval in *Sinarundinaria jaunsarensis*

(Naithani *et al.*, 2003) and 28-33 years in *Thamnocalamus falconeri*, coupled with short seed viability of 6-8 months, vegetative propagation is the only option to raise planting stock on regular basis. The present paper describes the propagation technologies developed at Forest Research Institute, Dehra Dun in *Thamnocalamus falconeri* (Dev Ringal). This is a relatively tall and the preferred Ringal hill bamboo species in the Garhwal Himalayas (Fig.1). Like all the Ringal bamboos, this species has thin culms unlike those of *Bambusa* and *Dendrocalamus*, and would not be expected to respond well to propagation techniques requiring production of rooted shoots from branches, such as ground layering, air layering. However, as noted by Stapleton (1994), there are varieties of *T. falconeri* that spontaneously produce aerial roots on branch bases, making them more suitable. The techniques investigated here are innovative for hill bamboos, relatively easy and can produce planting stock for any desired number of years sustainably. Sustainable production and utilization of bamboo for rural and tribal communities can provide them with a way out of poverty through provision of a stable source of income.



Fig 1. *Thamnocalamus falconeri* clump in nature

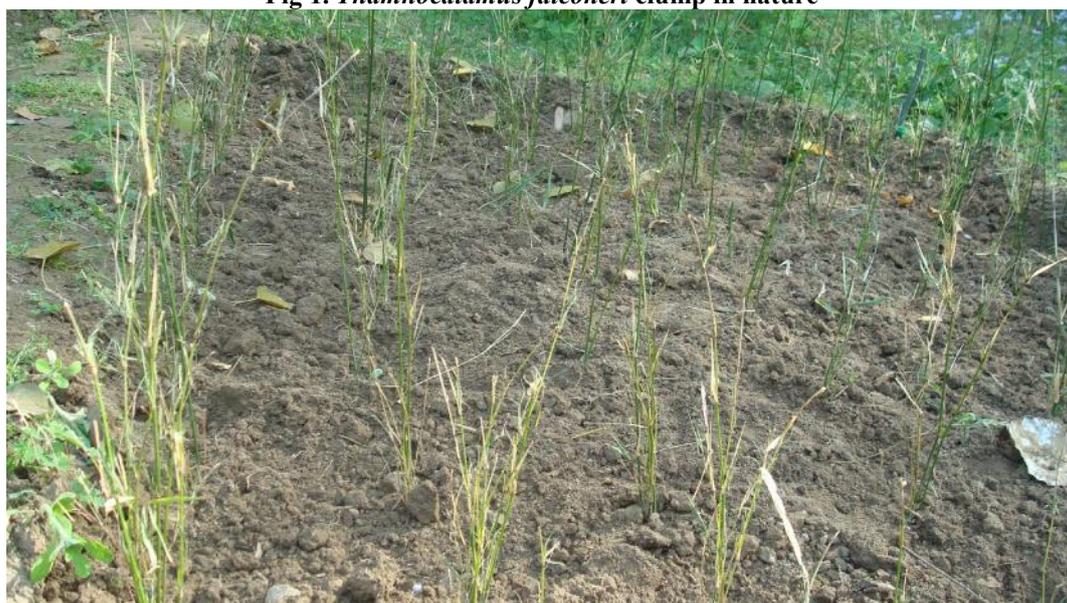


Fig 2. Offset planting in the field A and B

Material and Methods

The study was carried out at the Plant Physiology Discipline of Botany Division, Forest Research Institute, Dehradun India. The various vegetative propagation methods developed in *Thamnocalamus falconeri* (Dev Ringal) are highlighted below.

Offset planting

Offset planting is the traditional and conventional method of propagation of bamboos. An offset has only one rhizome and limited number of culm buds. This method although provides good results for sympodial and monopodial Ringal however, is not feasible for large scale planting due to limited number of rhizomes and tedious and costly method of extraction. Part clumps of *Thamnocalamus falconeri* were extracted from forests of Chopta and Mandal areas (Chamoli district). They were separated with sharp secateurs into single culm portions with rhizome and roots (Fig. 2) and planted at Khirsu Research Station (1934 masl) in nursery beds before onset of Monsoon. A total of 60 offsets were planted in 3 replicates. 90% per cent survival was achieved in this species. Young offsets (1-2 year) gave better response.

Clump planting

This method is similar to the offset planting except that in this method parts of the clump (4-6 culms with the rhizomes) are taken as propagule. This type of propagule can be termed as part clump or rhizome assembly. The rhizome in a clump propagule is not separated at the time of collection from soil else propagated as such from one place to another (Fig. 3). The method of extractions and planting was similar to offset planting. The culm part should have 3-4 nodes with viable branch buds. The profuse culm sprouting takes place from rhizome buds resulting into a wider clump. A total of 50 part clumps were planted in beds at Khirsu in the months of June- July. Almost 100% success was achieved in this species when planted in monsoon.



Fig 3. Clump planting

Rhizome planting

Rhizomes of Ringal were extracted from wild in forests of Chopta –Mandal areas (Fig.4). Every rhizome has buds to proliferate. Small pieces of rhizome were separated with the help of sharp secateurs or knife. The rhizome pieces were planted in trays or beds (normal garden soil) with nodes facing upwards. A total of 60 rhizome pieces were planted in three replicates of 20 each. They were watered regularly. After 15-20 days, new sprouts started proliferating from nodes with profuse rooting which were then planted in the field before onset of monsoon. 85% of rhizomes produced plants.



Fig 4. Collection of rhizomes

Ground Layering

Six months to one year old peripheral culms of *Thamnocalamus falconeri* clump were buried in the ground in 6-9 inches deep furrows. The soil was covered nicely on furrows so that the culms were tight in position and not disturbed. A total of 40 peripheral branches were buried. The furrows were kept moist by regular watering. In 5-6 months, there was profuse sprouting and rooting on the nodes. The proliferated shoots were extracted from the soil separated into individual propagules and planted in polypots having 2:1:1 (Soil: Sand and Manure) (Fig. 5). Initially they were acclimatized in shade and then shifted to natural conditions after a week. 80 % of the peripheral branches developed propagules. This is the easiest method of propagation of this Ringal species but is limited to the supply of few peripheral shoots. Alternatively, the lower most culms of this Ringal species were covered up to 3-4 nodes with a well rotten farmyard manure mixed soil and kept moist with watering. A total of 64 culms were covered with soil. After 2-3 months, root formation took place on covered nodes which were separated and planted in polypots containing 2: 1: 1 ratio of soil, sand and manure. Almost 100% culms rooted when the technique was applied in monsoon. The limitation of this method is that only few propagules can be generated although the rhizome of mother plant is not disturbed. New culm formation takes place from nodes of the buried mother rhizome after 15-20 days. This is a non destructive method of propagation.



Fig 5. Ground layering

Marcotting (Air layering)

In this technique, 1-2 year old culm cuttings were selected. The branches at the nodes were pruned with sharp pruners leaving 3-4 nodes intact. IBA 4000 ppm paste was applied on nodes which were then wrapped around with an admixture of moist garden soil and sphagnum moss. The air layers were strongly covered with minutely perforated polythene which was tied at both the ends (Fig.6). Only 20% rooting was obtained in 35 air layers. Successful rooted air layers were separated from the main culm and planted separately in polypots or in the field.



Fig 6. Air layering

To meet the large demand for planting stock of Ringals, propagation through culm cuttings and macro-proliferation were also investigated, and are discussed below in detail.

Propagation through culm cuttings

One to two year old culms from healthy mature clumps of *T. falconeri* were extracted from natural forest. One or two-noded cuttings were prepared from culms with the help of sharp secateurs and treated with various growth regulators (IBA, NAA, IAA) in concentrations of 500ppm. The experiment was designed in Randomized block design with three replicates, each replicate having 20 ramets. The experiment was repeated every four months i.e. 3 seasons. The culm cuttings were planted horizontally in beds or trays (normal garden soil) keeping nodal buds in lateral position as shown in (Fig.7 A-C). The trays should be kept in shade till sprouting and rooting. Regular watering and weeding is practiced. Within 1-2 months, new sprouts were visible followed by rooting. The beds were kept moist avoiding over-watering. The cuttings were pre-treated with a fungicide Bavistin/ Emisan (0.1%) and beds sprayed with chloropyrophos. The branch cuttings rooted were carefully uprooted from the rooting medium (Nursery bed and earthen pots) after three months (90 days) and observations were made on rooting percentage, number of roots, root length, number of sprouts and sprout length. The rooted propagules were transferred in polypots containing 2:1:1 mixture as cited above. Initially they are acclimatized in shade house for a couple of days (1-2 weeks) with proper watering and then kept in open conditions. After a span of 6-8 months when profuse sprouting had occurred, the propagules were separated by macro-proliferation technique and replanted in polypots which after a span of two weeks were planted in the field at Magra (1834 masl).



Fig 7. Rooting of culm cuttings



Fig 8. Macro proliferation of seedlings

Seedling macro-proliferation Technique

Young seedlings of *Thamnocalamus falconeri* were pricked out from the natural forest floor of Chopta – Mandal (Chamoli Forest Division) and brought to Khirsu Research Station. The seedlings were transplanted into polypots in the first week of August 2009 and watered regularly. Each polypot was earlier filled with mixture of sieved soil, sand and farm yard manure in 2:1:1 ratio weighting nearly 4 kg in each polypot. One young seedling was planted in each of the polypots which were kept in shade for a week. All the seedlings planted in polypots established well and then the same were shifted in the open under direct sunlight. The emergence of new growth was observed regularly and new sprouts were observed within 15-20 days. In the first week of April, the seedlings having culms, rhizome, and roots were carefully removed from the polypots. Each proliferating culm along with some rhizome section and roots was separated and again planted in fresh polypots for further growth and development. At the time of separation of propagules due care was taken to ensure that each separated propagule retains one rhizome with buds and roots as suggested by Banik (1985). The separated propagules were again planted in polypots filled with the same mixture as mentioned above. Out of these freshly planted propagules, 1/6 propagules were retained for future multiplication purposes in the nursery whereas, 5/6 propagules were available for field planting and were planted in Forest Department Nursery at Magra, Mussoorie. The whole of the technique is illustrated in Fig 8A-C.

Analysis of variance (ANOVA)

All the data pertaining to rooting and subsequent growth was loaded in Microsoft Excel and subjected to analysis of variance using Genstat statistical package (Genwin 3.2 version). In the analysis of variance for studied parameters, the mean values of each replication were estimated. For comparison of different means of different treatment, the critical difference (CD) were calculated based on student's t test at $p < 0.05$ level. Critical Difference (CD) value was calculated by Schiff's method and is based on F-Statistics (Schiff's, 1959), CD is the minimum variance permissible between the means of treatments for grouping them as statistically same.

Result and Discussion

The results pertaining to three important methods are presented and discussed below:

Macro-proliferation

The data recorded on the number of propagules produced, diameter of culms, etc. recorded at eight months period is reflected in Table 1. It was observed that sprouting occurred after 15-20 days of planting. A total of 280 culms were produced from 50 planted seedlings within an average of 5.6 culms per seedling which is comparatively low. The propagules grew vigorously to reach an average height of 43.8 cm and diameter of 1.98 mm. Average number of roots per propagule was 10.7 with average length of 14.9 cm. Similar results were also recorded by Kumar (1992) in *Bambusa arundinacea*. The tiller number increased with the passage of time and average number of culms was recorded 5-6 in six months period (Fig. 6). Kumar and Pal (1994) reported production of 25 propagules from one mother sapling of seed origin after two multiplications in a year in case of *B. tulda* while 165, 160, 159, 149 and 60 numbers of propagules were obtained in respect of *B. bambos*, *B. balcooa*, *D. hamiltonii*, *B. nutans* and *B. tulda* respectively within one year (Dubey *et al.* 2008). In a similar study in *D. hamiltonii* (Kumar *et al.* 1992) and *B. bambos* (Kumar 1992) reported production of 16 and 49 number of propagules after 12 months respectively. In *Bambusa tulda* three proliferated seedlings has been obtained in 9 months by Banik (1985) who also suggested that such process of seedling multiplication should not be continued for long time (e.g. not more than 10 years in *Bambusa tulda*) as the time gap between the last multiplication and flowering gets shorter. The advantage of this technique is that proliferated seedlings remain small in size and hence easy to transport. The technique developed would overcome the scarcity of planting stock of this sympodial bamboo for future plantation and conservation strategies. This technique is low cost, simple and has direct application in the field, but can only be initiated at the time of flowering and seed production.

Table 1. Number of propagules produced after eight months of planting and their growth parameters.

Number of seedlings planted	50
Number of seedlings survived	50 (100%)
Total number of culms after 8 months	280
Average number of culms per seedling	5.60
Average length of each culm (cm)	43.8 cm
Average diameter of each culm (mm)	1.98 mm
Average number of nodes in each culm	4.30
Average number of roots per seedling	10.7
Average length of each root	14.9

Rooting of culm cuttings

Rooting response of branch cuttings of *Thamnocalamus falconeri* was studied under natural (open sunlight) conditions for one year (March 2010 to February 2011). The rooting behaviour with respect to number of roots per cutting, root length (cm), rooting percentage (%), number of sprouts and sprout length (cm) were recorded during this period under different treatments and seasons and analyzed by Analysis of Variance (ANOVA).

Interactive effects of Seasons (S) and Treatments (T) (SxT):

Sprouting percentage

Sprouting from nodes started within 9-11 days and the root development started after 45-60 days of culm planting. The statistical analysis revealed a significant ($P < 0.05$) effect in all seasons (Table-2). In

summer season, maximum (80%) sprouting was noticed in the cuttings treated with IBA 500 ppm followed by control and minimum (61.67%) in NAA 500 ppm. In rainy season maximum (73.33%) sprouts were recorded in the cuttings treated with IBA 500 ppm while, minimum (58.33%) sprouts were recorded in the cuttings treated with NAA 500 ppm. No sprouting was recorded during winter season (November-February) planted cuttings in any of the treatments. Overall, the maximum sprouting percentage (80%) was recorded in summer season with IBA 500 ppm. Treatment had highly significant influence ($P < 0.001$) on mean number of sprouts in relation to season (Table-2). In summer season maximum (4.33) number of sprouts were noticed in IBA 500 ppm treated cuttings and minimum (2.50) in NAA 500 ppm. In rainy season, maximum (4.50) number of sprouts are recorded in IBA 500 ppm treated cuttings whereas, minimum (2.83) in NAA 500 ppm. In winter season no sprouts were recorded in any of the treatments. Overall, the maximum numbers (4.50) sprout were recorded in rainy season cuttings when treated with IBA 500 ppm. It was evident from the analysis that all the treatments had highly significant influence ($P < 0.001$) on mean sprout length per cutting in relation to season (Table-2). In summer season maximum (12.49 cm) sprout length was noticed in the cuttings treated with IBA 500 ppm and minimum (9.52 cm) in NAA 500 ppm. In rainy season, maximum (15.95 cm) sprout length was recorded in the cuttings treated with IBA 500 ppm followed by (11.55 cm) in IAA 500 ppm and minimum (11.03 cm) in untreated cuttings. In winter season, no sprouting was recorded. Overall, the maximum sprout length (15.95 cm) was recorded in rainy season when treated with IBA 500 ppm.

Season	Treatment	Characters					
		Mean No. of roots	Mean root Length (cm)	Mean rooting %	Mean No. of Sprouts	Mean sprout Length (cm)	Mean sprouting (%)
March-June	Control	2.16	7.18	58.33	2.83	11.39	78.33
	IAA500ppm	3.00	7.36	48.33	3.17	10.21	66.67
	IBA500ppm	4.00	9.69	55.00	4.33	12.49	80.00
	NAA500ppm	2.50	8.42	41.67	2.50	9.52	61.67
	Mean	2.19	8.16	50.85	3.20	10.90	71.65
July-October	Control	3.16	8.40	48.33	3.17	11.03	63.33
	IAA500ppm	3.50	9.74	36.67	3.50	11.55	68.33
	IBA500ppm	4.83	11.14	41.67	4.50	15.95	73.33
	NAA500ppm	2.83	7.99	33.33	2.83	11.24	58.33
	Mean	3.85	9.31	40.00	3.5	12.44	65.83
Nov.- Feb.	Control	0	0	0	0	0	0
	IAA500ppm	0	0	0	0	0	0
	IBA500ppm	0	0	0	0	0	0
	NAA500ppm	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
Significance		***	*	***	*	***	*
CD		0.706	1.094	5.58	1.172	1.175	7.170

NS=Non Significant, *=Significant at 5%, ***= Significant at 0.01%

Rooting percentage

The rooting of cuttings is influenced by many external and internal factors, which have been known for a long time and excellent reviews on this subject have appeared from time to time (Allen and McComb 1955; Leopold 1960; and Hyun 1967). Of the external factors, season plays the most important role on adventitious rhizogenesis. The variation in treatments among different seasons in relation to rooting percentage was highly significant ($P < 0.001$). In summer season, maximum (58.33%) rooting were noticed in untreated cuttings followed by (55%) in IBA 500 ppm. Minimum (41.67%) rooting was observed in the cuttings treated with NAA 500 ppm. In rainy season maximum (48.33%) rooting was also recorded in untreated cuttings followed by (41.67) in IBA 500 ppm treated cuttings and minimum (33.33%) rooting NAA 500 ppm. However, in winter season no rooting was recorded. Overall, the maximum rooting (58.33 %) was recorded in summer season in untreated control cuttings (Table-2). Agnihotri and Ansari (2000) also reported that cuttings collected in February and April showed significantly maximum root induction and growth of adventitious roots in *B. bambos* and *D. strictus*. Singh *et al.* (2002) reported that single nodal culm and culm branch cuttings of *B. nutans* collected in the month of April and May and treated with IBA were effectively good for large scale vegetative propagation. In autumn season (September-November), maximum (52.2%) rooting was recorded in the cuttings treated with IBA 200 ppm while minimum (21.83%) rooting was recorded in the cuttings treated with NAA 200 ppm. which is non confirmatory to the results by Agnihotri and Ansari (2000) in *D. strictus* who exhibited steep decline in rooting percentage from the cuttings collected during August to January. Kumar *et al.*, (1997) reported that *Bambusa tulda* produces adventitious roots almost whole year, except in the month of December whereas *Dendrocalamus strictus* did so for six months i.e. from February to July only. Raveendran *et al.* (2010) observed in *Dendrocalamus brandisii* that the rooting occurs during all the seasons and of the three seasons summer months were found to be the best. Thus the result of season to rooting response in bamboos also establish this fact that low temperature during winters completely inhibits the rooting and sprouting because of the low metabolic activities and resumes growth on return of favourable season. The interactive effect of season and treatment on the mean number of roots revealed a highly significant effect (0.01% level) in all seasons except November planted cuttings (winter season). In summer season, maximum (4.00) number of roots were noticed in the cuttings treated with IBA 500 ppm followed by (3.00) in the cuttings treated with IAA 500 ppm. Minimum (2.16) roots were observed in untreated cuttings. In rainy season, maximum (4.83) number of roots were also recorded in cuttings treated with IBA 500 ppm however, minimum (2.83) roots were recorded in the cuttings treated with NAA 500 ppm. As regards winter season, no rooting was recorded. Overall, the maximum number of roots per cutting (4.83) was recorded in rainy season with IBA 500 ppm treated cuttings. The treatment effect is significant at 5% level in all seasons with regard to mean root length (Table-2). In summer season maximum (9.69 cm) root length was noticed in the cuttings treated with IBA 500 ppm followed by the NAA 500 ppm (8.24 cm). Minimum (7.18 cm) root length was observed in untreated cuttings. In rainy season, maximum (11.14 cm) root length was recorded in the cuttings treated with IBA 500 ppm, however, minimum (7.99 cm) root length was recorded in the cuttings treated with NAA 500 ppm. In winter season no rooting was recorded. Overall, the maximum root length (11.14 cm) was recorded in rainy season with IBA 500 ppm.

Several investigations were made earlier by Nautiyal *et al.* (1991); Nautiyal and Rawat (1994); Sorin *et al.* (2005) on rooting behaviour of cuttings. It is well established fact that all auxins IAA, IBA and NAA generally stimulate adventitious root formation, but in our studies it is interesting to note that maximum rooting was observed in untreated control cuttings (Fig. 7C) followed by IBA. Similar observations were also recorded earlier in *Dendrocalamus giganteus* (Nautiyal *et al.*, 2007); in *Bambusa vulgaris* var. *striata* (Razvi and Nautiyal, 2009) and in *Bambusa vulgaris* 'Wamin' (Razvi *et al.*, 2011). Although maximum rooting was observed in untreated (control) cuttings, revealing thus

that this species does not require any rooting hormone treatment, however the efficacy of hormones was noticed in other rooting parameters where an increase in number of roots and root length was discernible in IBA 500 ppm treated cuttings.

Macro-proliferation of rooted cuttings

The number of propagules produced from each node at various stages of separation is reflected in tabular form (Table 3). It is observed sprouting commenced 9-11 days after planting, followed by root initiation. Only 17 % of the cuttings produced sprouts at both nodes with the formation of 4.5 sprouts in the initial stage. As 60% of cuttings rooted, but only 10% rooted at both nodes, it is much more productive to plant single-node cuttings, rather than 2-noded cuttings. The rooted cuttings when transplanted in polypots after separation of nodes in the month of July 2010 (Monsoon) showed 80% survival. The tiller number increased gradually with the passage of time and reached an average number of 5.0 sprouts /node after 9 months of planting. Starting with 100 binodal cuttings, 280 propagules were obtained at this stage. The propagules were separated and planted in the field. With survival of 80%, 224 propagules were produced in February 2011 i.e. 12 months after planting. Periodic observations are being recorded. So far after 20 months of planting (8 months after separation), an average of 8.5 sprouts with a maximum culm length of 103cm and average 3.29 mm collar diameter are observable (Table 3). With a multiplication rate of 8.5, the expected propagules at this stage are 1904 after two multiplications. Kumar and Pal (1994) reported production of 25 propagules from one mother sapling of seed origin after two multiplications in a year in *B. tulda* while Dubey *et al.* (2008) reported on an average 180, 165, 160,159,149 and 60 numbers of propagules / node in respect of *B. vulgaris*, *B. bambos*, *B. balcooa*, *D. hamiltonii*, *B. nutans* and *B. tulda* respectively after 4 multiplications.

Table 3. Number of propagules produced at various stages of culm cuttings with macro-proliferation in *T. falconeri*

a	No. of two noded culm cuttings planted	100 (200 nodes)
b	Date of planting	March 2010
c	Number of cuttings rooted	60
d	Number of cuttings rooted at both nodes	10
e	Total number of rooted nodes (c+d)	70
f	Average number of sprouts at each node	4.5
g	Number of nodes separated and planted in polypots	70
h	Date of planting	July 2010
i	Number of propagules survived in polypots	56 (80%)
j	Average number of sprouts/node at this stage in polypots	5.0
k	Date of separation	Feb. 2011
l	Total number of survived new propagules separated out of culm cuttings (ixj)	280
m	Number of survived new propagules separated out of culm cuttings after planting in field in Feb. 2011	224 (80%)
n	Average number of sprouts/propagule in the field	8.5
	Average height	103 cm
o	Average collar diameter	3.29mm
p	Date of observation	Oct 2011 (20 months after cutting planting)
q	Expected number of survived new propagules separated out	1904 propagules

	of propagules proliferating in beds (mxn)	
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Conclusion

Ringal hill bamboo species are eroding fast from Garhwal Himalayas, affecting lives of farmers and artisans who are solely dependent on natural resources for their livelihood. To meet their day to day requirements, large scale planting is thus required. Most of the Ringal species flower at long intervals and their seeds have short viability hence they cannot be raised directly from seed on an ongoing basis. Therefore, for production of planting material, development of vegetative propagation methods is the need of the hour. Unfortunately no appropriate propagation technologies are reported in any Ringal species which could be used to enhance and conserve the Ringal stocks in sufficient quantity. We have developed unconventional technologies which are new and hitherto not reported in relation to *T. falconeri*. Conventionally, the species can be propagated through offsets and clump divisions. Propagation through these techniques yields limited number of plants, depending on size of the mother clumps, and hence they are not suitable for large scale plantations. We have demonstrated that culm cuttings, rhizome cuttings, air layering, and ground layering are also successful in this species from forests of Chopta and Mandal areas. In order to meet demands for planting stock on a sustainable basis, repeated macro-proliferation of small plants derived from seedlings or culm cuttings were found to be the most productive techniques and are recommended. The methods described here are easy, cost effective and farmer-friendly. Use of plant growth regulators to stimulate rooting is not necessary. Further investigations are required to assess the uses, site requirements, and establishment techniques of the different hill bamboo species. Accurate identification of the species and varieties, along with their distribution and conservation status, and which propagation techniques are appropriate for them all would also be very useful. How the planting of these bamboos can be achieved in the current social and land-use situation also needs to be investigated, given the high pressures on land and the poor forest management practices that have led to their decline in the first place.

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References

- Agnihotri, K.; Ansari, S.A. 2000. Adventitious rhizogenesis in relation to seasonal variation, size of culm branch cuttings and IAA treatment in Bamboos. *The Indian Forester*, **126** (9), 971-984.
- Allen, R.M.; McComb. 1955. Uber Factoren die Bewurze lung der Steckling von der *Populus deltoids*. *Barti Beein Flussen, Zentralbi Forshwesen*, **74**, 199-220.
- Anon, 1981. Wealth of India, vol. II(B), CSIR, New Delhi, India, 1989, p.13.
- Banik, R.L. 1985. Techniques of bamboo propagation with special reference to pre-rooted and pre-rhizomed branch cuttings and Tissue culture. *Proc. Int. Bamboo Workshop, Hangzhou, China.*, Oct. 6-14, pp. 160- 169.
- Campbell, J.J.N. 1988. Note on Sino- Himalayan bamboo species. *II Intl. bamboo conf. 7-9 June, Anduze, France*, pp 1 105.
- Dubey, M.; Das, P.S.; Choudhary, R. 2008. An investigation into macro-proliferation of some selected bamboo species of Assam. *The Indian Forester*, **134**(3), 367-378.
- Goraya, G.S.; Jishtu, V.; Kapoor, K.S.; Pal, M. 2003. Mass flowering of montane bamboos in Himachal Pradesh: Ushering in the new millennium. *The Indian Forester*, **129**(8), 1013-1020.
- Hyun, L.S. 1967. Physiological differences among trees with respect to rooting-XIV. *IUFRO-Kongress Munchen, Section 22*, pp. 168.
- Kapur, K.S. 1991. Economically useful plants of Majauri-Kirchi forest tract (Jammu and Kashmir), J. *Econ. Tax. Bot.*, **14**, 534.
- Kumar, A. 1992. A New Technology for Mass Production of field Planting Stock of *Bambusa arundinacea* through Macro Proliferation. In: Zhu Shilin *et al.*, ed. Bamboo and its Use, International Symposium on Industrial Use of Bamboo: 7-11 December 1992, China and International Tropical Timber Organization, Yokohama and Chinese Academy of Forestry, Beijing pp. 56-60.
- Kumar, A.; Pal, M.; Kumar, S. 1992. Mass production of field planting stock of *Dendrocalamus hamiltonii* vegetatively through macro-proliferation. *The Indian Forester*, **118**(9), 638-646.
- Kumar, A.; Pal, M. 1994. Mass propagation of *Bambusa tulda* through macro-proliferation for raising industrial and commercial plantations. *The Indian Forester*, **120**(2), 152-157.
- Kumar, A.; Dhawan, M.; Gupta, B.B. 1997. Vegetative propagation of *Bambusa tulda* and *Dendrocalamus strictus* using growth promoting substances. *The Indian Forester*, **114**, 569-575.
- Leopold, A. C. 1960. Auxin and plant growth. University of California Press, Berkeley.
- Naithani, H.B. 1993. *Contributions to the taxonomic studies of Indian bamboos*. Ph.D. Thesis H.N.B.Garhwal University, Srinaga.
- Naithani, H.B.; Pal, M.; Lepchaa, S.T.S. 2003. Gregarious flowering of *Thamnocalamus spathiflorus* and *T. falconeri*, bamboos from Uttaranchal, India. *The Indian Forester*, **129**(4), 517-526.
- Nautiyal, S.; Rawat M.S. 1994. Macropropagation of Teak (*Tectona grandis* L.F.). *The Indian Forester*, **120**(2), 146-151.
- Nautiyal S.; Uma Singh; Gurumurti, K. 1991. Rooting response of branch cutting of Teak (*Tectona grandis*) as influenced by season and growth hormones. *The Indian Forester*, **117**(4), 249-255.
- Nautiyal, S.; Bhandari, H.C.S.; Prakash, R. (2007). Mass Propagation of *Dendrocalamus giganteus* through branch cuttings. *Indian Forester* **133**(12):1695-1698.
- Razvi, S.; Nautiyal, S. 2009. Mass propagation of *Bambusa vulgaris* (green) through juvenile branch cuttings: A new technology. *The Indian Forester*, **135**(11), 1585-1587.
- Razvi, S.; Nautiyal, S.; Prakash, R; Ajaz Bhat 2011. Studies on multiplication of *Bambusa vulgaris* cv. Wamin through juvenile branch cuttings. *The Indian Forester*, **137**(1), 264-266.

- Raveendran, V. P.; Seethalakshmi, K.K.; Jijeesh, C.M. 2010. Effect of season, position of node and growth regulating substances on adventitious root induction in an edible Bamboo, *Dendrocalamus brandisii* (Munro) Kurz. *The Indian Forester*, **136** (3), 231-243.
- Sharma, Y.M.L. 1980. Bamboos in the Asia-Pacific region. In: Lessard, G. and Choumard, A. (eds.) *Bamboo Research in Asia*, IDRC, 99-120.
- Singh, S.; Ansari, S.H.; Kumar, P. 2002. Clonal propagation of *Bambusa nutans* through culm and culm branch cuttings. *The Indian Forester*, **128**, 35-40.
- Sorin, C.; John, D.B.; Camus, I.; Ljung, K.; Kowalczyk, M.; Geiss, G.; Mckhann, H.; Garcion, C.; Vaucheret, H.; Sandberg, G.; Bellini, C. 2005. Auxin and light control of adventitious rooting in *Arabidopsis* require ARGONAUTE1. *Plant Cell*, **17**, 1-17.
- Stapleton, C.M.A. 1994. *Bamboos of Bhutan*. Royal Botanic Gardens, Kew, UK.
- Troup, R.S. 1921. The Silviculture of Indian Trees. Volume 1. *Oxford University Press, Oxford.*, pp. 228-296.

Human urine boosts bamboo biomass yield (*Phyllostachys viridiglaucescens*)

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Abstract

Collected separately from faeces, urine is directly usable as fertilizer for agriculture. The pioneer research linking human urine and bamboo plantations (bbP) demonstrated that human urine can fertilize efficiently *Phyllostachys viridiglaucescens* (*P. viridiglaucescens*) at high continuous fertilization rates (HCFR). From a wastewater point of view, the treatment corresponds to absorption of pollution by bamboo while for bamboo plantations (bbP) it is fertilization. Among the investigated aspects in that pioneer work (cf. footnote 3) during the 2 years of experiment at HCFR, bamboo biomass was produced, which is the concern in the present paper. Its growth throughout the experiment duration was observed and further analysed by counting, measuring and weighting. The results show that the growth pattern of bamboo (*P. viridiglaucescens*) alternated by growing season and resting period did not vary after urine-based fertilization. HCFR influences positively bamboo biomass productivity. In year 1 (y1), as a consequence of early treatment (before plants establishment in reactors), HCFR provoked a non linear positive response in fertilized groups of reactors, as each group did produce shoots, whereas no shoot emerged in unfertilized group. In year 2 (y2), a linear positive response due to HCFR was observed and the number of shoots correlated with biomass by weight. From y1 to y2, productivity increased and was respectively 236% for shooting and 154% for biomass by weight. HCFR did not influence linearly the increase in culm diameter, although on average, maximum culm diameter (D_{max}) increased by 27% from y1 to y2.

1. Introduction

Human urine is keeping on drawing more attention in the recent time, especially in the field of Resource-oriented Sanitation (RoS): for handling and treatment with different techniques including storage/direct reuse, continuous/intermittent treatment, concentration, crystallisation, struvite precipitation (Kirchmann and Pettersson 1995; Behrendt et. al. 2002; Niederste-Hollenberg 2003; Esrey et. al. 2004; Gulyas et. al. 2004; Harada et. al. 2006; Aguilar 2011; Beler-Baykal et. al. 2011), and as fertilizer in several countries on many vegetables (Kärrman et. al. 1999; Mnkeni et. al. 2008; Pradhan 2010) with successful results. Nevertheless, before (Ndzana and Otterpohl 2009), literature about human urine linking to bamboo plantations (bbP) was inexistent. That pioneer paper reported on the use of human urine as fertilizer on bamboo plantations and the conducted experiments demonstrated that urine² can be removed/treated by *P. viridiglaucescens* –thus bamboo plantations- at

² The overall concept was introduced earlier in (Ndzana and Otterpohl 2008) for the treatment of Resource-oriented Sanitation (RoS) waste streams (Greywater, Yellowwater, Brownwater and Rainwater) by bamboo plantations. The resulting treatment of the waste streams by bamboo corresponds to fertilization of bamboo plantations from agricultural point of view.

continuous basis. The applied treatment –which corresponds to fertilization for bamboo plants-, was investigated in several aspects³. This paper is a partial analysis of the biomass produced during the 2 years of experiments; the first evoked issue relates the quality of growth of *P. viridiglaucescens* due to high continuous fertilization rates (HCFR) applied, by answering the question whether the intrinsic growth behaviour -alternated by growing and resting period- are disturbed? Biomass yield (counting, measuring and weighting) comparison between fertilized and unfertilized plants is performed, and further analysed within fertilized groups of reactors in relation to fertilization rates (FR). The progress of the number of shoots throughout experiment's duration is elucidated month after month and year after year [year1 (y1) and year2 (y2)]. Comparative analysis between maximum diameters (D_{max}) of emerged shoots in respective groups of reactors according to FR is performed as well.

2. Material and methods

2.1. The experimental site

The experiment was set in the interior court of authors' Institute in Hamburg. Hamburg is located approximately 9 meter (m) above sea level. Annual precipitations range between 700 and 800 mm/a (millimetre per year). In general summer time is relatively hot and dry while winter time is cold and moist. During the two years of experiment the maximum (max) and minimum (min) temperatures with their corresponding relative humidity are shown in Table 2. A data logger was used to record continuously air temperature and moisture content throughout the duration of experiment (entire spectrum of data is not shown). The bamboo species tested is *Phyllostachys viridiglaucescens* (*P. viridiglaucescens*) that was chosen accordingly, as it can withstand temperature down to -22°C.

Table 2 Mean monthly hottest and coldest temperature and their corresponding air moisture content recorded in the course of the experiment

	Highest	Lowest
Air temperature (°C)	36.5	-10.9
Air moisture content (%)	27.6	69.0

The experimental plant was composed of 10 reactors divided into groups (cf. Table 2) according to FR. The reactors were aboveground. Each reactor is a huge container of 160 litre (L) and 53 centimetre (cm) deep. As bamboo grows within the first half meter in the soil, and even efficiently within the first 30 cm (An et. al. 1995; Li et. al. 1998; Widmer 1998), the 53 cm provided sufficient depth for growth. The bamboo plants were transplanted from a mature stand of the Botanic Garden Hamburg and roots balls were embedded in each reactor with additional self-composition soil of compost (85%), sand (10%) and crushed tree barks (5%). Culm height of bamboo was above 3.00 m. The reactors were placed in row to be under identical ecological conditions (sunshine, air temperature and moisture content, rainfall). The experimental site was relatively windy.

2.2. The analytical method

Author's urine was collected and used to mix the substrate. This ensured no pathogenic contamination and additionally no tablet was taken during the two years of experiment to avoid potential side effects

³ The main researched aspects included; substrate degradation, soil response to HCFR, quantitative and qualitative biomass production, nutrient uptake, influence of weed.

resulting from pharmaceuticals excreted with urine. Urine was mixed up with tap water (TW) to compose the substrate at nitrogen-based (N) nutrient loading rates (NLR expressed in kgN/ha/a) corresponding to FR presented on

Table 3. Each reactor was replicated for each FR. 3 times of feeding per week throughout the experiment's duration ensured continuous reuse of urine. The other 4 days were fed with TW on a necessity basis, in order to avoid drying of reactors, especially in summer hot days. The substrate was fed at the top of each reactor and the major volume remained in the reactors. Nevertheless, holes were made at the bottom of reactors and leachates collectors placed under them to collect the minor leaching volume that was further analysed⁴. Concerning FR which were taken from 300 kgN/ha/a and stepwise increased up to 1050 kgN/ha/a; (Kleinhenz and Midmore 2001) suggested N-based fertilization of 350 kgN/ha/a with mineral fertilizers in their review "Aspects of bamboo agronomy". Seven decades earlier, Oshima (1931) came up with the conclusion that no studies show that excessive fertilizer application reduces the biomass yield and quality. Therefore HCFR were chosen in order to investigate also the potential response of bamboo under high nutrient loads. It is worth to mention about the issue that to date, lack of practical guidance on application rates of fertilizer is pertinent, as most bbP are part of natural forest and thus not fertilized.

Table 3 Groups of reactors and their fertilization rates. G0 is the reference group and was fed only with tap water (TW).

	G0	G300	G600	G900	G1050
FR (kgN/ha/a)	0	300	600	900	1050

Standard statistical tools were used on the one hand to process recorded data relating urine and substrate and on the other hand to evaluate and interpret the results.

2.3. Urine characterization

1.1.1 Urine performed analyses

The analysed parameters were the following: Total Organic Carbon (TOC), Total Carbon (TC), Total Inorganic Carbon (TIC), Total Nitrogen (TN), Total Phosphorus (TP), potential Hydrogen (pH) and Electrical Conductivity (EC). Raw urine was analysed and the NLR applied in each reactor according to FR were found by dilution with TW. TOC, TC, TIC, TN were analysed by using the TOC/TN analyser multi N/C 3000 from Analytik Jena, Germany. P-PO₄ was analysed by using the Stannous Chloride method and TP was analysed by using Dr. Lange cuvette test. N-NH₄ was analysed by using a photometer. pH and EC were measured by using electrodes incorporating temperature readings; Microprocessor pH 196 for pH and LF konduktometer 191 for EC. Analyses were performed according to German standards (DIN⁵), which provide laboratory procedures of each analysis.

⁴ The results of leachates analysis showed insignificant leaching by volume and by nutrient content compared to the fed amounts. They are not shown in this paper that rather focuses on biomass production.

⁵ DIN stands for "Deutsche Institute für Norm". It is the most powerful German Institution making norms

1.1.2 Urine characteristics

As mentioned in (1.1.1), the characteristics of raw urine were measured and FR fed to the reactors were built upon them by dilution. Table 4 presents the characteristics of urine. fresh urine was continuously applied to avoid loss of N by volatilization in ammonia (NH₃)⁶. By applying fresh urine, loss of N is hindered, as hydrolysis processes that lead to NH₃ formation are lessened.

Table 4 Characteristics of raw urine. Values in the table are in mg/l, except for EC in mS/cm and pH without unit. SD is the standard deviation. TOC: total organic carbon, TIC: total inorganic carbon, TC: total carbon, TN: total nitrogen, TP: total phosphorus

	TOC	TIC	TC	TN	TP	pH [-]	EC
max.	6960	3030	7240	7530	1050	9.4	43.4
min.	1080	271	2590	2860	204	6.7	12.4
Mean	2507	1828	4335	5399	370	9.0	27.0
SD	1300	737	1035	1132	167	1.0	7.0
Samples number	37	37	37	37	37	37	37

37 samples were analysed in the course of experiment. The seven parameters analysed first demonstrate the high value of urine, especially in relation to nutrient content N and phosphorus (P), which are primary essential macronutrients for plant growth. But the fluctuation is also considerable when looking at SD. Actually nutrient content of urine is subjected to many factors among which diet is important. In winter time the tendency is to fat diet than in summer time where more fruits and vegetables are consumed and urine nutrient content varies accordingly (Heinonen-Tanski et. al. 2005; WHO 2006).

2.4. Bamboo yield analyses

The quantitative approach was used to analyse the bamboo biomass yield. It consisted by counting, measuring and weighting the biomass produced. By counting, emerging shoots from each group of reactors were numbered monthly and consequently annually for each experimental year. This resulted to cumulative shoots number analysis through the experiment duration. By measuring, the diameter of emerged shoots in each group of reactors was recorded. This enabled a comparison between max culm diameter (D_{max}) between y1 and y2 of experiment. (D_{max}) was measured at culm breast height (CBH). By weighting, the total emerged number of shoots in each group of reactors was weighted in y1 and y2. This led to biomass yield comparison between y1 and y2. The equipments used for measuring and weighting during the laboratory work comply with German Norms. Leaves were not considered in the weight of culms, as the experimental plant was exposed to strong winds.

3. Results

It was investigated whether fertilization with human urine affects the growth pattern of the tested bamboo species. Further aspects presented in this section included: (i) cumulative monthly number of shoots (which enabled the analysis about the yearly shoots plant yield), (ii) comparison between D_{max}

⁶ A strong unpleasant stripping smell from urine is an indication of NH₃ formation and vaporization and consequently a sign of loss of N. Hydrolysis processes in urine are causing-agents of this. By using fresh urine hydrolysis is considerably lessened and consequently N losses are minimized, thus handling is without smell.

of each group of reactors in y1 and y2, (iii) mean yearly shoots production per group, and (iv) the yielded biomass weight in each group of reactors for y1 and y2.

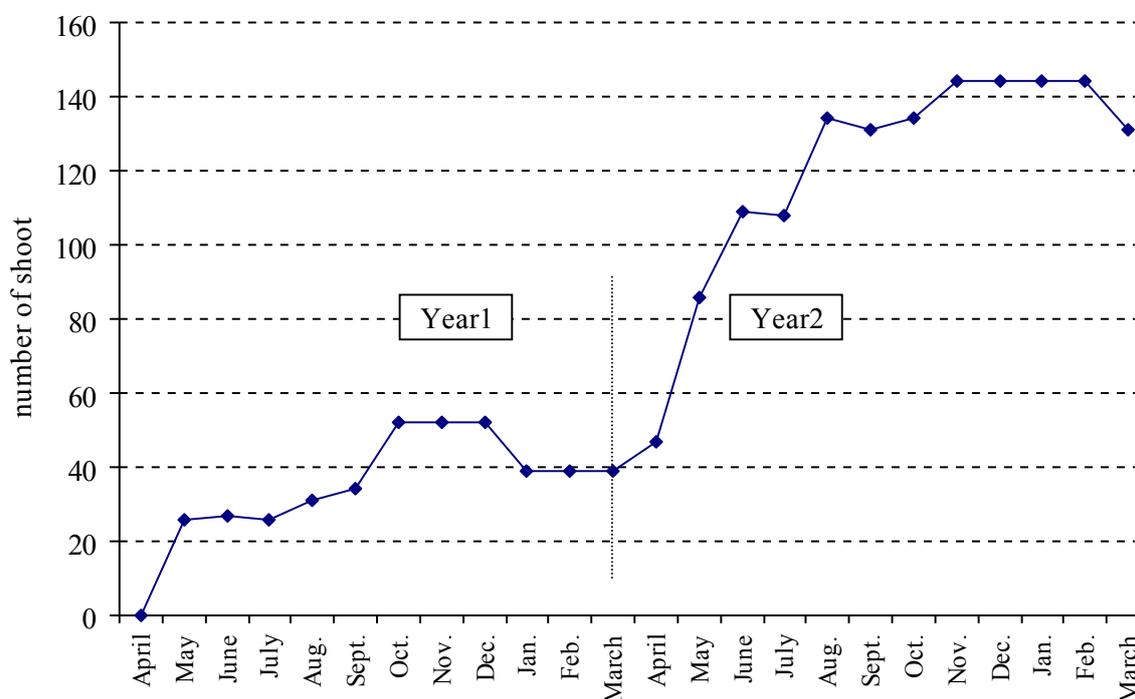


Figure 1 Cumulative monthly number of shoots

3.1. Does urine fertilization influence the growth pattern of *P. viridiglaucescens* (growing season and resting period)?

This question arisen because in y1, -just two months following plant setting-, first shoots were observed only in fertilized groups of reactors and not in unfertilized (control group; fed with TW). It is in the growing season in y2 that shoots also emerged from unfertilized group. The issue was therefore to understand whether urine-based HCFR influence the growth behaviour alternated by growing season and resting period. At primary⁷ approach, visual observation of plants throughout experiment duration provides answers here with respect of growing and resting period.

Bamboo plants were embedded in the reactors in March 2008; between end of winter time and spring. This corresponds roughly to the beginning of the vegetation period in Hamburg (growing season). In y1 (2008), first shoots emerged 2 months later in May (Figure 1) and shooting period occurred until October, when plant growth starts to cease, as the air temperature is going down and consequently cooling soil. A number of culms died during the winter (vegetation resting period). The second

⁷ At secondary approach, ongoing further investigations are looking at deeper levels whether HCFR with urine-based substrate influence intrinsic growth morphologically (culm growth, height, diameter) and anatomically (nutrient content in bamboo components tissue) of *P. viridiglaucescens* –and thus bamboo plants–.

shooting period in y2 started in April 2009 (Figure 1), together with the vegetation period. Shooting in y2 went on until November 2009, simultaneously with cease of vegetation growth.

3.2. Cumulative monthly number of shoots and yearly plant yield

Figure 1 presents the cumulative shoots production of the plant month per month after application of substrate. In y1, the shoots production increased from 26 (May 2008) at the beginning of the shooting period to 52 (Oct. 2008) and stabilized at that value until Dec. 2008 before decreasing and stabilizing at 39 between Jan. and March 2009. In y2, the number of shoots increased strongly between April and Nov. 2009 from 39 to 144 and stabilized at that value up to Feb. 2010, before slightly decreasing to 131 at the end of experiment. Therefore the yearly shoots plant yield increased from 39 (y1) to 131 (y2) as shown on Figure 2. This makes an increase of 236% in productivity from y1 to y2.

3.3. Mean yearly shoots production per group

When looking separately at groups of reactors in relation to FR (Figure 3), in y1, the number of shoots produced varies from 0 at 0 NLR (only tap water) to 6 at 600 NLR, irrespectively to FR. This makes an average production per group of 4 shoots in y1. In turn, in y2 a trend of linear increase in productivity is noticeable from 5 at 0 NLR to 25 at 1050 NLR, making therefore an average production of 13 shoots per group in y2. Furthermore in both years of experiments, the minimum yearly number of shoots produced is found at 0 NLR; respectively (resp.) 0 and 5. Looking for relationship between shoots production per group and FR in y2 enabled to establish linear regression coefficient of 0.88 (cf. linear regression equation and coefficient on Figure 3).

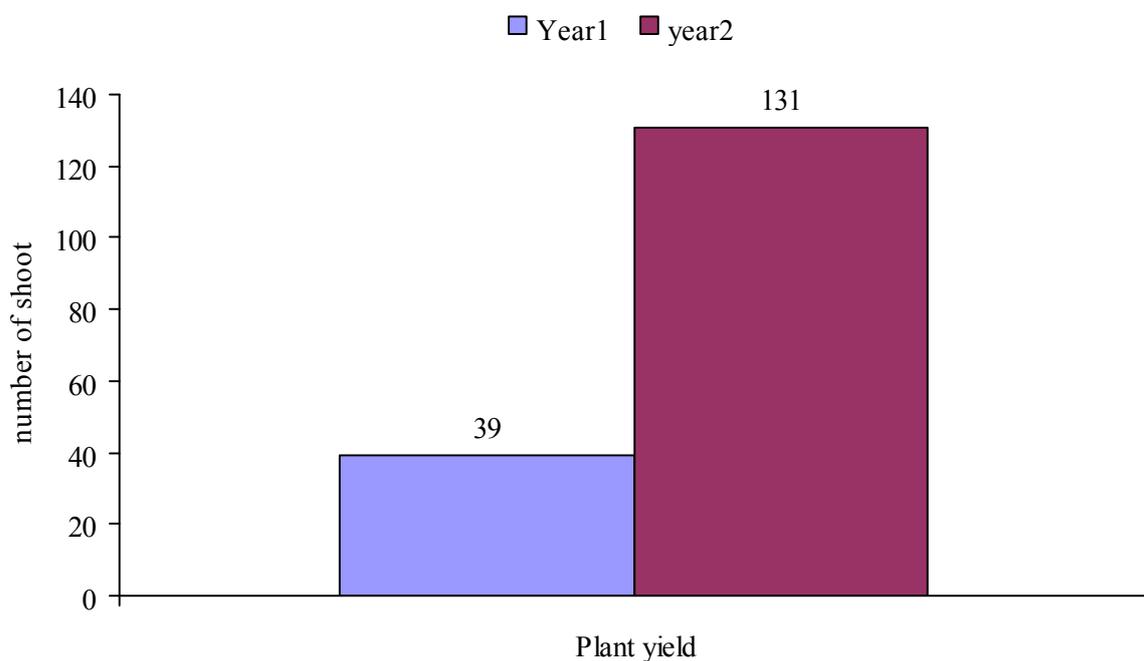


Figure 2 Yearly shoots plant yield

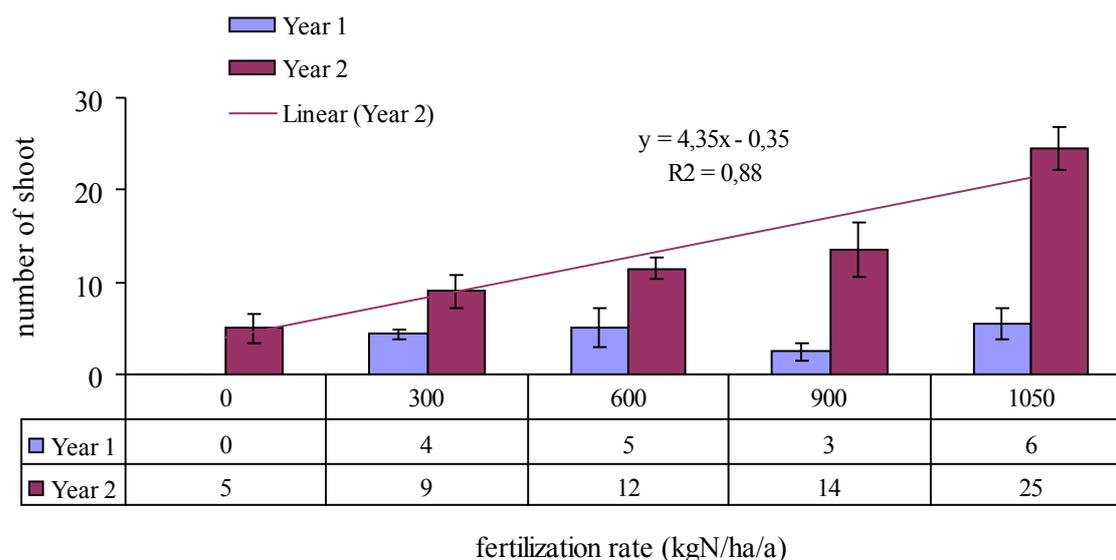


Figure 3 Mean yearly shoots production per group

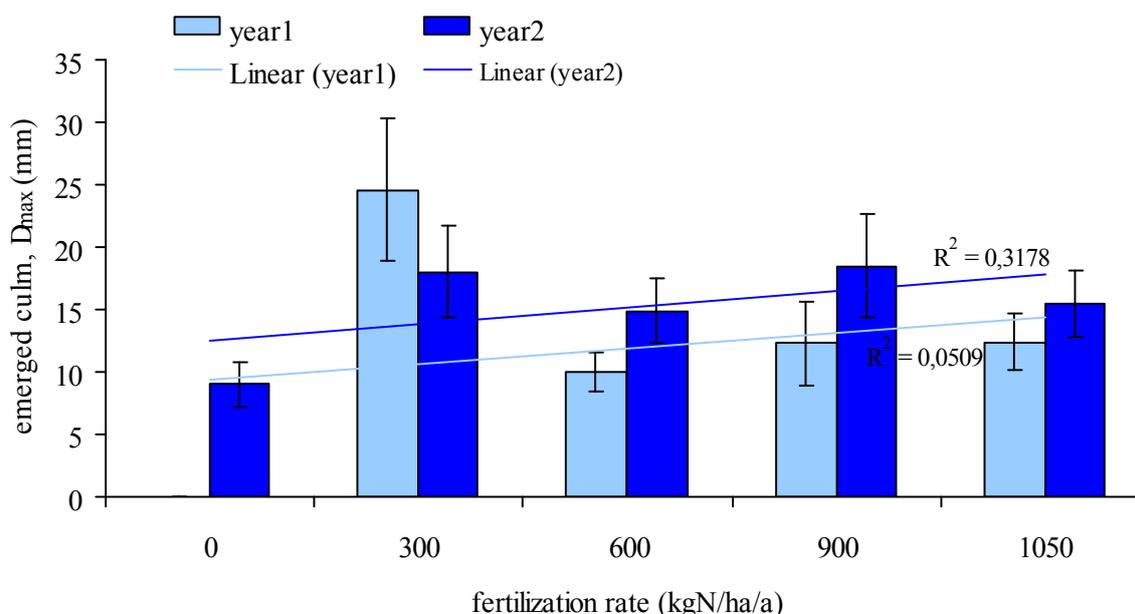


Figure 4 Comparison of maximum diameter (D_{max}) between emerged culms in year1 and year2

3.4. Comparing maximum emerged culm diameter between year1 and year2

The comparison between y1 and y2 of emerged shoots max diameter (D_{max}) was performed with respect of FR (cf. Figure 4); in y1, D_{max} varies from 0 at 0 NLR to 24.6 mm at 300 NLR, whereas in y2 min D_{max} is 9.0 mm at 0 NLR and max D_{max} is 18.5 mm. Both series of measured data vary independently on FR. No correlation could be established between D_{max} per group for both years (cf. linear regression coefficients near to 0 on Figure 4). Same as for mean yearly shoots production per group the min D_{max} in both years is found at 0 NLR (only tap water). The overall max D_{max} of plant was found in y1; 24.6 mm at 300 NLR. Nevertheless, within each FR a trend of greater D_{max} is found in y2 than y1 and the min D_{max} are found at 0 NLR (only tap water). An average D_{max} of 11.9 mm and 15.2 mm was calculated resp. for y1 and y2. This makes an increase of 27% in culm diameter from y1

to y2.

3.5. Biomass by weight

The yielded biomass by weight in relation to FR is shown on Figure 5. In y1, it varies from 0 at 0 NLR to 16.50 tons dry matter per hectare (tDM/ha) at 300 NLR, whereas in y2 from 3.26 at 0 NLR to 25.83 tDM/ha at 1050 NLR. A trend of linearity is observed only for y2; the same linear coefficient as for yearly number of shoots produced per group in y2 was found for biomass by weight (cf. Figure 3 for number of shoots and Figure 5 for biomass weight). The average biomass weight was resp. 5.2 and 13.2 tDM/ha, which makes an increase in productivity of 154% from y1 to y2.

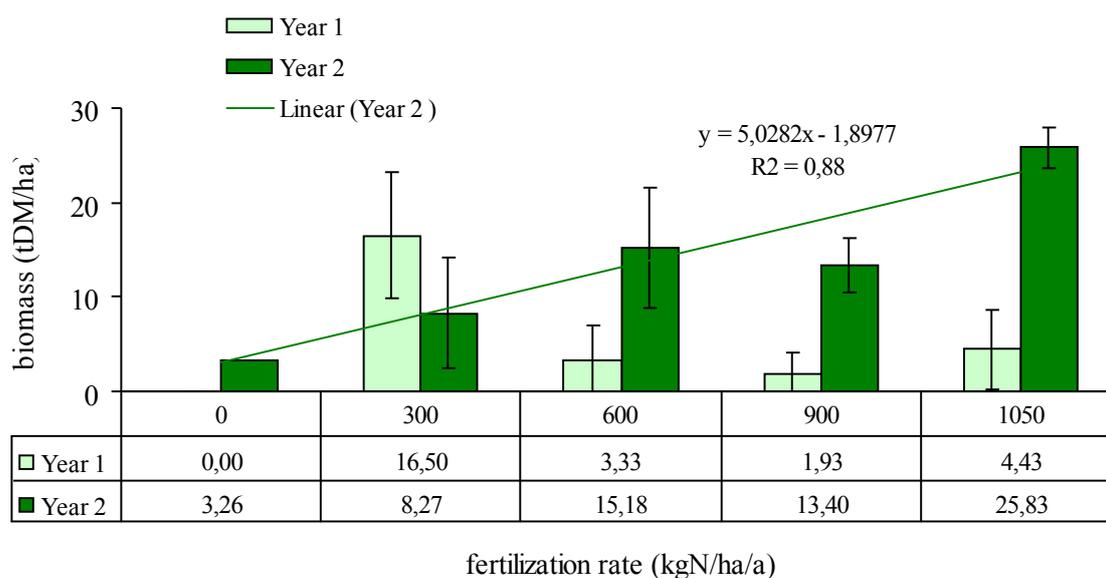


Figure 5 Yearly biomass by weight per group of reactors

4. Discussion

4.1. Urine-based HCFR and growth pattern of *P. viridiglaucescens*

By shooting just 2 months after embedment in the year of planting, *P. viridiglaucescens* shows rapid rooting in the reactors. It was observed that shooting seasons of both years 1 and 2 occurred together with the natural vegetation period⁸ in Hamburg. Also resting periods of bamboo growth corresponded to vegetation ceasing periods. Indications of this was the simultaneous of both occurrence and cease resp. for shooting and weed in each reactor. The shooting season in y2 was one month longer than the previous one; according to recorded data on data logger, Nov. 2009 was warmer than Nov. 2008; the average monthly temperature for resp. period was 9.3 and 7.0°C. This probably prolonged the vegetation period in general, as thus of bamboo growth so that shooting continued until November 2009 (Figure 1). Weed growth in each reactor was an indication of soil fertility; soil promoted plant growth although highly loaded from nutrient in the substrate. Therefore, HCFR did not impact on the growing pattern of *P. viridiglaucescens*; the shooting season and resting period fitted to natural vegetation periods of plants. The effect of urine nutrient is consequently responsible of early shooting observed in only fertilized groups of reactors in y1 (the point is discussed just below in 4.2).

4.2. Effects of HCFR on biomass by counting and measuring

In y1 shooting was observed only in fertilized groups of reactors (no shoot emerged in group fed only with TW). This suggests that nutrient contained in substrate fed was the driving force for shooting. Without nutrient from FR, growth is weakened and in this particular case inexistent (shoots and consequently biomass by weight at 0 NLR). When looking inside fertilized groups that produced shoots in y1, the rate of mortality (13 shoots representing 25% losses) is max; the normal losses in bamboo plantations reported in (Mohan 1997) are between 5.5 and 25.5%. The average shoots production per group of 4 is within the limit reported in (Liese 1985): up to 10 shoots. These effects observed in y1 are most probably because plant operation started just after bamboo plants were embedded in the reactors; the plant did not established first before feeding commences. Thus, a non-linear positive response can be stated; though HCFR provoked emergence of shoots in y1, but not with respect of FR and max mortality was observed. In y2 the hypothesis of linear positive response is to admit; increase of nutrient through HCFR resulted to consequently number of shoots. Mortality strongly decreased at 9%. The average shoots production is beyond the limit of 10 shoots; 13 shoots, which confirms the positive linear response of *P. viridiglaucescens* according to FR. The title of the paper is therefore justified. Concerning emerged shoots max diameter, in general the FR did not provoke a linear increase in diameter for both years in each group of reactors, although a trend of increase can be stated between y1 and y2; increase of 27% in max diameter from an average of resp. 11.9 mm to 15.2 mm. The trend of increase in diameter is in line with the well-known statement that; with plantation age, the emerged shoots increased in diameter from year to year until plantation maturity is attained. With respect to this point, a still opened issue would be the research towards fertilizing effect on growth acceleration towards D_{max} attainment of a given species.

⁸ The natural vegetation period spans from March to September/October depending on soil temperature as a function of ecological conditions (mainly air temperature and moisture content).

4.3. Effects of HCFR on biomass by weight

The biomass by weight followed the trend described in 0 relating shoots production. Consequently to no shoot at 0 NLR in y1, also no biomass at 0 NLR could be weighted in y1. In y1 the biomass by weight follows the non-linear positive response hypothesis evoked in 0. In y2 the biomass by weight correlates with HCFR applied to plants, because plants were established already. The same linear regression coefficient for shooting ($R^2 = 0.88$) was found for biomass yield by weight in y2. Therefore, biomass by weight and number of shoots production correlated in y2. Both biomass by weight in y1 and y2 are within the ranges of biomass production; a max value of 150 t/ha is reported in (Midmore 2009), most probably with a species grown in native region. It can therefore be stated that the HCFR did affect positively biomass by weight.

5. Conclusion

HCFR did not influence the growth pattern of *P. viridiglaucescens*; the growing season and resting period fit to the vegetation period. Nutrient content of urine impacts positively on biomass yield: without substrate (in fact its urine nutrient content) *P. viridiglaucescens* grows poorly and results consequently in poor biomass production. In turn the growth is boosted with nutrient from the substrate; each fertilized group produced superior biomass than unfertilized group and within fertilized groups, the yield grew increasingly according to FR for both number of shoots and biomass by weight. Correlation between these two latter parameters was found in y2 only. This is most probably due to early application of HCFR immediately after embedding plants in the reactors. In the second year plants were already established in reactors. This suggests starting with fertilization of bamboo plantations after onsite establishment. In turn, D_{max} remained independently on HCFR, although an increase on average in diameter from y1 to y2 was calculated. As overall conclusion; at a time where the world watches its resources progressively decreasing for instance nutrient for agriculture (especially P), urine is an infinite alternative at hand of every human being and community for supplying agriculture with nutrient. Applying it at high continuous rates on *P. viridiglaucescens* resulted in increase of productivity by shooting and weight. The title of this paper is therefore justified for *P. viridiglaucescens* and thus, for bamboo plantations.

References

- Aguilar, M.J. 2011. Fertilizer NPK from human urine and olive oil mill wastewaters. Journal of Water Reuse and Desalination. Vol 1 No 3 pp 152–159. IWA Publishing 2011. Abstract read on the Internet at www.iwaponline.com on 05.12.2011
- An, Q.-N.; Wang, J.-P.; Zhang, X.-M.; Du, W.-Y.; Wu, M.-H.; Hu, X.-Q. 1995. Study on fertilization in *Phyllostachys nidularia* forest. *J. Bamboo. Res.* 14, 73-80
- Behrendt, J.; Arevalo, E.; Gulyas, H.; Niederste-Hollenberg, J.; Niemiec, A.; Zhou, J.; Otterpohl, R. 2002. Production of value added products from separately collected urine. *Water Science & Technology* Vol 46 No 6-7 pp 341–346. IWA Publishing 2002
- Beler-Baykal, B.; Allar, A.D.; Bayram, S. 2011. Nitrogen recovery from source-separated human urine using clinoptilolite and preliminary results of its use as fertilizer. *Water Science and Technology* 63.4. 2011
- Calvert, P.; Morgan, P.; Rosemarin, A.; Sawyer, R.; Xiao, J.; Winblad, U.; Simpson-Hébert, M. 2004. Ecological sanitation revised and enlarged edition. Stockholm Environment Institute, 2004 Stockholm, Sweden
- Excreta and greywater use in agriculture. The World health Organization. WHO Library Cataloguing-in-Publication Data, France

- Gulyas, H.; Bruhn, P.; Furmanska, M.; Hartrampf, K.; Kot, K.; Lüttenberg, B.; Mahmood, Z.; Stelmaszewska, K.; Otterpohl, R. 2004. Freeze concentration for enrichment of nutrients in yellow water from no-mix toilets. *Water Science and Technology* Vol. 50 N° 6 pp 61-68. IWA Publishing 2004
- Harada, H.; Shimizu, Y.; Miyagoshi, Y.; Matsui, S.; Matsuda, T.; Nagasaka, T. 2006. Predicting struvite formation for phosphorus recovery from human urine using an equilibrium model. *Water Science & Technology* Vol 54 No 8 pp 247–255. IWA Publishing 2006
- Heinonen-Tanski, H.; Sjöblom, A.; Fabritius, H.; Karinen, P. 2007. Pure human urine is a good fertiliser for cucumbers. *Bioresource Technology* 98 (2007) 214–217
- Kärrman, E.; Jönsson, H.; Gruberger, C.; Dalemo, M.; Sonesson, U. 1999. Management of wastewater and organic waste – systems analysis. In the Proceedings of Managing the Wastewater Resource, 4th International Conference for Ecological engineering for Wastewater Treatment, As, Norway, 1999 June 7-11
- Kirchmann, H.; Pettersson, S. 1995. Human urine - Chemical composition and fertilizer use efficiency. *Fertilizer Research* 40: 149-154, 1995. Kluwer Academic Publishers. The Netherlands
- Kleinhenz, V.; Midmore, D.J. 2001. Aspects of Bamboo Agronomy. *Advances in Agronomy*, Volume 74. 2001 by Academic Press. 0065-2113/01. Queensland, Australia
- Li, R.; Werger, M. J. A.; During, H. J.; Zhong, Z.-C. 1998. Carbon and nutrient dynamics in relation to growth rhythm in the giant bamboo *Phyllostachys pubescens*. *Plant Soil* 201, 113-123
- Liese, W. 1985. Bamboos – biology, silvics, properties, utilization. *GTZ Publications* N° 180. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), 1985 Eschborn, Germany
- Midmore, D.J. 2009. Bamboo in the global and Australian contexts. In the Proceedings of the Workshop Silvicultural management of bamboo in the Philippines and Australia for shoots and timber. Los Banos, the Philippines, 2006, Nov.22-23. Australian Centre for international Agricultural Research (ACIAR), Australia
- Mnkeni, P.N.S.; Kutu, F.R.; Pardon, M.; Austin, L.M. 2007. Evaluation of Human urine as source of nutrients for selected vegetables and maize under tunnel house conditions in the Eastern Cape, South Africa – *Waste Management and Research* 2008(26), 132-139
- Mohan, C. 1997. Diseases of bamboos in Asia. Technical Report. International Network for Bamboo and Rattan (INBAR). Beijing, China
- Ndzana, J.E.; Otterpohl, R. 2008. Innovatives Regenwasser Management mit Bambusplantagen für europäische Wohngebiete. In the Proceedings of the „20. Norddeutsche Tagung für Abwasserwirtschaft und Gewässerentwicklung“. Lübeck, 2008 May 21 - 22, pp. 169-175
- Ndzana, J.E.; Otterpohl, R. 2009. Urine Reuse as Fertilizer for Bamboo Plantations. Peer Reviewed Paper. In the Proceedings of the International Conference on Nutrient Recovery from Wastewater Streams. Vancouver, 2009 Mai 10 - 13, pp. 687 - 696. IWA Publishing 2009
- Niederste-Hollenberg, J. 2003. Nährstoffrückgewinnung aus kommunalem Abwasser durch Teilstromerfassung und –behandlung in urbanen Gebieten. Dissertation, Technische Universität Hamburg-Harburg, Publikationen von Hamburger Berichte zur Siedlungswasserwirtschaft 44. 2003, Hamburg, Deutschland
- Oshima, J. 1931. The culture of Moso bamboo in Japan, Part I: The plant, its uses, and how to start a Moso plantation. *Journal of American Bamboo Society* 3, 3-32
- Pradhan, S.K. 2010. Yield and quality of vegetables fertilized with human urine and wood ash. PhD Thesis. University of Eastern Finland. Publications of the University of Eastern Finland. Finland, 2010
- WHO, 2006. Guidelines for the safe use of wastewater, excreta and greywater. Volume 4

Widmer, Y. 1998. Soil characteristics and *Chusquea* bamboos in the *Quercus* forests of the Cordillera de Talamanca, Costa Rica . Bull. Geobot. Inst. Eth. 64, 3-14

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Biogenic volatile organic compound emissions from bamboo: Exploring patterns of diversity across species

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Abstract

Emissions of biogenic volatile organic compounds (BVOCs) from plant leaves play significant roles in biological and atmospheric processes. BVOCs emissions can involve a diverse number of compounds and are an important method of plant signaling. However, some emissions of volatile compounds can negatively impact air quality at a regional scale. In order to better understand the role of BVOCs in plant physiology and chemical ecology, and to better predict how these emissions may alter air quality, the underlying relationships between these diverse compounds must be addressed. Comparisons between biogenic emissions from plants have been difficult in the past, as surveys have tended to focus on a limited number of compounds, and analytical techniques have lacked the ability to detect or separate compounds within functional groups. Additionally, closely related species typically emit similar compounds, making the reasons that plants emit certain BVOCs over others difficult to elucidate.

We have identified the bamboos as a novel system for studying BVOC emission because they emit a diverse range of compounds and emission of isoprene, a well-conserved compound, is widely variable across species. Between 75-196 individual compounds were identified using two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC TOF-MS) from 12 species of bamboo, and one other species of grass. BVOCs emissions were analyzed by comparing patterns of compound class emission after assigning each compound to class based on its functional groups. Using non-metric multidimensional data scaling, we mapped the relationship between bamboo plants based on their compound class composition. We found significant differences in overall compound class composition between species that do and do not emit isoprene, suggesting there is a relationship between isoprene emission and the pattern of BVOC emissions observed in the bamboos. Overall, BVOC composition varies significantly amongst species of bamboo and may differentially impact chemical ecology and atmospheric chemistry.

Keywords

Biogenic volatile organic compounds, bamboo, isoprene, GCxGC TOF-MS, chemical ecology

Abbreviations

Biogenic volatile organic compounds (BVOCs)

Green leaf volatiles (GLVs),

International Union of Pure and Applied Chemists (IUPAC)

Non-metric multidimensional scaling (NMDS)

Photosynthetically active radiation (PAR)

Secondary organic aerosol (SOA)

Solid phase microextraction (SPME)

Two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC TOF-MS)

Volatile organic compound (VOC)

Introduction

Carbon emissions from plants, known as biogenic volatile organic compounds (BVOCs), are a significant source of atmospheric carbon. BVOCs comprise 95% of the total global volatile organic compound (VOC) emissions (Loreto et al 2008). Biogenic sources of carbon can be emitted in a wide range of structural forms with various degrees of volatility and reactivity. Common plant-based biogenics include terpenes, alkenes, alkanes, alcohols, ethers, esters, and acids (Kesselmeier and Staudt 1999). Depending on their chemical structure, BVOCs may be long-lived or very reactive in the atmosphere.

Because of the magnitude and diversity of BVOCs generated in plant tissues, the release of these compounds can have significant impacts atmospheric chemistry. Ozone generation, secondary organic aerosol formation, and extended lifetimes of other pollutants can all occur as a result of BVOC emission (Arneth et al 2008; Papiez et al 2009). Tropospheric ozone levels can increase as a result of BVOC oxidation in the presence of nitrogen oxides. Ozone is known to have negative effects on human health, and causes damage to lung tissues in humans and animals and leaf tissues in plants (United States Environmental Protection Agency). Secondary organic aerosols (SOA), a form of particulate matter that influences regional visibility and temperatures, can be formed if larger BVOC molecules aggregate (Papiez et al 2009). Atmospheric peroxides break down BVOCs, and depletions of peroxides in the presence of large amounts of BVOCs may extend the lifetime of other greenhouse gases in the atmosphere (Arneth et al 2008).

Isoprene (2-methyl 1,3-butadiene, C_5H_8), a reactive molecule composed solely of carbon and hydrogen, is one of the most significant BVOCs. Isoprene is the most abundant non-methane BVOC, and 711 Tg y^{-1} are emitted from vegetation spanning a wide range of plant groups (Harley et al 1999; Ashworth et al 2010). The bond structure of isoprene makes it very reactive in the troposphere, where isoprene is quickly oxidized by peroxide radicals, leads to significant increases in tropospheric ozone, and ultimately broken down to CO_2 and water (Sharkey et al 2008; Ashworth et al 2010). Because isoprene is widespread, reactive, and can create by-products which are detrimental to human health, its emission has been well characterized in a number of model plant systems often associated with large-scale monocultures, including poplar, oak, and eucalyptus. Though emissions of isoprene are found in plant groups that are phylogenetically dispersed throughout the plant kingdom, it is typically well-constrained within a given plant group, making comparisons between the physiology of isoprene emitting and non-emitting plants difficult to obtain (Harley et al 1999; Sharkey et al 2008). Functionally, isoprene has been shown to increase with light and temperature, and has been hypothesized to help plants combat temperature and ozone stress, though the question as to why plants make isoprene is still remains unanswered (Fortunati et al 2008; Sharkey 2009).

The range of BVOCs emitted by plants extends beyond isoprene to structurally and functionally diverse categories of compounds that can play important roles in chemical ecology, plant-insect and plant-plant communication. When wounded, many plants emit green leaf volatiles (GLVs), some of which are responsible for the characteristic “fresh cut grass” smell of leaves. GLVs include a variety of oxygenated C_6 through C_8 compounds like aldehydes and alcohols. The presence of GLV emission is associated with physical damage to the lipid membranes of leaves as a result of stress or in response to herbivory (Holopainen 2004).

Compounds in the terpenoid family, of which isoprene makes up a single unit, are widely emitted by plants and are important signaling molecules. Monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$), are

fragrant compounds can exist in a number of structural forms which serve a variety of ecological functions (Kesselmeier and Staudt 1999; Duhl et al 2008). Despite the importance and diversity of BVOCs, measurements of leaf-level emissions are typically constrained to a limited set of compounds due to availability of standards or sensitivity of instrumentation (Duhl 2008; Ortega and Helmig 2008). As a result, studies of emissions are constrained to compounds like isoprene and the potential combined effects of other BVOC compounds and their impacts on ecology or atmospheric chemistry are not considered.

We have identified the bamboos as a novel system for studying BVOC emission because, unlike most isoprene emitting plants, bamboos do not emit isoprene uniformly within their clade. A survey of isoprene emission in 75 species in 25 genera found that basal isoprene emission rates range from 0 – 47 nmol isoprene m⁻² sec⁻¹ (Melnychenko and Rosenstiel, unpublished). This pattern of variation exists across genera and within a genus at the species and cultivar levels.

Because bamboos do not uniformly emit isoprene, we hypothesized that other emissions of BVOCs would vary within the clade as well. The full range of BVOC emissions in 12 bamboo species and one other grass species have been analyzed alongside isoprene emission and are presented here.

Materials and Methods

Bamboo growth conditions

Twelve species of bamboo within six genera of the subtribe Bambusoideae were cultivated at Portland State University in the Research Greenhouse facility. Study species were chosen based on preliminary surveys of isoprene emission in bamboos and selected to represent a range of basal isoprene emission rates found in plants phylogenetically dispersed within the Bambusoideae. One member of Arundinoideae, *Arundo donax* var. ‘Candy cane’, was also included in this study. For some genera we selected multiple representative species within a genus that varied according to leaf characteristics or basal isoprene emission rate (Table 1).

Genus and species	Subfamily	Abbreviation	Relative isoprene emission level	Basal isoprene emission rate (nmol isoprene m ⁻² sec ⁻¹)	Leaf color	Habit
<i>Arundo donax</i>	Arundinoideae	AdV	High	8.524	Variegated	Clumping
<i>Arundinaria gigantea</i>	Bambusoideae	AG	None	0.542	Green	Running
<i>Bambusa ventricosa</i>	Bambusoideae	Bve	High	6.244	Green	Clumping
<i>Bambusa ventricosa</i> ‘Kimmei’	Bambusoideae	BveV	High	5.724	Variegated	Clumping
<i>Fargesia rufa</i>	Bambusoideae	Fr	None	0.410	Green	Clumping
<i>Phyllostachys aurea</i>	Bambusoideae	Pa	High	7.477	Green	Running
<i>Phyllostachys edulis</i>	Bambusoideae	Pe	None	0.864	Green	Running
<i>Phyllostachys nigra</i>	Bambusoideae	Pn	High	10.443	Green	Running
<i>Pleioblastus chino</i>	Bambusoideae	PLc	None	0.294	Green	Running
<i>Pleioblastus chino</i> ‘Murakamianus’	Bambusoideae	PLcmV	None	0.876	Variegated	Running
<i>Pleioblastus chino</i> ‘Vaginatus Variegatus’	Bambusoideae	PLcV	None	0.386	Variegated	Running
<i>Sasa kurilensis</i>	Bambusoideae	Sk	None	0.418	Green	Running
<i>Sasa kurilensis</i> ‘Simofuri’	Bambusoideae	SkV	High	2.136	Variegated	Running

Table 1. Grass species used in this study for comparison of BVOC emissions. ‘Habit’ refers to the vegetative method of rhizome growth.

A minimum of five plants per species were supplied by Bamboo Garden Nursery in North Plains, OR, and were transplanted into 10-15 gallon pots upon arrival to the facilities at Portland State University. Plants were grown at 22° C during the day, and 15°C at night for 8 months prior to this experiment. High-intensity discharge lamps were used from 6 am to 10 pm daily, and provided an average of 250 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$ of photosynthetically active radiation (PAR) to the bamboo plants. Plants were watered every other day and fertilized with an organic nitrogen, phosphorus and potassium supplement once every three weeks.

Isoprene flux measurements

Measurements of *in situ* isoprene flux were made during August of 2011 from intact, attached leaves on greenhouse plants that were brought into the laboratory. A leaf was placed in the light-controlled cuvette (LI-6400, LiCor Inc. Lincoln, NE, USA) and was equilibrated at a flow of 200 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ at 1000 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$ PAR for 10 minutes prior to sampling. Two milliliters of the effluent air stream was sampled from the cuvette using a syringe and then injected into a RGD2 Gas Chromatograph with Reducing Gas Detector. The isoprene peak was identified and quantified using an authentic standard.

BVOC sample collection

Eighty-four leaf samples were collected for BVOC emission profiling during the months of November and December 2010 from four individuals per species. Leaves that were third from the apex of a branch, in good condition and fully exposed to light were selected for this study. Individual leaves were cut at the petiole with an ultra-sharp razor one to three hours prior to sampling for BVOC analysis. Each leaf was placed in a clean 40 ml vial and capped with a new Teflon backed silicon septa. Samples in vials were purged for 4 minutes at a flow of 50ml min^{-1} with lab air passed through a hydrocarbon trap to scrub ambient VOCs.

“Dark” samples (62 leaves from 13 species): After sample vials were purged a clean, conditioned solid phase microextraction (SPME) fiber in an SPME assembly (Sigma-Aldrich, St. Louis, MO, USA) was inserted through the septa and the fiber was exposed to the leaf for 60 minutes. “Light” samples (21 leaves from 7 species): Individual samples were incubated under a cool light source set at 1000 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$ PAR for 20 minutes. At the end of the light incubation period, the SPME fiber was inserted into the vial and exposed to the leaf for 40 minutes. After exposure to each leaf sample, the SPME fiber was inserted into the gas chromatograph injector for 10 minutes.

GCxGC TOF-MS

Two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC TOF-MS) using a 4D Leco Pegasus GCxGC TOF-MS (Leco, St. Joseph, MI, USA). GCxGC TOF-MS allows compounds to be separated and represented in two dimensions; in the primary dimension compounds are separated within a column according to volatility or weight, in the secondary dimension compounds are separated according to their polarity. The TOF-MS detector allows for excellent qualitative identification of compounds based on their unique spectrum of fragment masses. A

summary of GCxGC TOF-MS methodology and conditions used for BVOC analysis is given in Pankow et al (2012). An example of a typical GCxGC TOF-MS chromatogram is shown in Figure 1.

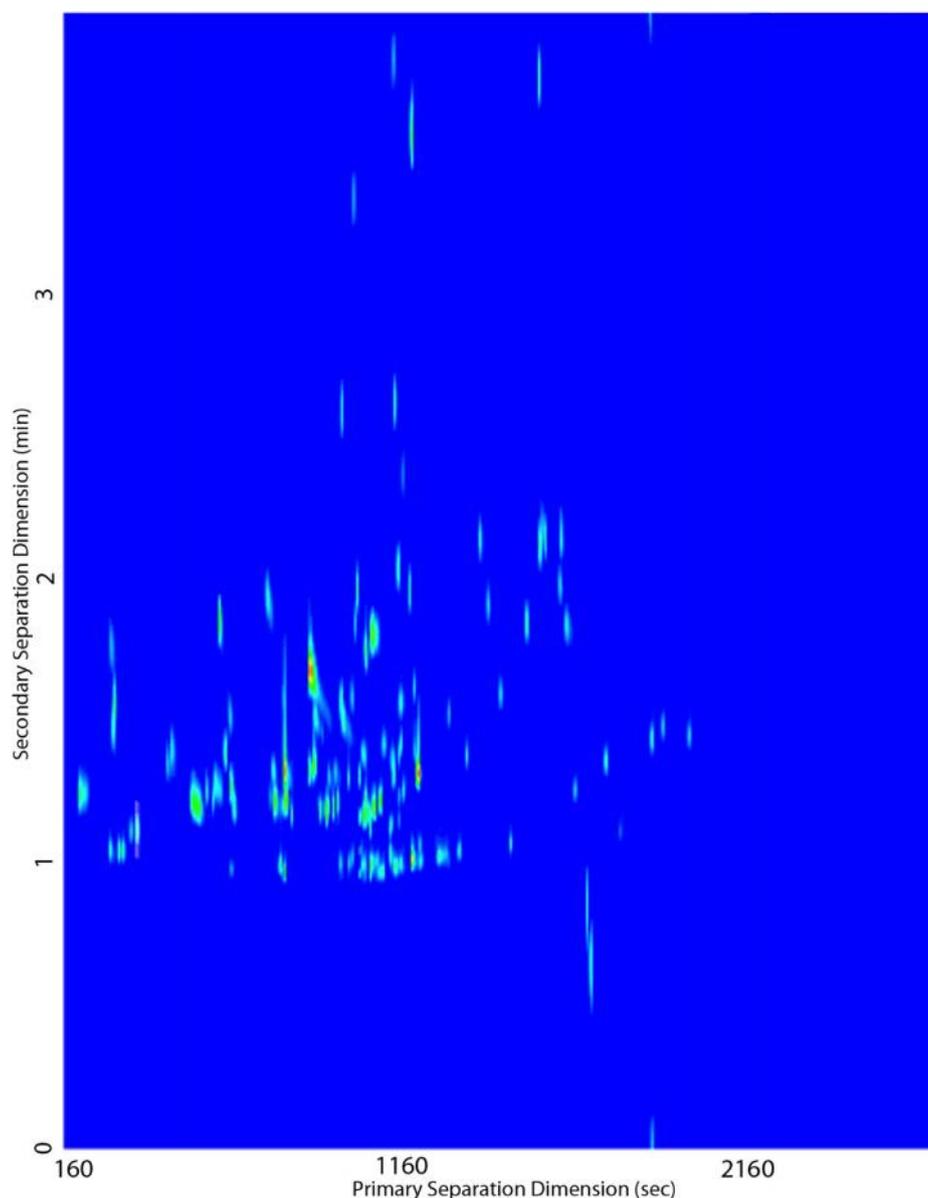


Figure 1. Example of a two-dimensional contour chromatogram generated using a GCxGC TOF-MS system. Data collected from a leaf of *Arundinaria gigantea*, a non-isoprene emitting species of bamboo.

Conditions were set up according to Pankow et al (2012) with minor modifications. The injector was set at 225°C splitless injection, and for 3 minutes Helium carrier gas passed over the fiber and moved the sample into the column at a flow of 1 ml/min. The primary column was a DB-VRX, 45 m, 0.25 mm I.D., 1.4 m film (Agilent, Santa Clara, CA, USA). After samples traveled the GCxGC modulator employed a trap with cold gas from LN₂, followed by a hot pulse at 20°C for release onto the secondary column, composed of Stabilwax, 1.5 m, 0.25 mm I.D., 0.25 m film (Restek, Bellefonte, PA, USA). Each modulation occurred every 4 seconds, with a 0.9 second hot pulse between modulations. The GC oven was set at 45°C for 5 minutes, then stepped at 10°C/min to 175°C and was held at 175°C for 2 minutes, then stepped at 4°C/min to 240°C and was held at 240°C for 10 minutes. Each leaf took

approximately 1 hour to prepare for BVOC sampling, and 1 hour to cycle through the GCxGC TOF-MS.

Analysis of Individual Compounds

Each sample from the GCxGC TOF-MS was analyzed using Pegasus ChromaTOF software that identifies individual peaks in the two-dimensional space, and compares the mass spectra of each peak to a NIST library compound identification system. Peaks with a signal to noise ratio lower than 200 were automatically discarded. Silicon, which is a product of SPME degradation, was deleted from all samples prior to statistical analysis. Compounds found in blanks were deleted if the Peak Area was within two to three times of that found in the blanks. The spectra of each compound was compared to the NIST library match to check for mis-identifications and to combine peaks which labeled twice or exceeded the four second modulation slice. Peak Area was used as a proxy for abundance for each compound. Peak area is based on the magnitude of the peak, however the sensitivity and response of the TOF-MS detector can vary from compound to compound, and therefore Peak Area is considered as relative abundance rather than a quantitative value.

A total of 1076 distinct compounds were emitted in at least one of the 84 samples. Because such a broad range of compounds was emitted, each compound could not be classified using authentic standards, and so NIST library identification of spectra was used. Traditionally, studies of BVOCs focus on the emission of a well-characterized, small suite of compounds (Kesselmeier and Staudt 1999). To retain the diversity and magnitude of BVOCs emitted from the bamboos, the data was compiled into groups of compounds, or compound classes. Each BVOC found in a sample was assigned to a single Compound Class based on the priority assigned to functional groups by the International Union of Pure and Applied Chemists (IUPAC). Nomenclature and the structure of each compound were used to classify compounds into one of 18 Compound Classes (Table 2). The Peak Area of individual compounds were summed according to their compound classes.

Compound Class	Criteria for class	Compound Class	Criteria for class
Acid	“acid” in name	Furan	“furan” in name
Alcohol	-ol suffix	Halide	Contains Cl, Br, F, I
Aldehyde	-al, -yde suffix	Hemiterpene	C ₅ H ₈
Alkane	-ane suffix	Ketone	-one suffix
Alkene	-ene suffix	Monoterpene	C ₁₀ H ₁₆
Alkyne	-yne suffix	Nitros	Contains N
Dioxy.Monoterpene	C ₁₀ H ₁₆ O ₂	Oxy.Monoterpenes	C ₁₀ H ₁₆ O
Ester	“ester, -ate suffix	Sesquiterpenes	C ₁₅ H ₂₄
Ether	-ide suffix	Sulfurs	Contains S

Table 2. Criteria for assigning compound class to each compound. Terpenoids (Hemiterpenes, Monoterpenes, Sesquiterpenes), Halides, Nitros, and Sulfurs were assigned based on formula rather than on IUPAC nomenclature.

Statistical analyses

A Student's t-test was performed to test for any correlation found between the total number of compounds found in each sample to its isoprene emission level (High vs. None). Data was square root transformed to normalize residuals, and all assumptions of equal variance were met. (JMP Statistical Software; SAS institute Inc., Cary, NC, USA).

To analyze the relationships between compound classes within and across samples, multivariate statistical approaches were used. The total Peak Area of each compound class was considered to be a separate response variable for each individual leaf sample. Initially, a correlation matrix was generated to examine the relationships between different compound classes across all samples in the entire dataset. The data was visually examined and then square root transformed to normalize the distribution of the compound classes. The correlation matrix was used to determine whether any two compound classes were correlated with one another within the dataset.

Vegan and MASS libraries were used to run non-metric multidimensional scaling (NMDS) using metaMDS. In our ordination, each leaf was considered a separate sample and analyses were run against the entire compound class composition of the leaf. NMDS plots were created for all combined samples and for the Light treatment and Dark treatment alone. The NMDS algorithm was run 20 times for each ordination with a different starting configuration each time. The final ordination was chosen based on the configuration with the lowest stress value (badness-of-fit). NMDS ordinations were generated and analyzed in two dimensions and did not exceed a stress level of eleven. Analysis of Similarity (ANOSIM) was run on the output from the combined NMDS analysis, and on the Light NMDS and Dark NMDS plots individually. The Null Hypothesis of the ANOSIM assumes no difference between leaves of different species.

All multivariate statistical analyses were performed on compound class data in R statistical software (<http://cran.stat.ucla.edu/>).

Results

No significant difference was found between the number of BVOCs emitted between isoprene emitters and non-isoprene emitters ($p=0.1932$) (Table 3). Isoprene emission presence or absence was coded according to the relative emission rates found Tables 1 and 3.

Genus 'Cultivar'	species	Total number of compounds (average) n=4	Isoprene emission rate (nmol isoprene m ⁻² sec ⁻¹) n=12	Isoprene Emission Level
<i>Phyllostachys</i>	<i>nigra</i>	195	10.443	High
<i>Arundo</i>	<i>donax</i>	141	8.524	High
<i>Phyllostachys</i>	<i>aurea</i>	150	7.477	High
<i>Bambusa</i>	<i>ventricosa</i>	75	6.244	High
<i>Bambusa</i>	<i>ventricosa</i> 'Kimmei'	105	5.724	High
<i>Sasa</i>	<i>kurliensis</i> 'Simofuri'	156	2.136	High
<i>Pleiolblastus</i>	<i>chino</i> 'Murakamianus'	176	0.876	None
<i>Phyllostachys</i>	<i>edulis</i>	154	0.864	None
<i>Arundinaria</i>	<i>gigantea</i>	140	0.542	None
<i>Sasa</i>	<i>kurilensis</i>	117	0.418	None
<i>Fargesia</i>	<i>rufa</i>	123	0.410	None
<i>Pleiolblastus</i>	<i>chino</i> 'Vaginatus Variegatus'	196	0.386	None
<i>Pleiolblastus</i>	<i>chino</i>	169	0.294	None

Table 3. Compound number and isoprene emission rate for each species. Isoprene emission measurements were taken *in situ* from intact leaves attached to greenhouse specimens.

A correlation matrix was used to determine the relationships between each pairwise grouping of compound classes collectively for all samples. Strong positive correlations were found between Alcohols and Alkenes ($R=0.74$), Alcohols and Aldehydes ($R=0.62$), and Monoterpenes and Sesquiterpenes ($R=0.70$). Isoprene was treated as an individual compound class so that the relationship of isoprene to other classes could be determined. No significant correlation between isoprene and any other individual compound class was found.

A NMDS plot was generated for all samples combined, regardless of light treatment (Figure 2). Each point on the plot represents a single leaf sample, and the placement of the point in the ordination is determined by its overall compound class composition, and the relationship of that class composition to each other sample. NMDS plotted for light and dark species separately show similar clustering of isoprene emitting and non-emitting plants.

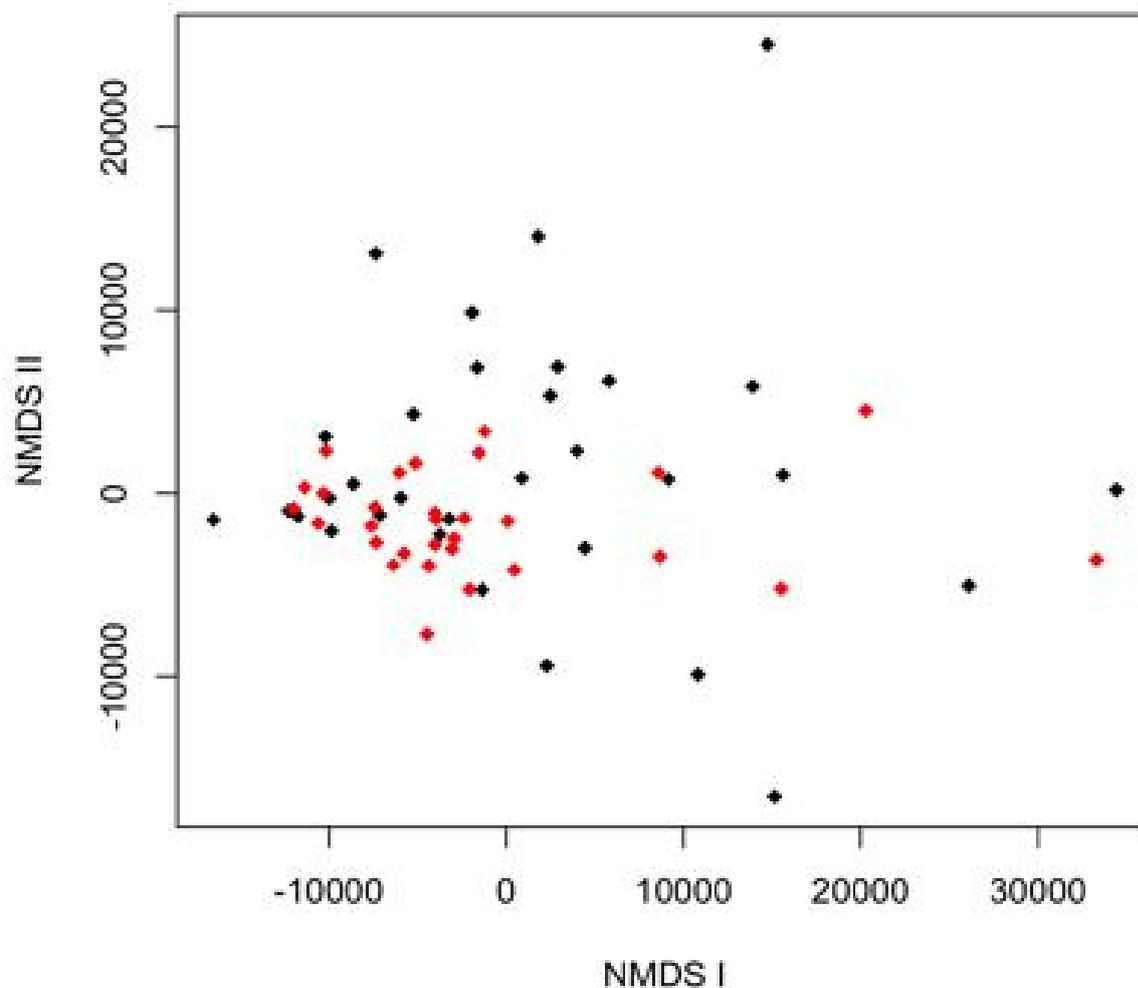


Figure 2. NMDS plot showing all bamboo (Light and Dark treatments). Each point represents a single leaf sample. Points in red indicate samples from isoprene emitting species, points in black from non-isoprene emitting species.

The NMDS plot in Figure 2 was regenerated to show the abundance of sesquiterpenes emitted by each leaf (Figure 3). Each circle represents the same data point from Figure 2, and the size of the circle is directly proportionate to the amount of sesquiterpenes emitted by that sample. Points in the upper right quadrant, identified as non-isoprene emitting plants, show the highest abundance of sesquiterpene emission.

An ANOSIM was generated to explore the difference in compound classes composition between isoprene emitting and non-emitting bamboo (Figure 4). The y-axis represents the distance between data points in the space, and the boxplots approximate the range of variation found within the data. The emissions of biogenic compound classes were significantly different between plants that do and do not make isoprene ($R=0.035$, $P=0.044$). Next, an ANOSIM was run to determine if compound class composition was also different amongst the thirteen species of grass surveyed in this study (Figure 5). The difference was significant ($R=0.173$, $P=0.004$) when all species were considered collectively.

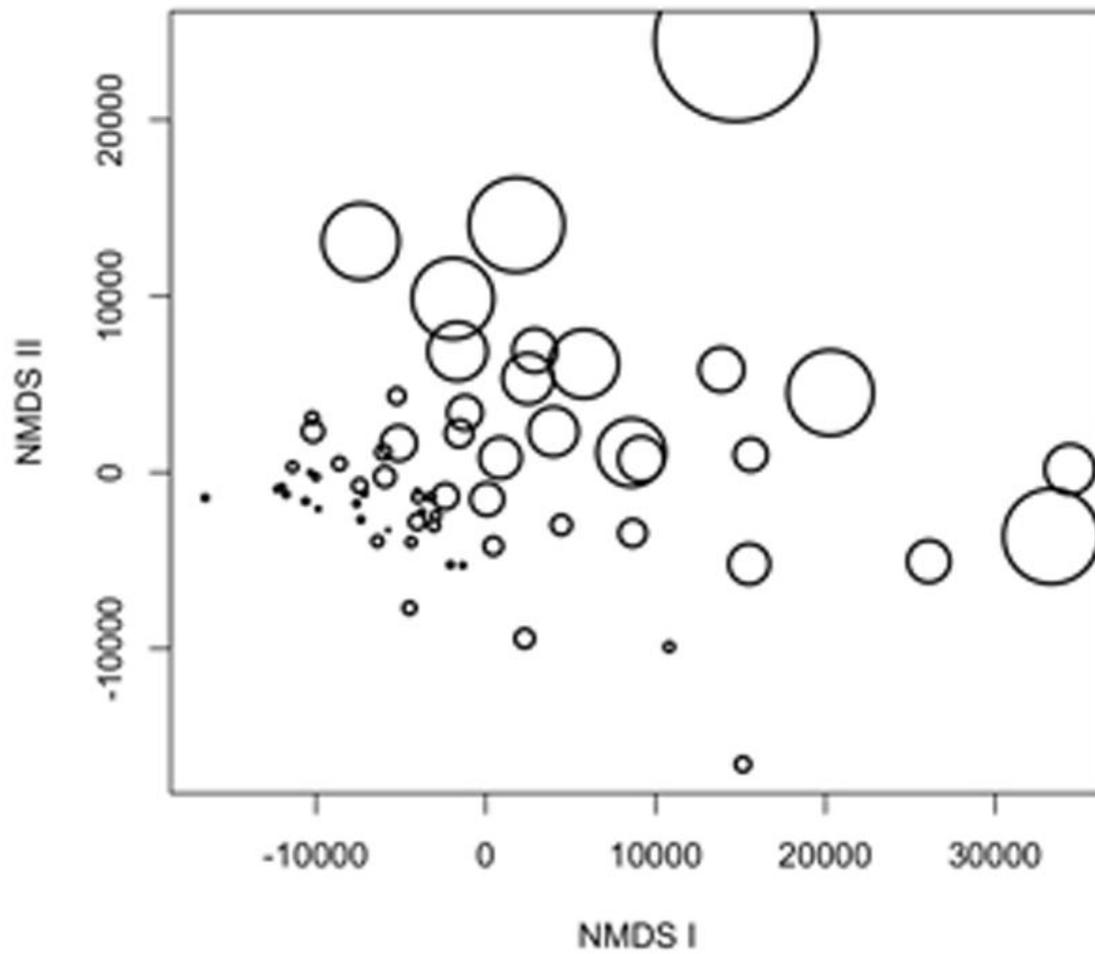


Figure 3. NMDS plot of all samples. Each point represents the same configuration seen in Fig. 2. Circles represent the relative abundance of sesquiterpenes emitted for each leaf sample.

Discussion

Plants emit a range of volatile organic compounds, including isoprene. Generally, isoprene is conserved within plant taxa making analysis of variance and BVOC correlates with this variance difficult (Harley et al 1999; Sharkey et al 2008). Our data show that across bamboo species isoprene emission is variable and that the composition of other volatile organic compounds varies with isoprene emission (Figure 4). The NMDS ordination is created by examining the entire suite of BVOCs emitted across the bamboos in our study. Though no single compound class is strongly correlated with isoprene, an observable pattern of isoprene emitters vs. non-emitters exists within the ordination space. This indicates that there is an underlying difference in the suite of BVOCs emitted from bamboos that do or do not make isoprene.

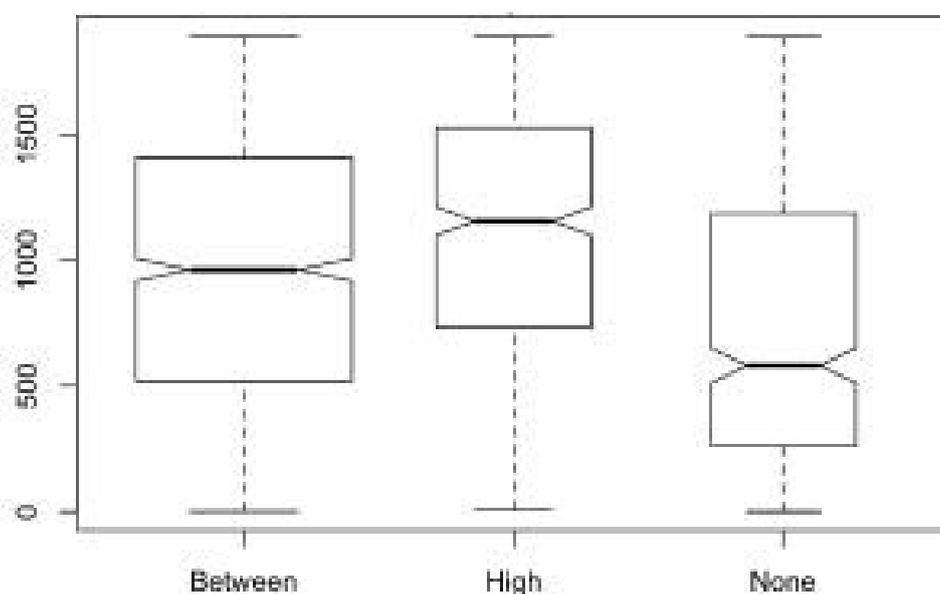


Figure 4. ANOSIM boxplot of differences in compound class composition between high and non-isoprene emitting species. “Between” shows entire range of dataset, “None” refers to non-isoprene emitting plants. N=24 “High”, N=28 “None”. R=0.035, P=0.044

Variations in other groups of compounds are also responsible for the patterns observed in the ordination, though the ecological relationships between these compound classes are still poorly understood (Figure 3). These results are interesting because, while isoprene is by far the most abundant BVOC emitted globally, it is not considered to play a distinct role with regard to interactions in chemical ecology. Compounds such as sesquiterpenes (Kesselmeier and Staudt 1999; Duhl 2008) play crucial roles in signaling between plants, and towards pollinators and insect predators. Our results suggest that isoprene emission may come at the cost of more ecologically relevant compounds, such as sesquiterpenes (Figure 3). Plants that emitted little to no isoprene showed the highest amounts of sesquiterpenes present within their total compound class composition. These results suggest the presence of a tradeoff between leaf isoprene emission, which is often associated with heat stress, and leaf sesquiterpene emission, which is known to aide in plant defense systems (Kesselmeier and Staudt 1999). The ecological or physiological implications of this shifting pattern of BVOC emissions within the Bamboos are currently unknown.

In addition to variations in BVOCs observed across our bamboos, within a given genus the overall emission of BVOCs may vary significantly (Figure 4). Differences were observed between species within the genera *Phyllostachys* and *Pleioblastus*, indicating that a purely phylogenetic approach to understanding BVOC emissions may not be appropriate in the bamboos.

We have identified the bamboos as a novel system for understanding the complex inter-relationships that may exist between the BVOCs emitted from plant leaves. Future studies will target the interactions between isoprene emissions and specific compounds, e.g sesquiterpenes. The quantity of carbon emitted by bamboos may be equally variable in addition to the compounds themselves, and while relative abundances between compound classes were variable, quantitative experiments need to be performed to elucidate these differences. We have shown here that the composition of BVOCs released in closely related bamboo species varies dramatically, providing the first glimpse of the remarkable diversity of BVOC emissions within the bamboos.

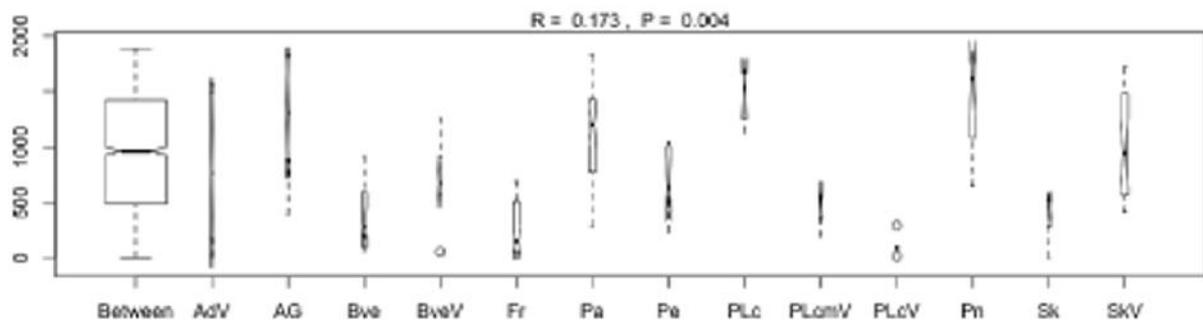


Figure 5. ANOSIM boxplot compound class composition across all species. Species abbreviations are shown. $N=4$, $R= 0.173$, $P=0.004$.

References

- Arneth A.; Monson R.K.; Schurgers G.; Niinemets U.; Palmer P.I. 2008. Why are estimates of global terrestrial isoprene emissions so similar (and why is this not so for monoterpenes)? *Atmospheric Chemistry and Physics* 8: 4605-4620.
- Ashworth K.; Wild O.; Hewitt C.N. 2010. Sensitivity of isoprene emissions estimated using MEGAN to the time resolution of input climate data. *Atmospheric Chemistry and Physics* 10: 1193-1201
- Duhl T.R.; Helmig D.; Guenther A. 2008. Sesquiterpene emissions from vegetation: a review. *Biogeosciences* 5: 761-777
- Environmental Protection Agency. www.epa.gov/air/urbanair/
- Fortunati A.; Barta C.; Brilli F.; Centritto M.; Zimmer I.; Schnitzler J.P.; Loreto F. 2008 Isoprene emission is not temperature-dependent during and after severe drought-stress: a physiological and biochemical analysis. *Plant Journal* 55: 687-697
- Harley P.C.; Monson R.K.; Lerdau M.T. 1999. Ecological and evolutionary aspects of isoprene emission. *Oecologia* 118: 109-123
- Holopainen, J. 2004. Multiple functions of inducible plant volatiles. *Trends in Plant Science* 9: 529-533
- Kesselmeier J.; and Staudt M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *Journal of Atmospheric Chemistry* 33: 23-88
- Loreto F.; Kesselmeier J.; Schnitzler J.P. 2008. Volatile organic compounds in the biosphere-atmosphere system: a preface. *Plant Biology* 10: 2-7.
- Ortega J.; Helmig D. 2008. Approaches for quantifying reactive and low-volatility biogenic organic compound emissions by vegetation enclosure techniques- Part A. *Chemosphere* 72: 343-364
- Pankow J.F.; Luo W.; Melnychenko A.N.; Barsanti K.C.; Isabelle L.M.; Chen C.; Guenther A.B.; Rosenstiel T.N. 2012. *Atmospheric Measurement Techniques* 5: 1-16
- Papiez, M.R.; Potosnak M.J.; Goliff W.S.; Guenther A.B.; Matsunaga S.N.; Stockwell W.R. 2009. The impacts of reactive terpene emissions from plants on air quality in Las Vegas, Nevada. *Atmospheric Environment* 43: 4109-4123
- Sharkey T.D.; Wiberly A.; Donohue A. 2008. Isoprene emission from plants: why and how. *Annals of Botany* 101: 5-18.
- Sharkey, T.D. 2009. The future of isoprene research. *Bulletin of the Georgian National Academy of Sciences* 3: 106-113

Visualisation and identification of endophytic bacterial communities of *Phyllostachys* and *Fargesia*

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Abstract

In the last decade, endophytes were shown to play a fundamental role in the physiology of the plant, and even to provide useful assistance for agricultural and phytoremediative purposes. Knowledge of the endoflora of multifunctional plant species such as bamboo, could in this respect enhance the already wide range of applications and possibilities these plants are being used for. In contrast with the possible benefits, however, almost nothing is known about the presence, let alone the functional activity, of endophytes of bamboo species. In our work we brought together several aspects of the bacterial communities inside bamboo species (more specifically, of several species of the genus *Phyllostachys*). Intact *P. humilis* and *Fargesia rufa* plants were used as a basis for the classic isolation strategy of the bacteria inside, to obtain pure cultures of endophytes. Identification of the obtained strains showed that both species contained *Bacillus* spp. as aboveground endophytes while underground cultivated endophytes of *P. humilis* were much more diverse and belonged to different genera: *Microbacterium* sp., *Leifsonia* sp., *Paenibacillus* spp., *Agrobacterium/Rhizobium* sp.; *Pseudomonas* spp., *Burkholderia* spp. Also, whole DNA was extracted (of plant and bacteria alike) from *P. atrovaginata*, *P. nigra* and *P. humilis* to go for a direct identification of possible bacterial sequences contained within. This analysis revealed the presence of *Microbacterium* spp. in *P. atrovaginata* and *P. nigra*, *P. humilis* and uncultured bacteria. Lastly, we used a modification of the classic technique of Cholodny (1934), who used glass microscopy slides to obtain patterns of bacterial soil communities, forming a micro-detailed landscape, which he later studied with different microscopic techniques. In our modification, plastic film strips made out of polyethylene terephthalate were used as a matrix for the attachment and direct growth of the bacterial communities, maintaining the original (*in situ*) spatial shape and structure of these communities. As such the method is instrumental for a better understanding of the cytoecology of endophytes, i.e. on the nature of the relation between plant cells and their endophytes.

Keywords

endophytes, bamboo, 16S rDNA

Introduction

For decades, scientists have investigated the interactions between micro-organisms and plants. This has led to the discovery of many useful symbiotic relations, such as the nodules on the roots of *Fabaceae* or on the leaves of *Myrsinaceae* and *Rubiaceae*. Overall, the coexistence of plants and their associated microorganisms demonstrates a complex variety of interactions (Compant et al. 2005, 2009; Schulz and Boyle 2006; Moshynets and Kosakivska 2010), from the rather independent colonies of bacteria (such as in the rhizosphere or the phyllosphere) to the very specific interrelations between the host plant and its internal, endophytic bacterial flora. In the latter case, the plant provides a suitable habitat and nutrients, while endophytes directly and indirectly stimulate the growth and development of plants (Mastretta et al. 2009). The main source of a plant's endoflora is the rhizosphere, although some endophytes are being transferred to the next generation through the seeds, and colonise the plant during germination (Weyens et al. 2009).

In the last decade, endophytes were shown to play a fundamental role in the physiology of the plant, and even to provide useful assistance for agricultural and phytoremediative purposes (Newman et al. 2005; Siciliano et al. 2001; Van Aken et al. 2004; Moore et al. 2006). Knowledge of the endoflora of multifunctional plant species such as bamboo, could in this respect enhance the already wide range of applications and possibilities these plants are being used for. In contrast with the possible benefits, however, almost nothing is known about the presence, let alone the functional activity, of endophytes of any bamboo species, with the sole exception of one study in *Phyllostachys edulis* (Han et al. 2009). Of course, one of the main problems in this line of research is the absence of good methodologies to investigate the endophytic bacterial communities. It is established that the functional activity of bacterial endophytes is determined by a variety of architectural properties and cellular interactions within the microbial community (Dworkin 1991; Caldwell et al. 1992; Costerton et al. 1994; Massol-Deya 1995). Classic methodological approaches such as cultivation and metagenomic analysis (analysis of the nucleotide composition of the pool sections 16S rRNA genes) are destructive, and therefore do not provide information about the spatial arrangement of the micro-organisms. Moreover, laboratory cultivation at best produces only a handful of species, and therefore does not permit us to define the size of the population (Ward et al. 1992), nor obtain an adequate view of the morphological, physiological and biochemical variations within the endophytic population (Deretic et al. 1994; Caldwell et al. 1997). Metagenomic analysis can only relate micro-organisms to the nearest related strains which were cultivated before, by using familiar rRNA sequences of known species, that were entered into the database (Ovcharenko and Kozyrovska 2008). In addition, the allocation of the entire pool of DNA has some methodological difficulties due to constraints in sequencing technology (Steffan et al. 1988; Leff et al. 1995). And while each of these methods in itself provides us with pieces of the general puzzle, what is lacking is a method that provides us with the spatial arrangement – say, the picture on the puzzle box, in order to obtain a more coherent view of the bacteria inside the plant.

In this paper, we bring together several aspects of the bacterial communities inside bamboo species (more specifically, of several species of the genus *Phyllostachys*). Intact plants were used as a basis for the classic isolation strategy of the bacteria inside, to obtain pure cultures of endophytes. Also, whole DNA was extracted (of plant and bacteria alike) to go for a direct identification. Lastly, we used a modification of the classic technique of Cholodny (1934), who used glass microscopy slides to obtain patterns of bacterial soil communities, forming a micro-detailed landscape, which he later studied with different microscopic techniques. In our modification, plastic film strips made with polyethylene terephthalate were used as a matrix for the attachment and direct growth of the bacterial communities, maintaining the original (*in situ*) spatial shape and structure of these communities.

Material and Methods

Plant material

In the current work bamboo plants *Phyllostachys humilis*, *P. atrovaginata*, *P. nigra* and *Fargesia rufa*, obtained via micropropagation approach by Oprins Plant NV, Rijkevorsel, Belgium, were used. Both *in vitro* plants and plants, obtained after hardening of the *in vitro* plantlets in soil for strike out months, were used for the experiments, respectively for the extraction of DNA and bacteria and for the application of the plastic film strips. Sterilization of the above-ground and underground surfaces of plants, grown in the substrate under non-sterile conditions, was done in accordance with Han et al. (2009), in a laminar flow as follows: 3 minutes in 70% ethanol, 5 minutes in 12% sodium hypochlorite solution, and 1 minute in 70% ethanol. Afterwards the tissues were washed five times with sterile distilled water. The whole sterilization procedure was done two times.

Isolation of endophytic bacteria from culms and rhizomes from bamboo

Endophytic bacteria from the above-ground parts of plants were collected by the following way, again according to Han et al. (2009). Surfaces of each piece of a plant were sterilized, and subsequently these parts were cut into small pieces and immersed in a volume with 5 mL of nutrient medium, either dextrose broth (5 g pepton C, 2 g proteose peptone No 3, 3 g peptone G, 3 g beef extract, 5 g dextrose, 5 g NaCl per liter) or Columbia broth (12 g pepton C, 5 g peptone A, 3 g east extract, 3 g beef extract, 1 g wheat starch, 5 g NaCl per liter). The material was incubated under shaking condition at 28 °C for 48 hours. Microorganisms were plated out to the corresponding solid media containing 1.5 % of agar and cultivated further at 28 °C for 24 hours. Pure culture isolates selected for subsequent molecular analysis were additionally cultivated on the corresponding dextrose and Columbia broths.

Plants grown in soil were used to collect rhizosphere bacteria as well. The rhizomes were first washed in distilled water to remove all soil particles. The plant surfaces were sterilized according to Han et al. (2009). Plant tissue samples of 1 g were cut up and ground in 10 mL of phosphate buffer saline (PBS, pH 7.4). This suspension was diluted in 10 and 100 times. 100 µl of each dilution was cultivated on LB and dextrose agar plates at 28 °C from 24 till 48 hours. Each colony was cultivated in the corresponding liquid medium, and the obtained biomass was used for DNA extraction.

Endophytes from *in vitro* plants were obtained in the following way. The biomass (0.5 g) was ground in 5 mL of PBS. This suspension was diluted in 10 and 100 times. 100 µl of each dilution was cultivated on LB and dextrose agar plates under 28 °C from 24 till 72 hours. Each colony was cultivated in the corresponding liquid medium, and the obtained biomass was used for DNA extraction.

All obtained colonies were stored as a stock culture by adding 20% glycerol to an overnight culture and kept at -80 °C.

DNA extraction from isolated bacteria and bamboo tissue

DNA extraction from bacterial cells was conducted according to Khanuya et al. (1999). DNA from bamboo tissue was extracted by freezing each specimen (0.25 g) in liquid nitrogen. Frozen bamboo tissue was then homogenized in 1 mL of ethanol using a Magnalyser (Roche, Germany). The concentration of DNA was measured by NanoDrop® (Thermo Fisher Scientific Inc., USA)

Identification of bacterial species

Fragments of the 16S rDNA gene were amplified using the universal forward primer 25f (5'-AAC TKA AGA GTT TGA TCC TGG CTC-3') and reverse primer 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Han et al. 2009), following the instructions of the High Fidelity PCR kit (Roche, Germany), either on the isolated DNA from bacteria or bamboo tissue, or, in some cases, directly on the bacterial cells during a colony PCR. The reaction regime was in all cases: denaturation 10 min under 94 °C; 35 cycles of: 45 s under 94 °C, 45 s under 62 °C, 1 min under 72 °C; 10 min under 72 °C. The product was visualized by gel electrophoresis in 0.8% agarose gel according Sambrook et al. (1989).

After PCR on isolated strains, both after colony PCR or after PCR on extracted DNA from isolated strains, the PCR products were sent directly for sequencing. PCR product sequencing was done using specific primers T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') and SP6 (5'-GAT TTA GGT GAC ACT ATA G-3') on the sequenator Applied Biosystems 3730 (USA). The sequence of the 16S rDNA fragments was analyzed using BLAST (Basic local alignment search tool) and the NCBI database (USA) (<http://www.ncbi.nlm.nih.gov/BLAST/>; Altschula et al. 1990). Positive identification was obtained when maximal identities of 98-99% and E-values of 0 were returned.

Modified Cholodny method for visualisation of bacterial communities

For the visualisation and further morphological and cytochemical analysis of the endophytic communities of bamboo, a modification of the classic method of Cholodny was used (as tested and tried by Moshynets et al. 2011). The original method used glass as a substrate for the attachment of bacterial communities, which was here replaced by slips of polyethylene terephthalate of 40 µm thickness. To avoid complete blocking of all water and solute transport through these slips, holes were provided with a diameter of on average 0.5 mm. The slips were then sterilized in 70% ethyl alcohol for 5 minutes. The surface of the bamboo culms was disinfected with 70% ethyl alcohol as well, and subsequently a longitudinal cut through the culm was made using a sterile scalpel blade. The plastic slip was pressed in the open culm and the wound with the plastic inside was covered with a sterile bandage. Every culm was outfitted with three slips at different heights, applied through the nodes or not. The exposure of the plastic strips lasted 4 months, while the plants were growing in the laboratory, under natural sunlight. Afterwards, the culms were cut in different pieces; the pieces with a plastic slip inside were stored at -80°C awaiting further treatment.

Morphological and cytological analysis

For further analysis, the plastic slips were fixed in 37% formaldehyde solution evaporation for 30 minutes. For cytochemical analysis the following dyes were used. Nucleic acids were visualised using either ethidium bromide (EB) in a concentration of 2 µg mL⁻¹ distilled water, applied for staining for 2-5 min at room temperature before CLSM (confocal laser scanning microscopy) (excitation: 488 nm, emission: 560 nm) or SYBR Green (SG), in a concentration 0.5 µg mL⁻¹ distilled water, and again with an exposure of 2-5 minutes at room temperature before microscopic analyses (excitation: 497 nm, emission: 520 nm). Polypeptides were stained with thiazine red R (TRR) in a concentration of 0.5 µg mL⁻¹ of distilled water, with an exposure time of 2-5 min before fluorescence microscopy (excitation: 510 nm, emission: 580 nm). Acridin orange (AO) was used for obtaining the total morphological pattern, and was applied in an aqueous solution of 5 µg mL⁻¹ with a staining time of 5 min (excitation: 488 nm, emission: 560 nm). Anti-bleach reagent was used (Johnson et al., 1982). The analyses were done using a confocal laser scanning microscope ZEISS AXIOSCOPE- 2 Plus and software LSM 5 PASCAL. Pre-efficiency staining was tested for fluorescence using a LM-2 microscope (LOMO,

Russia). Morphological analysis of sections of the culm occurred using electron microscopy on tissues which were dried, fixed in 37% formaldehyde solution vapors, and covered with gold. Scanning electron microscopy (SEM) occurred using SEMicroscopes Jeol JSM 35C and Jeol JSM 6060LA.

Table 1. Endophytic bacteria of *Bambusoideae* subfamily

Plant tissues	Phylum	Genera	Species	Bamboo species
Shoot	<i>Firmicutes</i>	<i>Bacillus</i>	<i>B. amyloliquefaciens</i>	<i>P. humilis</i> <i>F. rufa</i>
			<i>B. subtilis</i>	
			<i>B. mojavensis</i>	<i>P. humilis</i>
	<i>Actinobacteria</i>	<i>Mycobacterium</i>	<i>M. palustre</i>	<i>P. atrovaginata</i>
			<i>M. lentiflavum</i>	
			<i>M. avium</i> complex	<i>P. nigra</i>
			<i>M. arosiense</i>	
Uncultured bacterium clones				<i>P. humilis</i>
Root, rhizomes	<i>Alphaproteobacteria</i>	<i>Agrobacterium/Rhizobium</i>	sp.	<i>P. humilis</i>
	<i>Betaproteobacteria</i>	<i>Burkholderia</i>	sp.	
			<i>B. cepacia</i> complex	
	<i>Gammaproteobacteria</i>	<i>Pseudomonas</i>	<i>P. fuscovaginae</i>	
			<i>P. fluorescens</i>	
	<i>Firmicutes</i>	<i>Paenibacillus</i>	sp.	
			<i>P. chondroitinus</i>	
<i>Actinobacteria</i>	<i>Microbacterium</i>	<i>M. laevaniformans</i>		
	<i>Leifsonia</i>	sp.		
<i>In vitro</i>	<i>Betaproteobacteria</i>	<i>Achromobacter</i>	sp.	<i>P. humilis</i>
	<i>Gammaproteobacteria</i>	<i>Acinetobacter</i>	<i>A. calcoaceticus</i>	<i>P. atrovaginata</i>

Results and Discussion

Isolation and identification of bamboo endophytes

Two approaches were followed for the isolation and the identification of the bamboo endophytes. The first one consists of the cultivation of endophytic microorganisms isolated directly from different bamboo tissues (and subsequent identification using the 16S ribosomal RNA sequence), while the second one is based on direct extraction of total DNA from plant tissues and its subsequent analysis of the 16S ribosomal RNA sequences found in the extracts. 61 colonies were obtained from 1 g of underground plant parts; these were subjected to PCR and 26 of them were sequenced. 34 colonies were used for PCR after isolation from the *in vitro* tissues and they were sequenced as well. An overview can be found in table 1.

Identification of the strains isolated from *P. humilis* and *F. rufa* shows that both species contain the bacteria *Bacillus amyloliquefaciens* and *B. subtilis* as endophytic bacteria. *P. humilis* also contained the congeneric species *B. mojavensis*. In general, members of the genus *Bacillus* are widespread as endophytes (McInroy and Kloepper 1995; Bai et al. 2002; Melnick et al. 2008). These bacteria play an important phytoprotective role, by increasing the resistance of the plant against fungi (Wilhelm et al. 1998).

The cultivation of endophytes of the rhizome parts of the bamboo plants revealed a larger variety than in the culms. Among these were found: *Microbacterium laeviformans* and *Leifsonia* sp. (family *Microbacteriaceae*, phylum *Actinobacteria*), *Paenibacillus* sp. and *Paenibacillus chondroitinus* (family *Paenibacillaceae*, phylum *Firmicutes*), and finally several isolates related to the phylum *Proteobacteria*: one isolate *Agrobacterium/Rhizobium* belonging to the *Alphaproteobacteria*; two isolates, *Pseudomonas fuscovaginae* and *Pseudomonas fluorescens*, belonging to *Gammaproteobacteria*; and two isolates, *Burkholderia cepacia* complex and *Burkholderia* sp., belonging to the *Betaproteobacteria*.

Likewise, the endophytic population of *in vitro* tissues of *P. atrovaginata* and *P. humilis* was analysed using a cultivation step. This indicated the presence of *Acinetobacter calcoaceticus* in the tissues of *P. atrovaginata*, while isolates obtained from *P. humilis* corresponded to *Achromobacter* sp. Both bacteria belong to the phylum *Proteobacteria*, with *A. calcoaceticus* belonging to the *Gammaproteobacteria*, and *Achromobacter* sp. to the *Betaproteobacteria*. Such a reduction in the diversity of endophytic population of *in vitro* plants in comparison to plants grown in soil, perhaps, was the result of repeated cloning of plant material under sterile conditions (Podolich et al. 2007). The results obtained here coincide with the results of other authors. Endophytes of the subterranean parts of *Phyllostachys edulis* and *Zea mays* L. were more diverse, than the populations of the aboveground organs (Han et al. 2009; Bai et al. 2002), while endophytes of plants grown in soil were more diverse, than the endophytes of *in vitro* plants (Koskimaki et al. 2010; Podolich et al. 2007).

Other experiments were focused on total DNA extraction from culm tissues of young bamboo plants (grown in soil) of the species *P. atrovaginata*, *P. nigra* and *P. humilis*. Amplification and sequencing 16S rRNA fragments revealed several endophytic bacteria belonging to the *Mycobacteriaceae* family, (phylum *Actinobacteria*): *Mycobacterium palustre* and *M. lentiflavum* were found in *P. atrovaginata*; *M. avium* complex and *M. arosiense* were detected in *P. nigra*; there were a few uncultivable bacteria clones in *P. humilis*. Uncultivable endophytes of the *Mycobacterium* genus were also found in other plants belonging to the order of the *Poales* (White 1987; Koskimaki et al. 2010). Generally, mycobacteria can be considered as saprophytes, commensals and symbionts of animals, humans and protozoa. A few endophytic *Mycobacterium* spp. were detected in rice root tissues (Mano et al., 2007), wheat (Conn and Franco 2004) and peat moss (Katila et al. 1995). Rarely endophytic mycobacteria can be also found (Laukkanen et al. 2000; Koskimaki et al. 2010).

Visualization of the bacterial communities

To obtaining innovative data on the nature of the relation between plant cells and their endophytes, a modification of the method of Cholodny was used, based on plastic slips inserted in the culm of *P. atrovaginata* (Fig. 1). This method has already been applied successfully in a study of the architecture of microbial communities in the soil and rhizosphere (Moshynets et al. 2010; Moshynets et al. 2011).



Figure 1. A plastic slip inserted in the culm of *P. atrovaginata*

The maximal microbial growth in the endosphere was found on the level of the second and the third node of the culm. Characteristic features of microbial fouling on the whole were the absence of biofilm structures, such as those frequently found in microbial communities in soils (Costerton et al. 2004; Hall-Stoodley et al., 2004; Spiers et al. 2006; Ude et al. 2006). However, some areas were still covered with a layer of mucus-rich DNA, as evidenced by the cytochemical reaction after application of the DNA-specific dye SG (Fig. 2A).

In the lower part of the shoot, mostly fungal hyphae could be detected, mainly in association with bacteria, although there were also individual hyphae. At the bottom of the shoots, three hyphal morphotypes could be observed. Fig. 2A is an example of first morphotype: hyphae, up to 2 μm wide, covered with mucus containing relatively small amounts of DNA. These hyphae were usually not associated with bacteria. The second morphotype shows up to 7 μm wide hyphae, in association with bacteria (Fig. 2B). Associated bacteria were small in size (up to 1 μm), with a coccoid form, were located along the hyphae, which is explained by the high humidity at the site of exposure. The third morphotype presented thick septate hyphae, in close association with bacilliform bacteria (Fig. 2C).

In contrast, in the upper part of the culms, the growth of microorganisms was rarely observed. Hyphae of the third morphotype were predominantly found, linked to colonies of small bacteria, ranging from 0.5 μm and smaller. These colonies were often associated with hyphae and had mucous sheaths.

Individual micro-colonies were characterized by high metabolic activity, demonstrated by an intense coloration with TRR, due to the high concentration of protein in cells (Fig. 2D-F). The absence of

staining with EB dye, considered as an appropriate dye for a total visualization of fixed microbial cells in a natural microbial consortium (Walberg et al., 1999; Auschill et al., 2001), and low TRR coloration indicates a small quantity of nucleic acids in the cells and low metabolism level (Fig. 2G-I). Possibly, these bacteria may be in the low nuclei acid (LNA) ecological form. This LNA form is an alternative to high nuclei acid (HNA) form which can be characterized with low content of DNA and low metabolism level. LNA bacteria are viable and even can be cultivated under laboratory conditions (Servais et al. 2003; Longnecker et al. 2005).

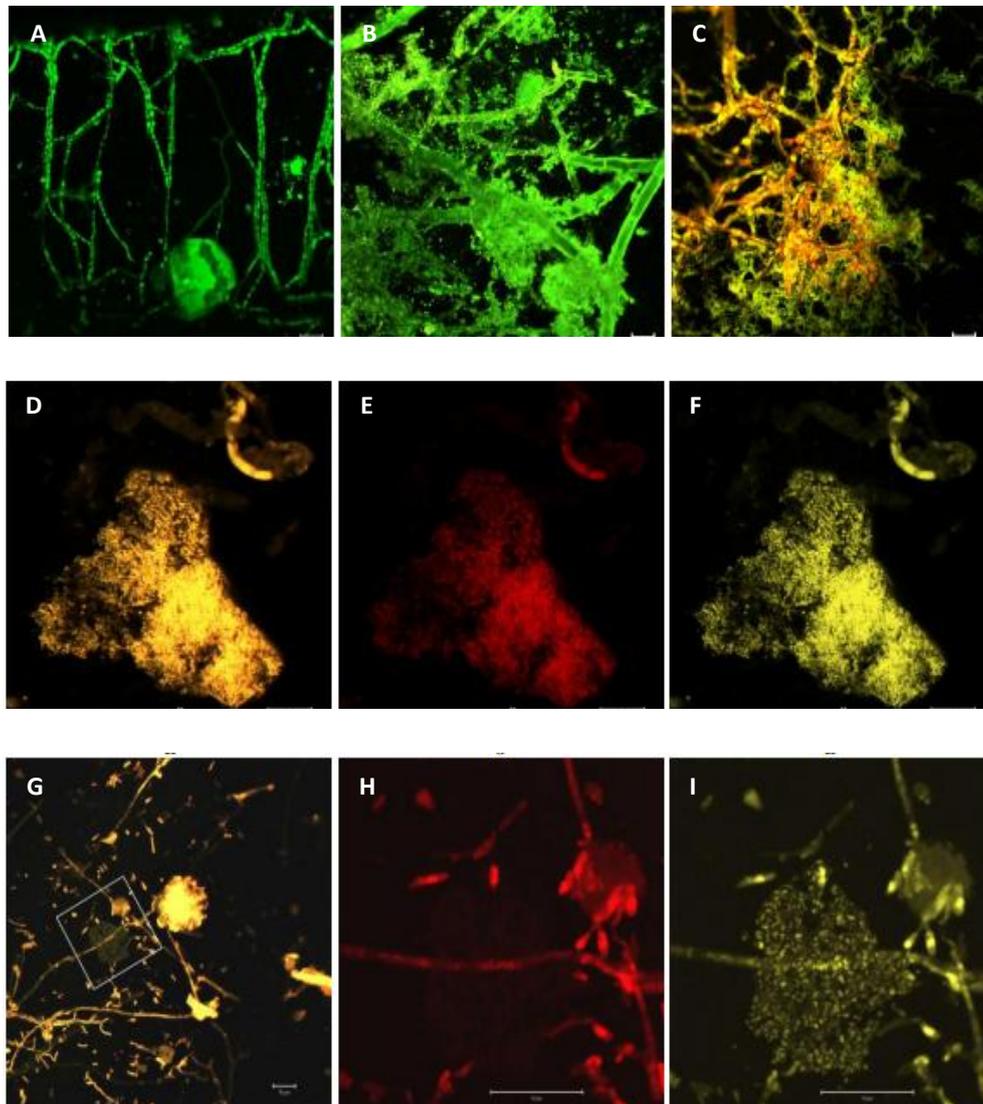


Figure 2. Endophytic microbial communities of *P. atrovaginata*, visualized using a confocal laser scanning microscope ZEISS AXIOSCOPE-2 Plus and software LSM 5 PASCAL. A, B, C – endophytes from the bottom part of the culm (the second - the third node), A, B – SYBR Green (SG) staining, C – acridin Orange (AO) staining; D, E, F - endophytes from the upper part of the culm (the fifth - the seventh node), D – ethidium bromide (EB) and thiazine red R (TRR) staining, E – EB staining, F – TRR staining; G, H, I – LNA bacteria associated with a hypha, G – EB and TRR staining, H – EB staining, I – TRR staining. Bars correspond with 10 μ m; bar in G is valid for A and D as well; bar in H is valid for B and E; bar in I is valid for F and C.

In the tissues of bamboo, endophytic bacteria were less common than fungal spores. Bacteria with a bacillary form were found in intracellular spaces (Fig. 3A-B) and vessels (Fig. 3C-D) within the bamboo tissue.

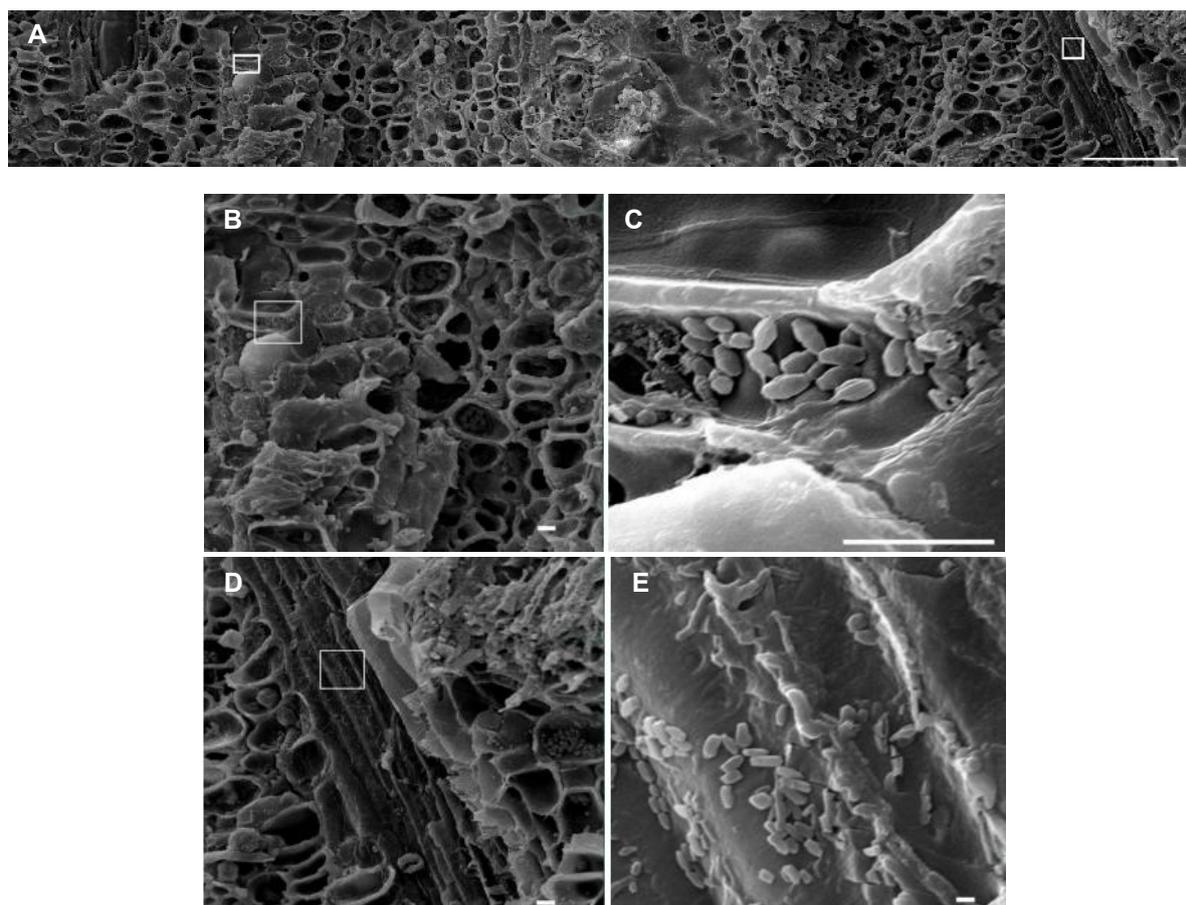


Figure 3. Endophytic bacteria in the bamboo culm tissues, visualized using SEMicroscopes Jeol JSM 35C and Jeol JSM 6060LA. A. General overview of the section, where bar corresponds with 10 μm . B, D. Detailed view of the plant tissue, as indicated in A. C, E. Detailed view of the bacterial colonies, as indicated in resp. B and D. Bar corresponds with 10 μm , except for E, where the bar corresponds with 1 μm .

These observations indicate that our modifications to the Cholodny method can be successfully applied for observation and analysis *in situ* and can be recommended for more profound studies of aspects of coexistence of endophytic microorganisms in plant tissues. In the future, the modified Cholodny method can be combined with modern molecular genetic methods, which will expand the range of usefulness of the method in the study of plant-microbe-relations.

It also needs to be remarked that the present study is purely qualitative and does not pretend to provide an exhaustive overview of the bacteria living inside bamboo tissue. To say the least, the design of the experiments discussed in this paper fails to take such things into account, and moreover, this is a task for which the pre-metagenomic methodology applied here does not suffice. Combination of the Cholodny visualization method with the use of fluorescently labeled DNA probes (for fluorescent *in situ* hybridization), however, might form the future basis for a much more thorough and quantitative study of the endophyte populations in bamboo.

References

- Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
- Auschill, T.M.; Artweiler, N.B.; Netuschil, L.; Brex, M.; Reich, E.; Sculean, A. 2001. Spatial distribution of vital and dead microorganisms in dental biofilms. *Archives of Oral Biology*, 46, 471–476.
- Bai, Y.; D'Aoust, F.; Smith, D.L.; Driscoll, B.T. 2002. Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. *Canadian Journal of Microbiology*, 48, 230–238.
- Caldwell, D.E.; Korber, D.R.; Lawrence, J.R. 1992. Confocal laser microscopy and digital image analysis in microbial ecology. *Advances in Microbial Ecology*, 12, 1–67.
- Caldwell, D.E.; Wolfaardt, G.M.; Korber, D.R. 1997. Do bacterial communities transcend Darwinism? *Advances in Microbial Ecology*, 15, 105–191.
- Cholodny, N.G. 1934. A soil chamber as a method for the microscopic study of the soil microflora. *Archives of Microbiology*, 5, 148–156.
- Compant, S.; Clement, C.; Sessitsch, A. 2009. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, 42, 669–678.
- Compant, S.; Duffy, B.; Nowak, J.; Clement, C.; Rarka, E.A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology*, 71, 4951–4959.
- Conn, V.M.; Franco, C.M.M. 2004. Analysis of the endophytic actinobacterial population in the roots of wheat (*Triticum aestivum* L.) by terminal restriction fragment length polymorphism and sequencing of 16S rRNA clones. *Applied and Environmental Microbiology*, 70, 1787–1794.
- Costerton, J.W., Lewandowski, Z., DeBeer, D.; Caldwell, D.; Korber, D.; James, G. 1994. Biofilms: the customized microniche. *Journal of Bacteriology*, 176, 2137–2142.
- Deretic, V.; Schurr, M.J.; Boucher, J.C.; Martin, D.W. 1994. Conversion of *Pseudomonas aeruginosa* to mucoidy in cystic fibrosis: environmental stress and regulation of bacterial virulence by alternative sigma factors. *Journal of Bacteriology*, 176, 2773–2780.
- Dworkin, M. Introduction in *Microbial cell-cell interactions*. 1991. Washington: American Society for Microbiology, DC, 1-6.
- Hall-Stoodley, L.; Costerton, J.W.; Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*, 2, 95–108.
- Han, J.; Xia, D.; Li, L.; Sun, L.; Yang, K.; Zhang, L. 2009. Diversity of culturable bacteria isolated from root domains of Moso Bamboo (*Phyllostachys edulis*). *Microbial Ecology*, 58, 363–373.
- Johnson, G.D.; Davidson, R.S.; McNamee, K.C., Russell, G.; Goodwin, D.; Holborow, E.J. 1982. Fading of immunofluorescence during microscopy: a study of the phenomenon and its remedy. *Immunological Methods*, 55, 231–242.
- Katila, M.L.; Iivanainen, E.; Torkko, P.; Kauppinen, J.; Martikainen, P.; Vaananen, P. 1995. Isolation of potentially pathogenic mycobacteria in the Finnish environment. *Scandinavian Journal of Infectious Diseases. Supplementum*, 98, 9–11.
- Khanuja, S.P.S.; Shasany, A.K.; Darokar, M.P.; Kumar, S. 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant Molecular Biology Reporter*, 17, 1–7.
- Koskimaki, J.J.; Hankala, E.; Suorsa, M.; Sannakajsa, N.; Pirtilla, A.M. 2010. Mycobacteria are hidden endophytes in the shoots of rock plant [*Pogonatherum paniceum* (Lat.) Hack.] (*Poaceae*). *Environmental Microbiology Reports*, 2(4), 619–624.

- Laukkanen, H.; Soini, H.; Kontunen-Soppela, S.; Hohtola, A.; Viljanen, M. 2000. A mycobacterium isolated from tissues cultures of mature *Pinus sylvestris* interferes with growth of Scots pine seedlings. *Tree Physiology*, 20, 915–920.
- Leff, L.G.; Dana, J.R.; McArthur, V.; Shimkets, L.J. 1995. Comparison of methods of DNA extraction from stream sediments. *Applied and Environmental Microbiology*, 61, 1141–1143.
- Longnecker, K.; Sherr, B.F.; Sherr, E.B. 2005. Activity and phylogenetic diversity of bacterial cells with high and low nucleic acid content and electron transport system activity in an upwelling ecosystem. *Applied and Environmental Microbiology*, 71, 7737–7749.
- Mano, H.; Tanaka, F.; Nakamura, C.; Kaga, H.; Morisaki, H. 2007. Culturable endophytic bacterial flora of the maturing leaves and roots of rice plants (*Oryza sativa*) cultivated in a paddy field. *Microbes and Environments*, 22, 175–185.
- Massol-Deya, A.A.; Whallon, J.; Hickey, R.F.; Tiedje, J.M. 1995. Channel structures in aerobic biofilms of fixed-film reactors treating contaminated groundwater. *Applied and Environmental Microbiology*, 61, 769–777.
- Mastretta, C.; Taghavi, S.; van der Lelie, D.; Mengoni, A.; Galardi, F.; Gonnelli, C.; Barac, T.; Boulet, J.; Weyens, N.; Vangronsveld, J. 2009. Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce Cd phytotoxicity. *International Journal of Phytoremediation*, 11, 251–267.
- McInroy, J.A.; Klopper, J.W. 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant and Soil*, 173 (2), 337–342.
- Melnick, R.L.; Zidack, N.K.; Bailey, B.A.; Maximova, S.N.; Gultinan, M.; Backmann, P.A. 2008. Bacterial endophytes: *Bacillus* spp. from annual crops as potential biological control agents of black pod rot of cacao. *Biological Control*, 46, 46–56.
- Moore, F.P.; Barac, T.; Borremans, B.; Oeyen, L.; Vangronsveld, J.; van der Leile, D.; Campbell, C.D.; Moore, E.R. 2006. Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. *Systematic and Applied Microbiology*, 29, 539–556.
- Moshynets, O.; Kosakivska, I.V. 2010. Phytosphere ecology: plant-microbial interactions. 1. Structure functional characteristics of rhizo-, endo- and phytosphere. *Bulletin of National Kharkov Agrarian University*, 2 (20), 19–36.
- Moshynets, O.; Koza, A.; Dello Sterpaio, P.; Kordium, V.; Spiers, A.J. 2011. Up-dating the Cholodny method using PET films to sample microbial communities in soil. *Biopolymers and cell*, 27(3), 199–205.
- Moshynets, O.V.; Shpylova, S.P.; Spiers, A.J.; Kosakivska, I.V. 2010. The phytosphere of *Brassica napus* L.: a niche for *Pseudomonas fluorescens* SBW25. *Reports of Academy of Sciences of Ukraine*, 12, 150–153.
- Newman, L.A.; Reynolds, C.M. 2005. Bacteria and phytoremediation: new uses for endophytic bacteria in plants. *Trends in Biotechnology*, 23(1), 6–9.
- Ovcharenko, L.P.; Kozyrovska, N.O. 2008. Metagenomic analiz mikroorganizmiv dovkilliya. Sprint Print, Kyiv, Ukraine.
- Podolich, O.V.; Ardanov, P.E.; Voznyuk, T.M.; Kovalchuk, M.V.; Danylchenko, O.V.; Lashevskyi, V.V.; Lyastchenko, S.A.; Kozyrovska, N.O. 2007. Endophytic bacteria from potato *in vitro* activated by exogenic non-pathogenic bacteria. *Biopolymers and Cell*, 23(1), 21–27.
- Sambrook, J.; Fritsch, E.F.; Maniatis, T. 1989. *Molecular cloning – a laboratory manual*. Second ed. Cold Spring Harbor Laboratory Press.
- Schulz, B.; Boyle, C. 2006. What are endophytes? In Schulz, B.J.E.; Boyle, C.J.C; Sieber, T.N. ed., *Microbial Root Endophytes*. Springer, Berlin, Heidelberg, pp.113.

- Servais, P.; Casamayor, E.O.; Courties, C.; Catala, P.; Parthuisot, N.; Lebaron, P. 2003. Activity and diversity of bacterial cells with high and low nucleic acid content. *Aquatic Microbial Ecology*, 33, 41–51.
- Siciliano, S.D.; Fortin, N.; Mihoc, A.; Wisse, G.; Labelle, S.; Beaumier, D.; Ouellette, D.; Roy, R.; Whyte, L.G.; Banks, M.K.; Schwab, P.; Lee, K.; Greer, C.W. 2001. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. *Applied and Environmental Microbiology*, 67, 2469–2475.
- Spiers, A.J.; Arnold, D.L.; Moon, C.D.; Timms-Wilson, T.M. 2006. A survey of A-L biofilm formation and cellulose expression amongst soil and plant-associated *Pseudomonas* isolates. In Bailey, M.J.; Lilley, A.K.; Timms-Wilson, T.M ed., *Microbial Ecology of Aerial Plant Surfaces*, CABI.
- Steffan, R.J.; Goksoyr, J.; Bej, A.K.; Atlas, R.M. 1988. Recovery of DNA from soil and sediments. *Applied and Environmental Microbiology*, 54, 2908–2915.
- Ude, S.; Arnold, D.L.; Moon, C.D.; Timms-Wilson, T.; Spiers, A.J. 2006. Biofilm formation and cellulose expression among diverse environmental *Pseudomonas* isolates. *Environmental Microbiology*, 8, 1997–2011.
- Van Aken, B.; Yoon, J.M.; Schnoor, J.L. 2004. Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp. associated with poplar tissues (*Populus deltoids x nigra* DN34). *Applied and Environmental Microbiology*, 70, 508–517.
- Ward, D.M.; Bateson, M.M.; Weller, R.; Rulf-Roberts, A.L. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. *Advances in Microbial Ecology*, 12, 219–289.
- Walberg, M.; Gaustad, P.; Steen, H.B. 1999. Uptake kinetics of nucleic acid targeting dyes in *S. aureus*, *E. faecalis* and *B. cereus*: a flow cytometric study. *Journal of Microbiological Methods*, 35, 167–176.
- Weyens, N.; van der Lelie, D.; Taghavi, S.; Vangronsveld, J. 2009. Phytoremediation: plant-endophyte partnerships take the challenge. *Current Opinion in Biotechnology*, 20, 248–254.
- White, J.F. 1987. Widespread distribution of endophytes in the *Poaceae*. *Plant Disease*, 71, 340–342.
- Wilhelm, E.; Arthofer, W.; Schafleitner, R.; Krebs, B. 1998. *Bacillus subtilis* as endophyte of chestnut (*Castanea sativa*) as antagonist against chestnut blight (*Cryphonectria parasitica*). *Plant Cell, Tissues and Organ Culture*, 52, 105–108.

Susceptibility of Bamboo to Fungi

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Abstract

Moulds and basidiomycetes were isolated from culms of several bamboo species from various countries and identified by DNA sequencing. Laboratory staining experiments of samples with pure cultures of blue-stain fungi revealed the colonization of the tissue with thick, brown hyphae, chlamydospores and penetration of lignified cell walls by transpressoria as it occurs also in wood cells. Degradation experiments with pure cultures of white -, brown - and soft- rot fungi using different test arrangements showed considerable mass loss particularly by white and soft rot. Micromorphology of fungal attack was demonstrated by transmission electron microscopy. Results improve the basic view on fungal damage of bamboo, which is needed for better utilization and suitable protection measures.

Keywords

Biodeterioration, Moulds, Blue Stain Fungi, Decay Fungi, Molecular Identification of Fungi, Micromorphology of Degradation

Introduction

Bamboo is an abundant natural product with excellent technological properties. However, the use of bamboo is restricted due to its susceptibility to deteriorating organisms and its low durability in general (Liese and Kumar 2003). Bamboo is rapidly colonized by moulds and blue stain fungi, particularly during overseas transport (Thang et al. 2009). Several publications deal with fungal decay of bamboo (Liese 1959, 1985; Banerjee and Mukhopathya 1962; Purushotham 1963; Abdurachim 1964; Wang and Hsieh 1968; Murphy et al. 1991; Razak et al. 2002; Hamid et al. 2003; Zhang et al. 2007; Schmidt et al. 2011). Micromorphology of fungal cell wall attack was reported for the white-rot mushroom *Lentinula edodes* (Kim et al. 2008) and the brown-rot fungus *Gloeophyllum trabeum* (Cho et al. 2008). More information will improve protection measures and lead to better utilization of bamboo. This paper gives an overview on laboratory experiments under controlled conditions.

Material and Methods

Fungal Isolation and Identification

Samples of bamboo culms were kindly provided by several colleagues in various countries and were sent to Hamburg by post. In most cases, culms had been in practical use so that the species was unknown. Some Thailand samples belong to *Bambus multiplex* and *Dendrocalamus asper*. *D. brandisii* was obtained from Costa Rica and *Phyllostachys glaucoviridescens* from Germany (see Table 1). Fungi were isolated by transfer of mycelia or spores from infected regions of the culm surface on malt extract (Oxoid 2%) agar (Oxoid 1.5%) plates and were brought to pure cultures by subsequent subculturing at room temperature. Molecular identification was mainly according to Schmidt et al. (2002). Briefly, DNA from mycelia and spores was extracted with the DNeasy Plant Extraction Kit (Qiagen). PCR program with the Qiagen *Taq* Core Kit and the ITS1/ITS4 primer of White et al. (1990) in the MJ Research thermocycler was 4 min at 98°C, 35 cycles of 30 sec at 94°C, 30 sec at 54°C and 45 sec at 72°C followed by 7 min at 72°C. Suitable PCR products after electrophoresis in 2% agarose gels (Biozym DNA agarose in TAE buffer) with the Mupid-ex system (Advance, Tokyo) were purified with the QIAprep Spin Miniprep Kit (Qiagen) and were sequenced by Eurofins MWG Operon. Species identification was performed via sequence comparison with the DNA data bases by BLAST (see Table 1).

Blue-stain Tests

The blue-stain fungi *Alternaria tenuissima*, *Alternaria alternata*, *Botryosphaeria subglobosa*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Pestalotiopsis microspora* and *Phoma macrostoma* deriving from bamboo were used for staining experiments, together with *Hormonema dematioides* and *Aureobasidium pullulans* from the Institute strain collection. Autoclaved bamboo samples placed on malt agar plates were inoculated with mycelial plugs from precultures and incubated at room temperature. After culture, small pieces from discoloured samples were embedded in PEG and cut to 10 µm sections with a microtome and visualized by light microscopy.

Degradation Tests

Degradation tests were done using culm sections of *Bambusa maculata*, *Gigantochloa atroviolacea* and *Phyllostachys pubescens*. Sample (3 x 1 cm) preparation and mass loss evaluation followed EN 113 (1996). Household preserving jars (500 ml) with 110 ml malt-agar were used as culture vessels for

the basidiomycetes and Abrams agar (Savory 1954) for the soft-rot fungi. Fungi used were derived from the laboratory strain collection and *Schizophyllum commune* from bamboo (see Table 1)

To investigate less active fungi, e.g. *S. commune* and *C. puteana*, in more detail and to imitate more natural conditions, degradation tests were also performed with bigger bamboo samples, which partly were placed on unsterile soil ('Fungus cellar-test'). Culm parts (25 cm) of *Arundinaria amabilis* (Vietnam), *Bambusa maculata* (Indonesia), *Dendrocalamus asper* (Indonesia), *Gigantochloa atroviolacea* (Indonesia), *Phyllostachys nigra* (Japan), *Phyllostachys nigra* 'Boryana' (China) and *Phyllostachys pubescens* (Germany) were longitudinally halved, dried at 103°C and weighed, dipped in tap water for two days and autoclaved at 121°C for 40 min. Metal tubs (120 cm long, 60 cm wide) were filled with 30 litres of unsterile compost soil from the institute garden. Samples were placed either on autoclaved wood supports or directly on the soil. The tubs were covered with panes of glass and the soil moistened weekly with sprayed tap water (see Figure 5).

Transmission Electron Microscopy of Decayed Bamboo Samples

Micromorphology of fungal degrading pattern of bamboo cell walls was examined by transmission electron microscopy (Kim et al. 2008)). Briefly, small pieces from the incubated samples were fixed in a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2), dehydrated in a graded acetone series and embedded in Spurr's epoxy resin (Spurr 1969). The embedded tissue was serially sectioned on an ultramicrotome (RMC, MTX) with a diamond knife. Ultrathin sections (100 nm in thickness) were examined with a Jeol 1200 transmission electron microscope at an accelerating voltage of 80 kV after staining with 0.5% (w/v) potassium permanganate (prepared in citrate buffer) to contrast the lignin component. Some sections were stained also with uranyl acetate and citrate.

Results

Identification of Fungi Colonizing Used Bamboo

Table 1 shows the fungi isolated from bamboo culms which had been in use in various countries.

Table 1. Fungi isolated from bamboo and identified by their rDNA-ITS sequence

Bamboo Origin	Deuteromycetes/Ascomycetes (number of strains)	Basidiomycetes (number of strains)
Ethiopia		<i>Schizophyllum commune</i> (1)
China	<i>Alternaria alternata</i> (1) <i>A. tenuissima</i> (1) <i>Arthrinium phaeospermum</i> (1) <i>Cladosporium cladosporioides</i> (2) <i>Dothiorella gregaria</i> (1) <i>Fusarium asiaticum</i> (1) <i>F. culmorum</i> (1) <i>F. zae</i> (1) <i>Nigrospora oryzae</i> (4) <i>Penicillium commune</i> (1) <i>P. chrysogenum</i> (1) <i>P. tricolor</i> (1) <i>P. variable</i> (1) <i>Phoma macrostoma</i> (1)	
Costa Rica		<i>Schizophyllum commune</i> (1)
Germany	<i>Trichoderma koningiopsis</i> (2) <i>T. viride</i> (3)	
Indonesia		<i>Cyathus stercoreus</i> (1)
Philippines	<i>Penicillium citrinum</i> (1) <i>P. sumatraense</i> (1)	
Thailand	<i>Aspergillus nomius</i> (1) <i>A. repens</i> (1) <i>Botryosphaeria subglobosa</i> (1) <i>Cladosporium cladosporioides</i> (2) <i>Epicoccum nigrum</i> (2) <i>Penicillium brevicompactum</i> (1) <i>P. citrinum</i> (2) <i>P. pinophilum</i> (1) <i>Trichoderma atroviride</i> (1) <i>T. koningiopsis</i> (1)	<i>Schizophyllum commune</i> (6)
Vietnam	<i>Apiospora montagnei</i> (2) <i>Arthrinium phaeospermum</i> (1) <i>A. sacchari</i> (3) <i>Aspergillus flavus</i> (5) <i>A. niger</i> (2) <i>Botryosphaeria subglobosa</i> (5) <i>Epicoccum nigrum</i> (4) <i>Penicillium bialowiezense</i> (1) <i>P. biourgeianum</i> (1) <i>P. brevicompactum</i> (2) <i>P. expansum</i> (1) <i>P. islandicum</i> (1) <i>Pestalotiopsis microspora</i> (1)	
Number of fungal isolates	67	9

150 strains were isolated and 76 isolates were identified. Most isolates were Deuteromycetes (moulds). Two basidiomycetes species *Schizophyllum commune* and *Cyathus stercoreus* were found. Figure 1 shows examples of mould appearance on malt agar.

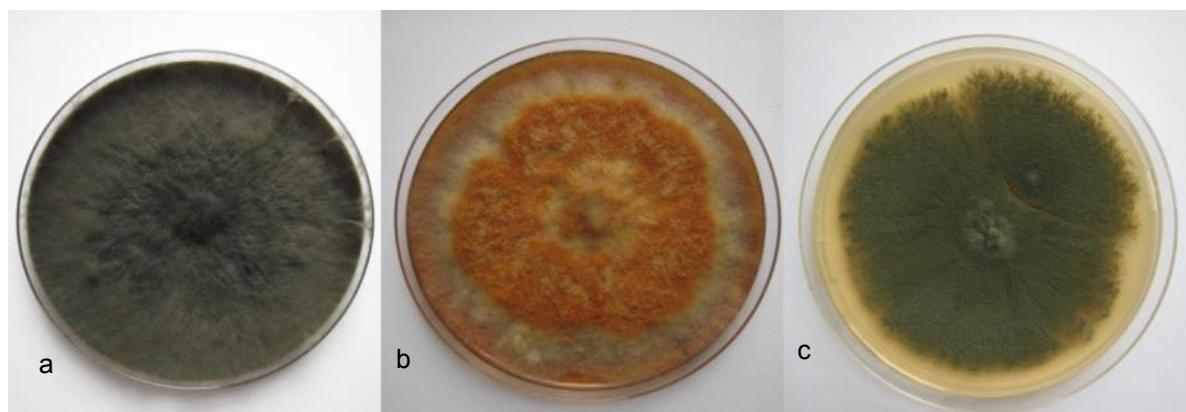


Figure 1. Moulds isolated from bamboo. a: *Botryosphaeria subglobosa*; b: *Epicoccum nigrum*; c: *Penicillium commune*

Table 2. Examples for comparing BLAST identification results based on rDNA-ITS, β -Tubulin and Calmodulin sequences

Fungal strain coding	ITS		β -Tubulin		Calmodulin	
	Species	Max. Identity (%)	Species	Max. Identity (%)	Species	Max. Identity (%)
D54	<i>Aspergillus nomius</i>	100	<i>A. nomius</i>	99	<i>A. nomius</i>	99
			<i>A. zhaoqingensis</i>	99	<i>A. zhaoqingensis</i>	99
D23-1	<i>Penicillium biourgeianum</i>	100	<i>P. biourgeianum</i>	100	<i>P. biourgeianum</i>	98
D19-2	<i>P. brevicompactum</i>	100	<i>P. brevicompactum</i>	98	<i>P. brevicompactum</i>	99
D36	<i>Penicillium</i> sp.	100	<i>P. phialosporum</i>	90	<i>P. variabile</i>	82
D80-2	<i>P. commune</i>	100	<i>P. commune</i>		<i>P. commune</i>	99
D46	<i>P. citrinum</i>	100	<i>P. citrinum</i>	100	<i>P. citrinum</i>	100
D9-1	<i>P. expansum</i>	100	<i>P. expansum</i>	99	<i>P. expansum</i>	97
D57	<i>P. verruculosum</i>	100	<i>Penicillium</i> sp.	87	<i>P. aculeatum</i>	87
	<i>P. aculeatum</i>	98			<i>P. pinophilum</i>	85
D79-1	<i>P. expansum</i>	100	<i>P. expansum</i>	100	<i>P. expansum</i>	97
D65-1	<i>P. polonicum</i>	100	<i>P. polonicum</i>	100	<i>P. commune</i>	92
					<i>P. thymicola</i>	92
					<i>P. echinulatum</i>	91

Some mould genera like *Aspergillus* and *Penicillium* contain very closely related species of which some have also various synonyms. Doubtful results may be obtained when only the ITS sequence is used for identification. Furthermore, the international DNA data bases are human-made and contain mistakes, like miss-identifications. Therefore, also β -Tubulin and Calmodulin sequences were obtained for those fungi and used for BLAST identification (Table 2).

Whereas some moulds like strains D23-1 and D19-2 revealed identical names with all three DNA sequences other fungi like D54 showed quite different naming. However, it has to be considered that some isolates are obviously not represented in the data bases by all three sequences like *Penicillium*

polonicum D65-1 and Calmodulin. We assume that most of the isolated and identified fungi are accidental infections under the respective use conditions of the culms.

Blue-stain Discolouration of Bamboo Samples

All bamboo samples inoculated with the blue-stain fungi *Alternaria tenuissima*, *Alternaria alternata*, *Aureobasidium pullulans*, *Botryosphaeria subglobosa*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Hormonema dematioides*, *Pestalotiopsis microspora* and *Phoma macrostoma* discoloured to grey-black-blue within four weeks of incubation.

The experimental set-up is demonstrated in Figure 2. Figure 3 shows examples of the light microscopic investigation of the attacked samples.

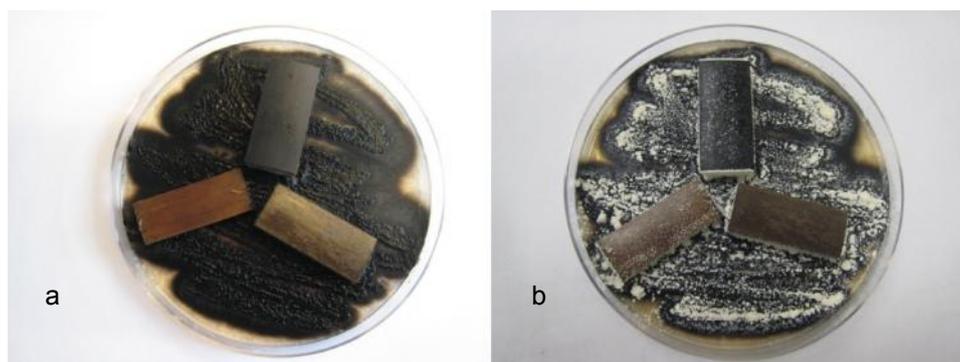


Figure 2. Cultures of the blue-stain fungus *Aureobasidium pullulans* with bamboo samples after 1 month (a) and 5 month (b) of incubation.

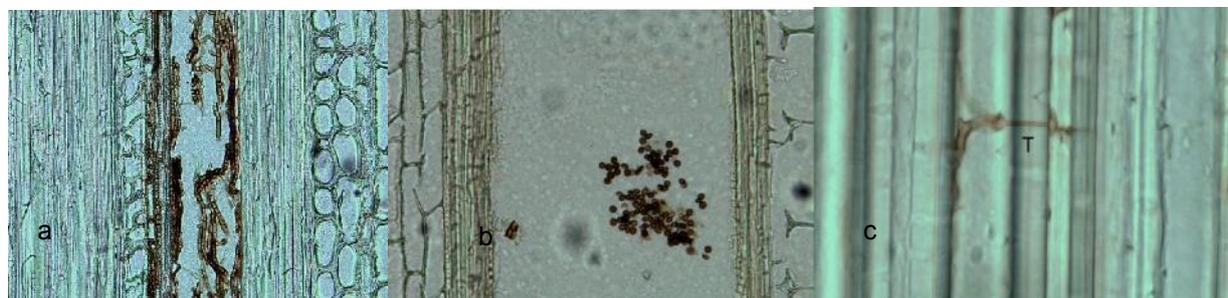


Figure 3. Colonization of bamboo by blue-stain fungi. a: Thick, brown hyphae of *Cladosporium cladosporioides* in a vessel of *Phyllostachys pubescens* (100x); b: brown chlamydospores of *Botryosphaeria subglobosa* in *Gigantochloa atroviolacea* (100x); c: transpressorium (T) of *Alternaria alternata* in *Bambusa maculata* (600x)

The tissue was colonized by the typical thick and brown hyphae and chlamydospores of blue stain fungi. In some cases, a transpressorium, a thin penetration hypha (Fig. 3 right), was observed. The transpressorium is the only fungal 'organ' by which blue stain fungi damage lignified cell walls (Liese and Schmid 1966).

Degradation of Bamboo by Fungi

Figure 4 shows the experimental set-up of decay tests performed in preserving jars.

Table 3 summarizes the % mass loss results after incubation.



Figure 4. a: Preserving jars with bamboo samples; b: samples on mycelium of *Trametes versicolor* after 1 week of incubation

Table 3. Decay of bamboo species (min., average and max. % mass loss of 3 replicates after 1 year of incubation in preserving jars)

Fungus	Rot type	<i>Bambusa maculata</i>	<i>Gigantochloa atroviolacea</i>	<i>Phyllostachys pubescens</i>
<i>Pleurotus ostreatus</i>	White	22.9.....35.7 28.2	8.5.....13.6 10.6	19.7.....22.3 21.0
<i>Schizophyllum commune</i> 87	White	2.7.....2.9 2.8	6.7.....6.8 6.7	4.7.....5.6 5.2
<i>S. commune</i> 98	White	1.6.....2.0 1.8	4.5.....7.3 5.6	3.6.....5.1 4.4
<i>Trametes versicolor</i>	White	60.2.....64.0 62.5	45.7.....55.4 51.6	44.5.....54.0 47.8
<i>Coniophora puteana</i>	Brown	2.8.....4.2 3.6	5.2.....5.9 5.6	4.6.....4.8 4.7
<i>Gloeophyllum trabeum</i>	Brown	1.8.....2.0 1.9	4.8.....6.7 5.7	4.3.....7.2 5.3
<i>Chaetomium globosum</i>	Soft	31.2.....32.7 31.8	7.6.....12.7 9.4	36.9.....39.7 38.0
<i>Paecilomyces variotii</i>	Soft	0.8.....1.4 1.2	3.3.....4.8 3.6	3.4.....4.2 3.9

Although the white rot species *Schizophyllum commune* is a common colonizer of bamboos (Liese 1985; Mohanan 1997; Liese and Kumar 2003), it caused only a minor level of decay (also reported by Suprati (2010) and Kim et al. (2011)). Of the other white-rotters, *Pleurotus ostreatus* showed medium degradation and *Trametes versicolor* caused the greatest mass loss of 62.5 %. The brown-rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* were relatively inactive, the latter contrasts to Lee et al. (2006) and Ma et al. (2010). Of the soft-rot fungi (cf. Leithoff and Peek 2001), *Chaetomium globosum* produced severe degradation (38%).

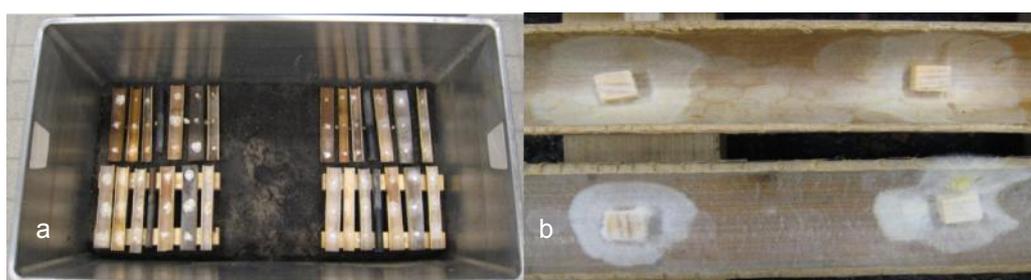


Figure 5. a: Fungus cellar-test with a metal tub and soil; b: samples with *Schizophyllum commune* after 1 week of incubation.

Table 4 summarizes the additional degradation results with the less active fungi *S. commune* and *C. puteana* obtained with the Fungus cellar test.

Table 4. Decay (% mass loss) and final moisture (% u) of bamboo samples in Fungus cellar test

Bamboo species	Tub	Soil contact	<i>Coniophora puteana</i> 1		<i>Schizophyllum commune</i> 87	
			Mass loss	Moisture	Mass loss	Moisture
<i>Arundinaria amabilis</i>	1	+	15.5	187	15.3	148
	1	-	38.6	41	10.8	31
	2	-	38.7	45	8.7	68
	2	-	39.0	56	7.1	40
<i>Bambusa maculata</i>	1	+	9.9	159	11.0	174
	1	-	20.4	39	5.1	28
	2	-	38.3	48	5.0	33
	2	-	34.3	52	3.6	37
<i>Dendrocalamus asper</i>	1	+	5.4	104	5.1	90
	1	-	29.3	42	4.3	28
	2	-	26.9	45	3.8	32
	2	-	28.5	50	4.3	31
<i>Gigantochloa atroviolacea</i>	1	+	6.1	95	6.1	95
	1	-	18.7	34	4.7	25
	2	-	41.6	58	3.0	38
	2	-	42.9	57	5.0	43
<i>Phyllostachys nigra</i>	1	+	9.7	112	16.4	126
	1	-	32.6	49	9.1	32
	2	-	39.7	65	6.6	40
	2	-	40.7	56	6.4	52
<i>Phyllostachys nigra</i> 'Boryana'	1	+	35.3	103	19.7	182
	1	-	38.8	54	6.7	26
	2	-	37.9	60	5.7	40
	2	-	38.8	46	5.1	35
<i>Phyllostachys pubescens</i>	1	+	6.3	61	6.3	63
	1	-	5.4	32	5.4	24
	2	-	38.3	42	5.7	31
	2	-	31.2	43	6.3	36

In contrast to results with small samples in the jar test, bamboo samples in the Fungus cellar were considerably more degraded by *C. puteana* (max. 43%) and *S. commune* (max 20%). Obviously, the moisture conditions in the Fungus cellar test influenced the activity of both fungi, whereby the white-rot fungus *S. commune* differed considerably from the brown-rot species *C. puteana*. *Schizophyllum commune* decayed samples with soil contact and thus with high water content (90-182 % u) more than samples without soil contact. In contrast, *C. puteana* produced a higher mass loss in samples located on wood supports and with lower water content (34-65 % u). Not shown experiments with samples incubated on vermiculite substrate with different water content also demonstrated the influence of moisture on decay.

The tested bamboos differed in susceptibility to fungi (Tables 3 and 4; also Suprati 2010). Furthermore, samples from young culms were more susceptible to decay than older ones (Hamid et al. 2003; Schmidt et al. 2011). Samples from culm top were more decayed than those from the bottom

(Suprati 2010; Schmidt et al. 2011). Sealing the crosscut ends forced the hyphae to penetrate through the epidermis and not through the vessels at the crosscut plane which reduced the rate of decay (Schmidt et al. 2011; also Kleist et al. 2002).

Micromorphological Studies of Degradation

Figure 6 shows examples for the micromorphological degrading patterns of bamboo cell walls. The tissue after brown-rot attack remained nearly intact (Figure 6a). Degradation of lignified cell walls by white-rot fungi (Figure 6b) of the erosion type begins in the lumen (Liese 1970). Excretion of enzymes caused wholes and openings in the cell wall. A conspicuous feature of bamboo is the frequent occurrence of hyphae in the interstitial region. The soft-rot decay (Figure 6c) resembles a late stage soft-rot in wood. Only the highly lignified middle lamella/primary walls and the tertiary wall remained; the secondary wall with the soft-rot hyphae inside was totally destroyed to residues. The findings extend previous work on the few micromorphological degradation studies of bamboo by wood-destroying fungi, which were only observed in the brown-rot species *Gloeophyllum trabeum* (Lee et al. 2006) and the white-rot mushroom *Lentinula edodes* (Ma et al. 2010).

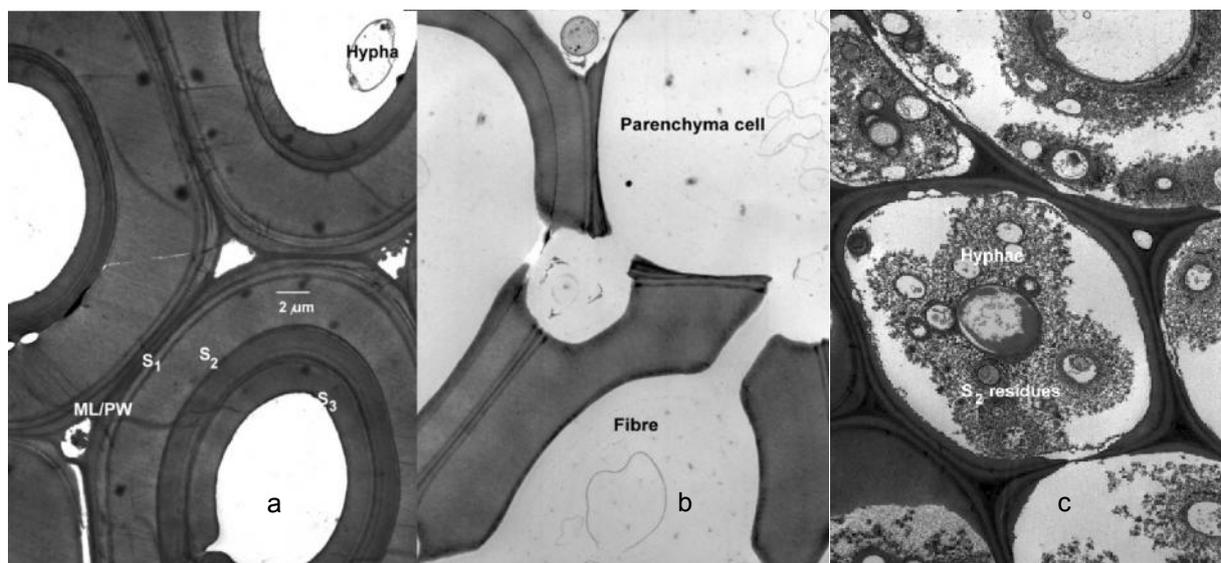


Figure 6. a: Early brown-rot symptoms in *Gigantochloa atroviolacea* by *Coniophora puteana*; b: medium white-rot decay in *Bambusa maculata* by *Trametes versicolor*; c: severe soft-rot degradation in *B. maculata* by *Chaetomium globosum*

In summary, the results show that all bamboo species investigated can be colonized by the various groups of fungi, moulds, staining and rot fungi. Considerable degradation occurs by white-, brown- and soft-rot fungi.

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References

- Abdurachim Martawidjaja, R.A. 1964. Bamboo preservation in Indonesia. *Rimba Indonesia*, 9, 66-76.
- Banerjee, S.; Mukhopadhyaya, S.A. 1962. A study on *Merulius similis* B. & FR. and the associated bamboo rot. *Oestereichische Botanische Zeitschrift*, 109, 197-212.
- EN 113. 1996. Determination of the toxic values of wood preservatives against wood destroying Basidiomycetes cultured on agar medium. Beuth, Berlin, Germany. 15 pp.
- Hamid, N.H.; Abd. Latif, M.; Sulaiman, O. 2003. Decay resistance of bamboo (*Gigantochloa scortechinii*) compared to 24 Malaysian hardwood. XII. World Forestry Congress 2003, Québec, Canada, www.fao.org/docrep/article/wfc/XII/0039-b4.htm
- Kim, J.S.; Lee, K.H.; Cho, C.H.; Koch, G.; Kim, Y.S. 2008. Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. *Holzforschung*, 62(4), 481-487.
- Kim, J.J.; Lee, S.S.; Ra, J.B.; Lee, H.; Huh, N.; Kim, G.H. 2011. Fungi associated with bamboo and their decay capabilities. *Holzforschung*, 65(2), 271-275.
- Kleist, G.; Morris, I.; Murphy, R. 2002. Invasion and colonization of bamboo culm material by stain and decay fungi. IRG Working Paper No. 02-10453. International Research Group on Wood Preservation, Stockholm, Sweden. 10 pp.
- Lee, K.H.; Cho, C.H.; Kim, Y.S. 2006. Micromorphology of bamboo fibers degraded by brown-rot fungus *Gloeophyllum trabeum*. IRG Working Paper No. 06-10576. International Research Group on Wood Preservation, Stockholm, Sweden. 9 pp.
- Leithoff, H.; Peek, R. 2001. Heat treatment of bamboo. IRG Working Paper No. 01-40216. International Research Group on Wood Preservation, Nara, Japan. 11 pp.
- Liese, W. 1959. Bamboo preservation and soft-rot. FAO Report to the Government of India 1106, 36 pp.
- Liese, W. 1970. Ultrastructural aspects of woody tissue disintegration. *Annual Review Phytopathology*, 8, 231-258.
- Liese, W. 1985. Bamboos - biology, silvics, properties, utilization. *Schriftenreihe Gesellschaft für Technische Zusammenarbeit*, Eschborn, Germany, 180, 132 pp.
- Liese, W.; Kumar, S. 2003. Bamboo preservation compendium. INBAR, Beijing, China, Technical Report 22. 231 pp.
- Liese, W.; Schmidt, R. 1966. Untersuchungen zum Zellwandabbau von Nadelholz durch *Trametes pini*. *Holz als Roh- und Werkstoff*, 24, 454-460.
- Ma, X.; Jiang, M.; Qin, D. 2010. The invasion channels of damage fungi in bamboo lumber. IRG Working Paper No. 10-10712. International Research Group on Wood Preservation, Biarritz, France. 7 pp.
- Mohanan, C. 1997. Diseases of bamboos in Asia. International Development Research Centre, New Delhi, India. 228 pp.
- Murphy, R.J.; Alvin, K.L.; Tan, Y.F. 1991. Development of soft rot decay in the bamboo *Sinobambusa tootsik*. *IAWA Bulletin n. s.* 12, 85-94.
- Purushotham, A. 1963. Utilization of bamboo. *Journal Timber Development Preservation Association*, 92, 2-19.
- Razak, W.; Hashim, W.S; Murphy, R.J. 2002. SEM observation on the decay of bamboo *Gigantochloa scortechinii* exposed in tropical soil. *Journal Tropical Forest Products*, 8, 168-178.
- Savory, J.G. 1954. Damage of wood caused by micro-organisms. *Journal Applied Microbiology*, 17, 213-218.
- Schmidt, O.; Grimm, K.; Moreth, U. 2002. Molecular identity of species and isolates of the *Coniophora cellar* fungi. *Holzforschung*, 56(6), 563-571.

- Schmidt, O.; Wei, D.S.; Liese, W. 2011. Fungal degradation of bamboo samples. *Holzforschung*, 65(6), 883-888.
- Spurr, A.R. 1969. A low viscosity embedding medium for electron microscopy. *Journal Ultrastructural Research*, 26, 31-43.
- Suprpti, S. 2010. Decay resistance of five Indonesian bamboo species against fungi. *Journal Tropical Forrest Science*, 22, 287-294.
- Thang, T.K.H.; Schmidt, O.; Liese, W. 2009. Environment-friendly short-term protection of bamboo against moulding. *Journal Timber Development Association India*, 55, 8-17.
- Wang, S.F.; Hsieh, R.C. 1968. Durability records of treated and untreated bamboo. *Cooperation Bulletin Taiwan Forest Research Institute, Taiwan* 15, 26 pp.
- White, T.J.; Bruns, T.; Lee, S.; Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A.; Gelfand, D.H.; Sninsky, J.J.; White, T.J. (eds.). *PCR protocols*. Academic Press, San Diego, 315-322.
- Zang, X.; Yu, H.; Huang, H.; Liu, Y. 2007. Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culm. *International Biodeterioration Biodegradation*, 60, 159-164.

Freezing avoidance in tropical Andean bamboos

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Abstract

Frost resistance was compared in five species of different life forms of the neotropical woody bamboo genus *Chusquea* growing along a cloud forest-paramo gradient, between 2250-4010 m asl in the Venezuelan Andes. *C. purdieana* and *C. serrulata* are viny bamboos of the upper montane cloud forest-treeline ecotone (2200-2700 m and 2450-2900, respectively); whereas *C. angustifolia*, *C. spencei* and *C. guirigayensis* are shrublike bamboos associated to swampy low elevation paramos (2525 m) in the first case, a broad range of paramo ecosystems (2670-3600 m) in the second, and dry high elevation paramos in the third (3800-4010 m). Intercellular ice nucleation and 50 % tissue injury temperatures were estimated in these five species under laboratory conditions in order to determine frost resistance mechanisms. No significant differences were observed among ice nucleation and tissue injury temperatures in any of these species, indicating that all avoid intercellular ice nucleation through supercooling (-12.3 to -10.1 °C). We conclude that variations in supercooling capacity and freezing injury are not related to life form, habitat or elevation and that freezing temperatures are not a determining factor in the altitudinal distribution of tropical Andean bamboos.

Keywords

supercooling capacity, tropical Andes, *Chusquea*, Bambusoideae, altitudinal gradients.

Introduction

Temperature is one of the main environmental factors that affect plant distribution. In the tropical Andes, life-form distribution usually responds to changing environmental conditions along altitudinal gradients, and those that reach the highest altitudes have adapted successfully to resist low temperature environments (Larcher 1995, Körner 1998).

In the Venezuelan Andes, low temperature resistance mechanisms have been described for giant rosettes (Goldstein et al. 1985), trees (Rada et al. 1985, Cavieres et al. 2000, García-Núñez et al. 2004; Azócar et al. 2007, Rada et al. 2009), dwarf shrubs (Squeo et al. 1991, Azócar 2006), herbs (Azócar et al. 1988; Squeo et al. 1991, Azócar 2006, Azócar and Rada 2006) and a wide variety of grasses (Márquez et al. 2006). Above treeline limits, freezing temperatures may occur any night of the year and surviving below zero temperatures is achieved either by frost tolerance (withstanding ice formation in intercellular spaces) or avoidance (withstanding temperatures well below zero without ice formation in any of the tissues) mainly through deep supercooling (Levitt 1972; Beck *et al.* 1982, 1984, Sakai and Larcher 1987, Pearce 2001). The former has been described as a mechanism utilized in harsher environments with prolonged below zero temperatures, whilst the latter, in less severe environments (Larcher 1995, Körner 1998, Sakai and Larcher 1987). In general, freezing avoidance represents the most common mechanism exhibited by woody species of the tropical high Andes (Squeo *et al.* 1991, Azócar 2006, Azócar and Rada 2006, Rada et al. 1985, 1987, 2009), given that periods below 0 °C may occur any night of the year, but remain close to zero lasting only a few hours (Monasterio and Reyes 1980, Azócar and Rada 2006). Avoidance through supercooling in leaves is achieved in tropical Andean giant rosettes and dwarf shrubs thanks to compact mesophylls with low apoplastic water contents (Rada et al. 1987, Azócar 2006, Azócar and Rada 2006). In other paramo woody species like *Polylepis sericea*, avoidance is achieved by the accumulation of osmotically active solutes and carbohydrates (Rada et al. 1985).

In contrast with woody life forms, herbaceous plants including grasses tolerate intercellular ice formation (Squeo et al. 1991, Márquez et al. 2006). Bamboos regarded as “woody grasses”, are associated to a diversity of high altitude tropical ecosystems, from cloud forests to high elevation paramos (Clark 1995, Monasterio and Molinillo 2003, Clark and Ely 2011). However, how this group responds to freezing temperatures remains unknown. Temperate Japanese bamboos of the genera *Sasa* and *Sasamorpha* avoid frost formation in foliage tissues through deep supercooling, reaching freezing temperatures between -22 and -15 °C (Ishikawa 1984, Sakai 1976, 1995, Tanaka 2002). In the tropical Andes, all of the high elevation bamboos belong to the genus *Chusquea* (Clark 1989, Clark 1995, Bussman 2004, Niño et al. 2006, Clark and Ely 2011), and the greatest species diversity may be found in Andean cloud forests, between 2400-2800 m a.s.l (Clark 1995). At higher altitudes (3000-4000 m asl), species diversity decreases markedly, and the viny life-form is replaced by a shrublike one. In the Venezuelan Andes the *Chusquea* species that grow along the upper cloud forest-paramo ecotone are: *C. purdieana* (2200-2700 m asl), *C. serrulata* (2450-2900 m asl), *C. angustifolia* (2525-2800 m asl), *C. spencei* (2670-3600 m asl) and *C. guirigayensis* (3800-4010 m asl). The first two species are upper cloud forest viny bamboos, whereas the remaining three species are shrublike and differ in their altitudinal distribution: *C. angustifolia* grows in swampy low paramos, *C. spencei* along the treeline-paramo ecotone, and *C. guirigayensis* exclusively in high elevation paramos.

At present no studies on the effect of freezing temperatures on high altitude bamboos have been conducted. Two main questions are addressed in this study: Firstly, how do Andean bamboos respond to freezing temperatures? One would expect the species of lower altitudes to be frost avoiders and those of higher elevations frost tolerant. Secondly, does freezing resistance increase in woody bamboos along the altitudinal gradient?

Materials and methods

Field sample collection and thermal analysis were carried out from 2008 through 2010. Fresh leaf samples were collected at different intervals, during both dry and rainy seasons, along an altitude gradient ranging from 2450 to 4010 m asl, comprising upper cloud forests, treeline ecotones, low and high paramos. Samples of *C. purdieana* and *C. serrulata* were collected at 2450 m asl in Monte Zerpa cloud forest (08° 38' 92" N and 71° 24' 63" W). *C. angustifolia* was collected in Las Piñuelas at 2525 m (N 8° 37' 35" and W 71° 24'), which constitutes the lowest paramo of the Venezuelan Andes. *C. spencei*, due to its broad altitudinal range of 930 m (Ely 2009), was collected at three different altitudes: paramos Las Coloradas (N 8° 28' and W 71° 57') at 2670 m, La Culata (N 08° 44' 99" and W 71° 04.17') at 3025 m asl, and La Aguada (N 8° 35' and W 71° 09') at 3320 m asl. *C. guirigayensis*, was collected in the páramo Piedras Blancas (N 8° 53' and W 70° 57'), at 4010 m asl. Mean annual rainfall at these five sites are: 2286 mm in Las Piñuelas, 2520 mm in Monte Zerpa, 1877 mm in Las Coloradas, 1780 mm in La Culata, 1573 mm, in La Aguada (Ely 2009) and 800-900 mm in Piedras Blancas (Kiyota 2011).

Species description

Extensive descriptions of the vegetative characters for these species may be reviewed in Clark (1989), Niño *et al.* (2004), Ely (2009) and Kiyota (2011). Vouchers of the five species collected are deposited in MERC, Instituto Jardín Botánico de Mérida, Faculty of Sciences, University of The Andes, Mérida, Venezuela.

Chusquea angustifolia (Soderstr. and C. Calderón) L.G. Clark. (*F. Ely et al. 2009*, v.s. 44 *MERC*). Shrub of variable height, 0.2-1.7 m high, that grows between 2520-2800 m asl. Foliage leaves pubescent, somewhat sticky, lanceolate, coriaceous, blades 6-8 cm long x 0.2-5 mm wide.

Chusquea guirigayensis Niño, L.G. Clark and Dorr. (*F. Ely et al. 2009*, v.s. 43 *MERC*). Miniature shrub, typically 20-50 cm high (exceptionally 120 cm). In Mérida, this species grows between 3800-4010 m asl. Foliage leaves glabrous, triangular to lanceolate, consistency markedly coriaceous, blades 1-2.5 cm long x 0.3-0.5 cm wide.

Chusquea purdieana Munro. (*F. Ely & Borregales 2006*, v.s. 15 *MERC*). Woody climber, usually 2-6 m high that grows between 2200-2700 m asl. Foliage leaves glabrous, membranous, blades 6.5-8.2 cm long x 3.8-4.2 cm wide.

Chusquea serrulata Pilger. (*F. Ely et al. 2006* v.s. 18 *MERC*). Woody climber, usually 3-6 m high, that grows between 2450-2900 m asl. Foliage leaves glabrous, linear, papery blades 18-30 cm long x 0.4-0.6 cm wide.

Chusquea spencei Ernst. (*F. Ely et al. 2006* v.s. 1 *MERC*). Shrub, ranging from 0.8-3 m high, growing between 2670-3650 m asl. Foliage leaves glabrous, linear-lanceolate, coriaceous, blades 5-14 cm long x 0.2-0.6 cm wide.

Climate measurements

Air temperature (°C) and relative humidity (RH) data from the last 10 years were considered, as well as measurements performed at these six localities; from February to October 2008 in Monte Zerpa, La Culata and La Aguada, and from May to November 2010 in Las Piñuelas, El Molino and Piedras Blancas. Data were registered every 15 minutes with portable data loggers at each site (*HOBO, Pro Series*. Onset, Massachusetts, USA). In all of the study sites, sensors were placed at 1.5 m above ground level, protected from direct full sunlight. These were also placed at ground level in the paramos Las Piñuelas, El Molino and Piedras Blancas.

Thermal analysis

The relationship between tissue ice nucleation and injury temperatures determine frost resistance mechanisms. If these two temperatures coincide well below zero, we refer to freezing avoidance. In frost tolerant plants, ice nucleation temperatures occur close to 0°C while injury at significantly lower temperatures.

Intercellular ice nucleation temperatures were measured in fresh leaves collected at the six sites. A minimum of four trials were carried out in n=18-20 samples per species. Leaf samples of all species were collected at different intervals of the dry (January through March) and the rainy (May through September) seasons. Sampling consisted of five young culms per species of different genets collected from culms separated at a minimum distance of 10 m. Culms were cut at ground level in the field, placed in water and the ends cut again under water to avoid formation of air bubbles in xylem vessels (Lei and Koike 1998). In the lab, culms were covered with black plastic bags and allowed to rehydrate overnight. A total of five leaf samples were placed in small glass test tubes, tightly sealed with rubber stoppers in order to avoid tissue moisture changes. Copper-constantan thermocouples were inserted in the tissue samples and temperature was continuously monitored with a 5-channel data logger connected to a PC. Test tubes were immersed in a refrigerated alcohol bath (*NESLAB*, mod. RTE-111). Temperature was then lowered progressively from 5 °C to -25 °C at a rate of approximately 7.5 °C/h and monitored continuously with specially designed software (*Planta-ICAE*). Intercellular ice nucleation was registered through the formation of exotherms, the result of an abrupt increase in temperature generated by heat released during the freezing process.

Determination of injury temperatures

50 % Injury temperatures were determined in these five species through the electrolyte leakage method used previously by Ishikawa (1984) and later modified by Lindén (2002). Electrical conductivity (μS) was measured in leaf tissues previously submitted to decreasing low temperatures, from 5 °C to -25 °C, and submerged in deionized water (with an initial electrical conductivity of 0 μS). Increases in electrical conductivity resulted from electrolyte leakage due to release of potassium ions as a consequence of cell wall rupture. Tissue samples were submitted to the same frost-inducing procedure described previously. This procedure was performed in n=18-20 samples per species. At 5 °C intervals, three tubes were withdrawn from the refrigerated bath, leaf samples removed from the tubes and placed in clean plastic containers with 15 mm³ of deionized water. The containers were then refrigerated at 6 °C during 48 h, and electrical conductivity was measured with an ExStik digital conductimeter, mod. EC500 (*Extech Instruments*, U.S.A). After measurements were performed, complete tissue rupture was induced by submerging samples briefly in liquid nitrogen and placing them again in their respective containers. These were refrigerated again for 48 h and electrical conductivity was measured afterwards. This last measurement corresponded to the electrical conductivity of samples after 100 % leakage had occurred. Tissue injury was estimated as the temperature at which 50 % leakage occurred through the following equation:

$$T_{50\%} = \frac{\text{Initial Electrical Conductivity}}{\text{Final Electrical Conductivity}} * 100$$

Initial Electrical Conductivity corresponded to the temperature to which the tissue was exposed before withdrawing the tube from the refrigerating bath, whereas Final Electrical Conductivity represented the electrical conductivity of the sample after inducing complete tissue rupture.

Data analysis

Ice nucleation temperatures were represented graphically in box plots (Sigma Plot, ver. 10.0), and average temperatures and standard deviation errors were estimated. U Mann-Whitney non-parametric tests were carried out to determine whether or not differences between intercellular ice nucleation and injury temperatures were statistically significant for each species. Kruskal-Wallis tests (*SPSS Statistics*, ver. 17.0) were performed to determine statistically significant differences regarding ice nucleation and injury temperatures among these five species.

Results

Climate characteristics

Below zero temperatures occurred only above 3000 m, and were relatively infrequent at treeline limits (3025-3320 m) where they remained very close to zero, being more frequent in the upper open paramo limits (3800-4010). The lowest night temperatures were registered in Páramo de Piedras Blancas followed by Páramo La Aguada (Table 1). The frequency of nights with freezing temperatures increased during the dry season (end of December through the end of March 2008) above 3000 m, representing a 10 % of the total of days registered at 3000, 18 % at 3320 m, and 41 % at 4010 m.

Table 1. Average air temperatures registered at the six study sites. Maximum and minimum temperatures registered are indicated in parentheses.

Study site	Average Temperature (°C)		Average Min. temperature (°C)		Average Max. temperature (°C)	
	Soil	Air	Soil	Air	Soil	Air
Monte Zerpa (2450 m)	ID	13.02	ID	8.03 ± 0.01 (6.53)	ID	17.06 (20)
Las Piñuelas (2525 m)	13 ± 0.03	14 ± 0.03	10 ± 0.2 (6.6)	8.5 ± 0.1 (4.6)	25 ± 0.6 (32)	20 ± 0.2 (25)
Las Coloradas (2670 m)	11 ± 0.02	12 ± 0.02	7.5 ± 0.2 (4.6)	9.0 ± 0.1 (7.0)	15 ± 0.2 (19)	17 ± 0.2 (21)
La Culata (3025 m)	ID	9.25 ± 0.07	ID	4.3 ± 0.65 (-0.16)	ID	8.8 ± 0.08 (19)
La Aguada (3320 m)	ID	7.6 ± 0.1	ID	2.3 ± 1.0 (-0.68)	ID	20 ± 0.06 (24)
Piedras Blancas (4010 m)	8.6 ± 0.1	6.2 ± 0.04	1.5 ± 0.1 (-12)	2.0 ± 0.1 (-4)	27 ± 1.0 (46)	15 ± 0.4 (21)

ID: insufficient data due to technical difficulties with the loggers

Intercellular ice nucleation and injury temperature

Both ice nucleation and 50 % injury temperatures were consistent in all of the trials. In these five species, average intercellular freezing temperatures varied between -12.1 and -10.1 °C, whereas average 50 % injury values ranged between -12.3 and -10.3 °C (Table 2). No statistically significant differences were observed between intercellular ice nucleation temperatures (exotherm formation temperature) and injury temperatures in any of the species along the altitudinal gradient, indicating that in these five *Chusquea* species, intercellular ice nucleation was avoided through a moderate supercooling capacity.

Intercellular ice nucleation temperatures did not decrease linearly along the altitude gradient in this genus (Figure 1); nor did they vary significantly between the two viny cloud forest species (*C. purdieana* and *C. serrulata*); nor amongst the latter and the paramo shrublike species growing at the lower and upper limits of this gradient (*C. angustifolia* at 2520 m, the genets of *C. spencei* at 2670 m, and *C. guirigayensis* at 3800-4010 m). However, significant differences were observed between the genets of *C. spencei* growing at lower and upper limits of its distribution range (2670 m vs 3025-3320 m), and among genets of *C. spencei* above 3000 m and the remaining four species (Figure 1, Table 2).

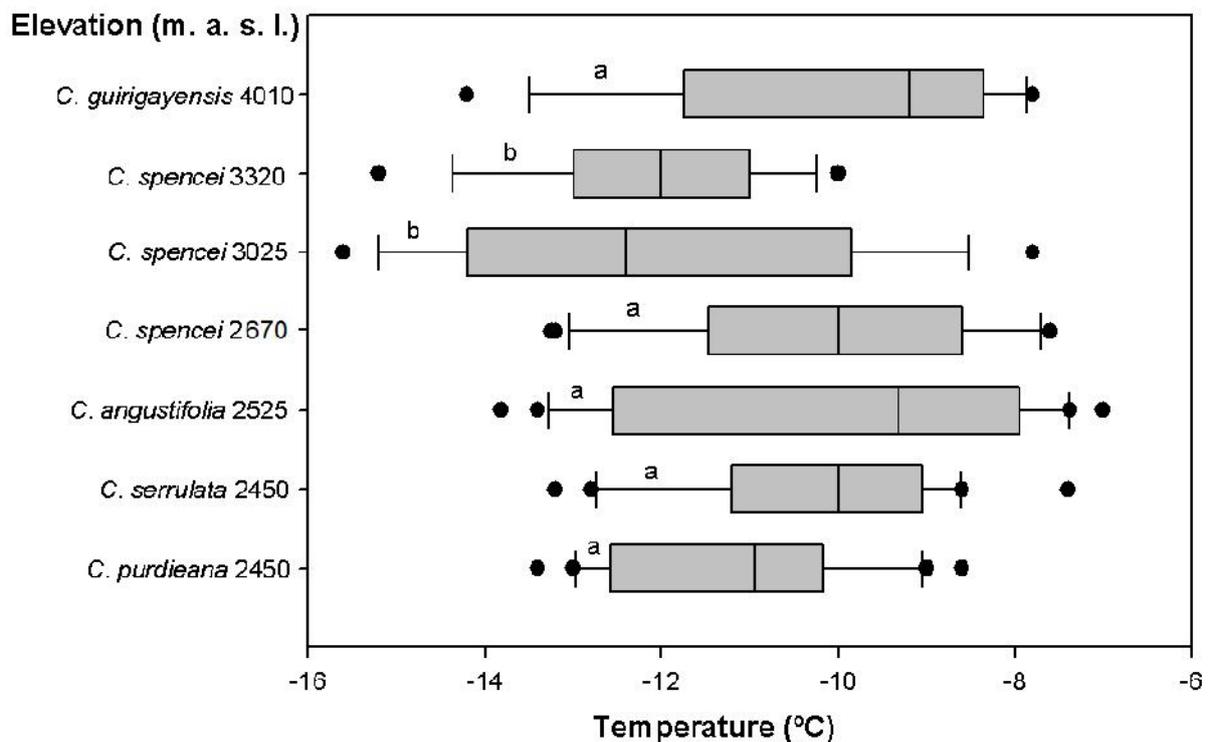


Figure 1. Relationship between ice nucleation temperatures and altitude for five *Chusquea* species along the 1560 m altitudinal gradient. Black circles represent extreme (high and low) values of ice nucleation temperatures in each case. Different letters depict significant differences ($p < 0.05$).

Table 2. Average intercellular ice nucleation and 50% injury temperatures measured in these five *Chusquea* species. Maximum and minimum temperatures are indicated in parenthesis.

Species/elevation (m.asl)	Intercellular ice nucleation temperature (°C)	50 % Injury temperature (°C)
<i>C. purdieana</i> (2450)	(-9.0) -11.0 ± 0.3 ^a (-13.4)	(-10.8) -11.5 ± 0.3 ^a (-12.4)
<i>C. serrulata</i> (2450)	(-9.0) -10.5 ± 0.3 ^a (-13.0)	(-9.6) -10.1 ± 0.4 ^a (-11.9)
<i>C. angustifolia</i> (2525)	(-7.4) -10.8 ± 0.2 ^a (-13.8)	(-11.4) -12.3 ± 0.3 ^a (-13.1)
<i>C. spencei</i> (2670)	(-7.6) -10.6 ± 0.4 ^a (-13.3)	(-8.4) -10.2 ± 0.4 ^a (-13.7)
<i>C. spencei</i> (3025)	(-7.9) -12.1 ± 0.5 ^b (-15.6)	(-9.8) -12.0 ± 0.2 ^b (-13.5)
<i>C. spencei</i> (3320 m)	(-10.4) -12.0 ± 0.3 ^b (-15.0)	(-9.8) -12.2 ± 0.3 ^b (-15.9)
<i>C. guirigayensis</i> (4010 m)	(-8.0) -10.3 ± 0.4 ^a (-14.2)	(-9.0) -10.6 ± 0.3 ^a (-12.0)

Different letters depict significant differences ($p < 0.05$).

Discussion

Chusquea is distinguished as the genus with the greatest diversity regarding species, habitats, life forms and altitudinal distribution in tropical Andean ecosystems (Clark 2001), yet above 3000 m, species diversity decreases markedly in this genus. These variations in species abundance suggest that freezing temperatures could be a determining factor in woody bamboo distribution in tropical mountain ecosystems. We had initially assumed that the bamboos growing below treeline limits (3000 m), which are not exposed to freezing temperatures, such as the viny species (*C. purdieana* and *C. serrulata*) and the genets of *C. angustifolia* and *C. spencei* of low paramo ecosystems (2520 and 2670, respectively) were altogether devoid of freezing resistance mechanisms in which case, ice nucleation and injury temperatures should have occurred very close to 0 °C, as described for other woody species of the upper cloud forest-paramo ecotone (Cavieres *et al.* 2000). Nevertheless, all five species avoided intercellular ice formation through a moderate supercooling, regardless of their life-form, habitat plant height, foliage leaf size or consistency. Supercooling capacity values were higher in these *Chusquea* species than those reported for other paramo woody species (Rada *et al.* 1985, 2009; Cavieres *et al.* 2000), and comparable to those reported for giant rosettes of the genus *Espeletia* (Goldstein *et al.* 1985, Rada *et al.* 1987). Another unexpected result was that neither intercellular ice nucleation nor 50 % injury temperatures varied significantly between the species situated at the upper and lower limits of the cloud forest-paramo gradient (2450 and 4010 m). Only *C. spencei* presented a slight increase in its supercooling capacity with increasing altitude, as the differences between the genets growing below and above 3000 m suggest. A possible explanation for the uniform supercooling capacity observed in these five species is that after the last glaciation, cloud forest and paramo boundaries suffered repeated displacements, with paramo ecosystems descending as low as 2000 m (Van der Hammen 1974, 1988, 2000, Salgado-Labouriau *et al.* 1977, 1992). In addition, minimum air temperatures have also increased considerably during the last decades, reducing the frequency of nocturnal frosts, as recent microclimate studies indicate (Monasterio and Reyes 1980, Azócar and Rada 2006, Azócar 2006, Ely 2009, Kiyota 2011).

Our results suggest that neither plant height nor elevation necessarily condition freezing resistance mechanisms in this group. *C. guirigayensis* with the smallest height and growing at the highest altitudes responds to freezing temperatures in the same manner as the other species studied. Freezing resistance mechanisms differ among herbaceous tussock grasses and strongly lignified grasses such as bamboos, regardless of whether they grow in temperate (Ishikawa 1984, Tanaka, 2002, Ashworth and Pearce 2001, Liu and Osborne 2008) or tropical climates (Márquez *et al.* 2006). Tussock grasses of the Venezuelan paramos tolerate extracellular freezing, with intercellular ice nucleation temperatures between -6.3 and -3 °C, and 50 % injury of foliage tissues occurring between -18 and -9.8 °C (Márquez *et al.* 2006). Tussock grasses are subjected to lower temperatures and for longer intervals; due to their proximity to the ground where the temperatures are lowest in the air-soil gradient, therefore foliage tissues are exposed to freezing temperatures in their early development, in contrast with woody bamboos, in which developing organs are protected from extreme temperatures by thick culm leaves until they have reached maturity (Ely 2009). In the Venezuelan paramos, where freezing air temperatures typically remain close to 0 °C and last for only a few hours, a moderate supercooling capacity combined with the protection of developing organs should be sufficient to impede the formation of intercellular frost in foliage tissues.

These results support the studies conducted in the Japanese species of *Sasa* and *Sasamorpha*, differing only in their greater supercooling capacity (-22 to -15 °C, Sakai 1976, 1995, Ishikawa 1984), which have likely evolved as an adaptation to the seasonal winters (Tanaka 2002). Based on these results we also conclude that the altitudinal distribution of *Chusquea purdieana*, *C. serrulata*, *C. angustifolia* and

C. spencei in the Venezuelan Andes is not conditioned by freezing temperatures, but more likely by other environmental factors not taken into account in the present study.

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References

- Ashworth, E.N.; Pearce, R.S. 2001. Extracellular freezing in leaves of freezing-sensitive species. *Planta*, 214, 798-805.
- Azócar, A.; Rada, F.; Goldstein, G. 1988. Freezing tolerance in *Draba chionophila* a “miniature” caulescent rosette species. *Oecología*, 75,156-160.
- Azócar, A.; Rada, F. 2006. Ecofisiología de plantas de páramo. Instituto de Ciencias Ambientales y Ecológicas (ICAE). Universidad de Los Andes. Mérida, Venezuela. Litorama. pp. 182.
- Azócar, A.; Rada, F.; García-Núñez, C. 2007. Functional characteristics of the arborescent genus *Polylepis* along a latitudinal gradient in the high Andes. *Interciencia*, 32, 663-668.
- Azócar, C. 2006. Relación entre anatomía foliar, forma de vida y mecanismos de resistencia a temperaturas congelantes en diferentes especies del páramo de Piedras Blancas. Undergraduate dissertation. Facultad de Ciencias. Universidad de Los Andes, Mérida, Venezuela. pp. 64.
- Beck, E.; Senser, M.; Scheibe, R.; Steiger, H.; Pongratz, P. 1982. Frost avoidance and freezing tolerance in Afroalpine “giant rosette” plants. *Plant, Cell and Environment*, 5, 215-222.
- Beck, E, Schulze, E, Senser, M, Scheibe, R. 1984. Equilibrium freezing of leaf water and extracellular ice formation in Afroalpine “giant rosette” plants. *Planta*, 162: 276-282.
- Bussman, R. 2004. Regeneration and succession patterns in African, Andean and Pacific tropical mountain forests: the role of natural and anthropogenic disturbance. *Lyonia*, 6: 93-111.
- Cavieres, L.; Rada, F.; Azócar, A.; García-Núñez, C; Cabrera, M. 2000. Gas Exchange and low temperature resistance in two tropical high mountain tree species from the Venezuelan Andes. *Acta Oecologica*, 21,203-211.
- Clark, L.G. 1989. Systematics of *Chusquea* Section *Swallenochloa*, Section *Verticillatae*, Section *Serpentes* and Section *Longifoliae* (Poaceae: Bambusoideae). *Systematic Botany Monographs*, 27, 1-127.
- Clark, L.G. 1995. Diversity and Distribution of Andean woody bamboos (Poaceae: Bambusae). In: Churchill, S. P., et al. ed.: *Biodiversity and Conservation of Neotropical Montane Forests*. pp. 501-512.
- Clark, L.G. 2001. Diversification and endemism in Andean woody bamboos (Poaceae: Bambusoideae). *Journal of the American Bamboo Society* 15 (1), 14-19.
- Clark, L.G.; Ely, F. 2011. Géneros de bambúes leñosos (Poaceae: Bambusoideae: Arundinarieae, Bambuseae) de Venezuela. *Acta Botanica Venezolánica* 34(1), 79-103.
- Ely, F. 2009. Respuesta ecofisiológica y diversidad genética de *Chusqueas* (Bambusoideae, Poaceae) de la Cordillera de Mérida. Doctoral dissertation. Facultad de Ciencias, Universidad de Los Andes. Mérida, Venezuela. pp. 211.
- Goldstein, G.; Rada, F.; Azócar, A. 1985. Cold hardiness and supercooling along an altitudinal gradient in Andean rosette species. *Oecologia*, 68. 147-152.

- Ishikawa, M. 1984. Deep supercooling in moist tissues of wintering *Sasa senanensis* and its mechanism in leaf blade tissues. *Plant Physiology*, 75, 196-202.
- Kiyota, B. S. 2011. Estructura morfoanatómica y resistencia al congelamiento en bambúes de páramo del género *Chusquea* (Bambusoideae: Poaceae). Undergraduate dissertation. Facultad de Ciencias, Universidad de Los Andes. Mérida, Venezuela. pp. 92.
- Körner, C. 1998. A re-assessment of high elevation treeline positions and their explanation. *Oecologia*, 115, 445-459.
- Larcher, W. 1995. *Physiological Plant Ecology*. 3rd Edition. Springer-Verlag. Berlin. pp. 504.
- Lei, T.; Koike, T. 1998. Functional leaf phenotypes for shaded and open environments of a dominant dwarf bamboo (*Sasa senanensis*) in Northern Japan. *International Journal of Plant Sciences*, 159, 812-829.
- Levitt, J. 1972. *Responses of plants to environmental stresses*. Academic Press, London. pp. 75-109.
- Lindén, L. 2002. Measuring cold hardiness in woody plants. Doctoral dissertation. Faculty of Agriculture and Forestry. University of Helsinki. Helsinki, Finland. pp. 57.
- Liu, M.Z.; Osborne, C.P. 2008. Leaf cold acclimation and freezing injury in C3 and C4 grasses of the Mongolian Plateau. *Journal of Experimental Botany*, 59, 4161-4170.
- Márquez, E.; Rada, F.; Fariñas, M. 2006. Freezing tolerance in grasses along an altitudinal gradient in the Venezuelan Andes. *Oecologia*, 150, 393-397.
- Monasterio, M; Reyes., S. 1980. Diversidad ambiental y variación de la vegetación en los páramos de los Andes venezolanos. In: Monasterio, M. ed.: *Estudios Ecológicos en los Páramos de los Andes Venezolanos*. Universidad de Los Andes. Mérida. Venezuela. pp. 47-91.
- Monasterio, M; Molinillo, M. 2003. Los páramos de Venezuela. In: Hofstede, R. et al. ed.: *Los páramos del mundo. Proyecto Atlas Mundial de los Páramos*. Global Peatland Initiative/NC-IUCN/Ecociencia. Quito, Ecuador. pp. 205-236.
- Niño, S.M.; Clark, L.G.; Dorr, L.J. 2006. Una nueva especie de *Chusquea* (Poaceae: Bambusoideae) de la Cordillera de Mérida, Venezuela. *Brittonia*, 58, 46-51.
- Pearce, R.S. 2001. Plant freezing and damage. *Annals of Botany*, 87, 417-424.
- Osborne, C. P.; Wythe, E. J.; Ibrahim, D. G.; Gilbert, M. E.; Ripley, B. S. 2008. Low temperature effects on leaf physiology and survivorship in the C3 and C4 subspecies of *Alloteropsis semialata*. *Journal of Experimental Botany*. doi:10.1093/jxb/ern062
- Rada, F.; Goldstein, G.; Azócar, A.; Meinzer, F. 1985. Daily and seasonal osmotic changes in tropical treeline species. *Journal of Experimental Botany*, 31, 989-10.
- Rada, F.; Goldstein, G.; Azócar, A.; Torres, F. 1987. Supercooling along an altitude gradient in *Espeletia schultzii* a caulescent giant rosette species. *Journal of Experimental Botany*, 38, 491-497.
- Rada, F.; García-Núñez, C.; Rangel, S. 2009. Low temperature resistance in saplings and ramets of *Polylepis sericea* in the Venezuelan Andes. *Acta Oecologica*, 35, 610-613.
- Sakai, A. 1976. Adaptation of plants to deposited snow. *Low Temperature Series*. B 34:7-76.
- Sakai, A. 1995. The plant distribution and environmental adaptation: from the tropics to the polar zone and desert. Asakura-Shoten, Tokyo. pp. 164.
- Sakai, A.; Larcher, W. 1987. Frost survival of plants. Responses and adaptation to freezing stress. In: Billings, WD, Golley, F, Lange, OJ, Olson, Remmert JSH. ed. *Ecological Studies*, vol. 62. Springer-Verlag, Berlin.
- Salgado-Labouriau, M. L.; Schubert, C.; Valastro, S. 1977. Paleocologic Analysis of a Late-Quaternary Terrace from Mucubaji, Venezuelan Andes. *Journal of Biogeography*, Vol. 4, No. 4, pp. 313-325.

- Salgado-labouriau M. L., Bradley, R. S.; Yuretich, R., Weingarten B. 1992. Paleoecological Analysis of the Sediments of Lake Mucubaji, Venezuelan Andes. *Journal of Biogeography*, Vol. 19, No. 3, pp. 317-327.
- Squeo, F.; Rada, F.; García, C.; Ponce, P.; Rojas, A.; Azócar, A. 1991. Freezing tolerance and avoidance in high tropical Andean plants, is it equally represented in species with different plant height? *Oecología*, 86, 378-382.
- Tanaka, N. 2002. Distribution and Ecophysiological Traits of Dwarf-Bamboo Species and Impact of Climate Changes in Japan. *Global Environment Research*, 6, 31-40.
- Van der Hammen, T. 1974. The Pleistocene changes of vegetation and climate in tropical South America. *Journal of Biogeography*, Vol. 1, No. 1, pp. 3-26.
- Van der Hammen, T. 1988. The Tropical Flora in Historical Perspective. *Taxon*, Vol. 37, No. 3, pp. 515-518.
- Van der Hammen, T. 2000. Aspectos de historia y ecología de la biodiversidad norandina y amazónica. 24 (91): 231-245.

Comparison of plant species richness between hedgerows and a bamboo field in Ireland.

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Abstract

The aim of the study was to investigate whether the presence of a bamboo biomass plantation, consisting of several exotic bamboo species (*Phyllostachys humilis*, *P. decora*, *P. bissetii*, *P. aurea* and *P. aureosulcata* ‘Alata’), has a negative influence on the indigenous rural flora around the plantation. The study was executed on a six year old bamboo plantation in county Dublin, Ireland. The bamboo plantation as well as 41 hedgerows on the same property, were inspected and scored to obtain full species lists. In total, 101 different species were found in the 42 samples. A Twinspan-analysis divided these samples in 11 groups, not showing a clear distinction between the bamboo field and the hedgerows. Based on the data recorded in the hedges, a species-area relation was drawn up, resulting in the equation $S = 2,366 A^{0,381}$. Using this equation the area around the bamboo field should only contain 37 species, instead of the 39 species found. Multiple Sørensen indices were used to make a pairwise comparison between every hedge and the bamboo field and resulted in an average of $0,41 \pm 0,10$. Given the fact that the bamboo field contains more species than expected and that the Twinspan-analysis could not make a clear distinction between the bamboo site and the neighbouring hedgerows, the conclusion of this study is that the presence of exotic bamboos in an agricultural setting is not disadvantageous for the floral biodiversity.

Keywords

bamboo, hedgerow, species richness, Twinspan-analysis

Introduction

The need for sustainable resources becomes more and more pressing in the face of a peak oil threat. Several novel sources of energy are being investigated nowadays, such as wind, water or solar energy, each with their advantages and disadvantages. One major quality of biomass as a replacement for mineral oil is all the more important – whereas the other sustainable sources for energy are solely applicable to a replacement of our energy supplies, biomass is able to replace fossil fuels as a major resource for other industries as well (for example, the plastics industry, which relies heavily on organic chemicals derived from cracked oil) (El Bassam et al. 1999). Also in the densely populated parts of the European Union, biomass will have to be introduced into the existing industrial production (Commission of the European Community 2006).

One candidate which is currently considered to be part of the European biomass crop portfolio is bamboo - or rather, the temperate species of the Bambuseae, a subfamily of the Poaceae. The whole group worldwide consists of more than 70 genera and about 1,450 species (Gratani et al. 2008). Bamboo is one of the fastest-growing plants on earth with reported growth rates of 100 cm in 24 hours (Farrelly 1984). Some of the largest timber bamboo can grow over 30 meters tall. However, the growth rate is dependent on local soil and climatic conditions as well as on the species. A more typical growth rate for many commonly cultivated bamboos in temperate climates is in the range of 3-10 cm a day during the growing period. This fast growth, together with a number of other possibly advantageous practical characteristics (see also Potters et al., in press) is of course what attracts investors in Europe, and serious efforts are now being undertaken to set up industrial networks to start using bamboo in different commercial sectors. The future of many branches of the industry clearly looks greener than ever (Scurlock et al. 2000, Potters et al. 2009, Potters et al. in press).

Nevertheless, it is of paramount importance that we do not combat one risk for the environment (the fossil fuels) with another (see also Stoeglehner and Narodoslawsky, 2009). Introducing large monocultures of biomass crops, threatening the existence of several local herbaceous plants, would irreparably damage the local biodiversity. Especially now, care for our biodiversity is needed, in a time period when it is already under heavy pressure due to the ongoing competition between the human population and the other species, trying to occupy the same areas to live in. On too many previous occasions, this care has been neglected, which has led to the establishment of many unwanted invasive plants in the European ecosystems: *Solidago canadensis* (Immel et al. 2011), *Buddleja davidii* (Ebeling et al. 2011), *Ambrosia artemisiifolia* (Gerber et al. 2011), *Fallopia japonica* (Shaw et al. 2011) and of course *Prunus serotina* (Closset-Kopp et al. 2011). Introduction of novel crops for biomass should therefore occur in such a way that local biodiversity is maximally protected.

One of the considerations to address before introducing bamboo into Europe as a novel energy and biomass crop, therefore concerns its exotic and invasive character. Indeed, bamboo species can be found in diverse climates, from cold mountains to hot tropical regions. They occur across East Asia (Bystriakova et al. 2003), in sub-Saharan Africa and in the Americas. Only (continental) Europe is not known to have any native species of bamboo (Huxley et al. 1992). Bamboo is in every definition of the word, an exotic plant group for the Old Continent.

The rapid spreading of the rhizomes of several species, linked to the fact that most of the growth reserves are being kept underground in these rhizomes, makes that a failure to contain bamboo plants to the plantation area (due to its spreading rhizomes) and therefore this might have an impact on the nearby (natural) ecosystems (Potters et al. 2009). Moreover, the dense canopy of bamboo, and its capacity to efficiently drain the water from a given area are further cause for concern for the

indigenous flora, and raise the question on whether it is safe to release plants of this group into the fields. Bamboo might therefore also be an invasive plant, with a devastating potential for endemic flora and fauna.

In this perspective the study aims to investigate this possible negative effect. We do this by comparing the plant species richness near a mature (6-7 y) bamboo field with the one in the hedgerows found in the same area.

Material en methods

Data collection

The area of interest is a bamboo field (40 x 100 m) near Ballyboughal in county Dublin, Ireland (53°31'N, 6°15'W) and the hedgerows on the same property (Figure 1). On this bamboo field, five temperate species have been planted, namely *Phyllostachys humilis*, *P. decora*, *P. bissetii*, *P. aurea* and *P. aureosulcata* 'Alata', who all spread by rhizomes and have their origin in the temperate regions of China. The plantation was for the largest part constructed in 2005. Plants were put in rows along the long side of the field, with strips of 5 m wide each dedicated to *P. humilis*, *P. decora* and *P. bissetii*, and a larger strip of 11 m wide used for *P. aurea*. *P. aureosulcata* has been added in an additional 5 m strip in 2007. The plants used had been produced via in vitro techniques in the laboratoria of Oprins Plant NV, Rijkvorsel, Belgium, and were planted after hardening up to a 2 L pot stage. The first year, the plants were only watered in summer and semozine was used to suppress upcoming weeds. The second year they also were only watered in summer and spot-treatment of emerging weeds was carried out. No more watering was deemed necessary from the third year onwards, and neither was spraying with herbicides. Van Goethem et al. (in press) discusses the agricultural yield of the field.



Figure 1: Picture of the property in Ireland (marked in red) and of the bamboo field on that property (marked in yellow). Adapted from Google Maps.

The perimeter of the bamboo field (with a width of 5 m) was chosen as a sampling site as well as 41 different hedgerows. These were defined using adjacent land use, management of the hedge and the dominant plant species in that part of the hedge as criteria. Once a hedgerow was identified, a complete list of plant species was recorded, by walking the two sides of the hedge and noting presence

or absence. In a second stage lengths and widths of the 41 identities were measured. The same way of working was used on the bamboo field. Plants were recorded along the four sides and the dimensions were noted as well. Plant identity was determined using Rose and O'Reilly (2006), Mennema (1994), Stace (2010) and Streeter et al. (2010).

In this way a dataset, with 42 samples and 101 different species was made. A summary of the data is given in tables 1 and 2.

Data processing

A part of this dataset, the list of species in every sample, was used to make a two-way indicator species analysis (Twinspan, Hill and Šmilauer 2005). Twinspan is a computer program designed for vegetation science, which classifies the samples according to the principal indicator species.

Based on the dataset it was also possible to count the total number of species in a hedgerow and to calculate the area of each sampling site (Table 1). Doing so, a species-area relation was drawn up, using the standard equation $S = cA^z$. For the bamboo field an area of 1400m² was used, as only the 4 sides were sampled (at a width of 5 m along every side) and not the whole surface.

Afterwards an overview was made to see how many species were common between the bamboo field and every hedge. These numbers were then used to calculate multiple Sørensen similarity indices by the following formula: $QS = 2C / A + B$ with A and B number of species in samples A (a hedge) and B (the bamboo field) and C number of common species. In this way it was possible to tell the extent to which the flora around the bamboo field resembled the community in the hedges nearby.

Results

Twinspan

The Twinspan-analysis couldn't make a clear separation between the hedges and the bamboo field and resulted in 11 groups (Figure 2), that are more or less related to each other.

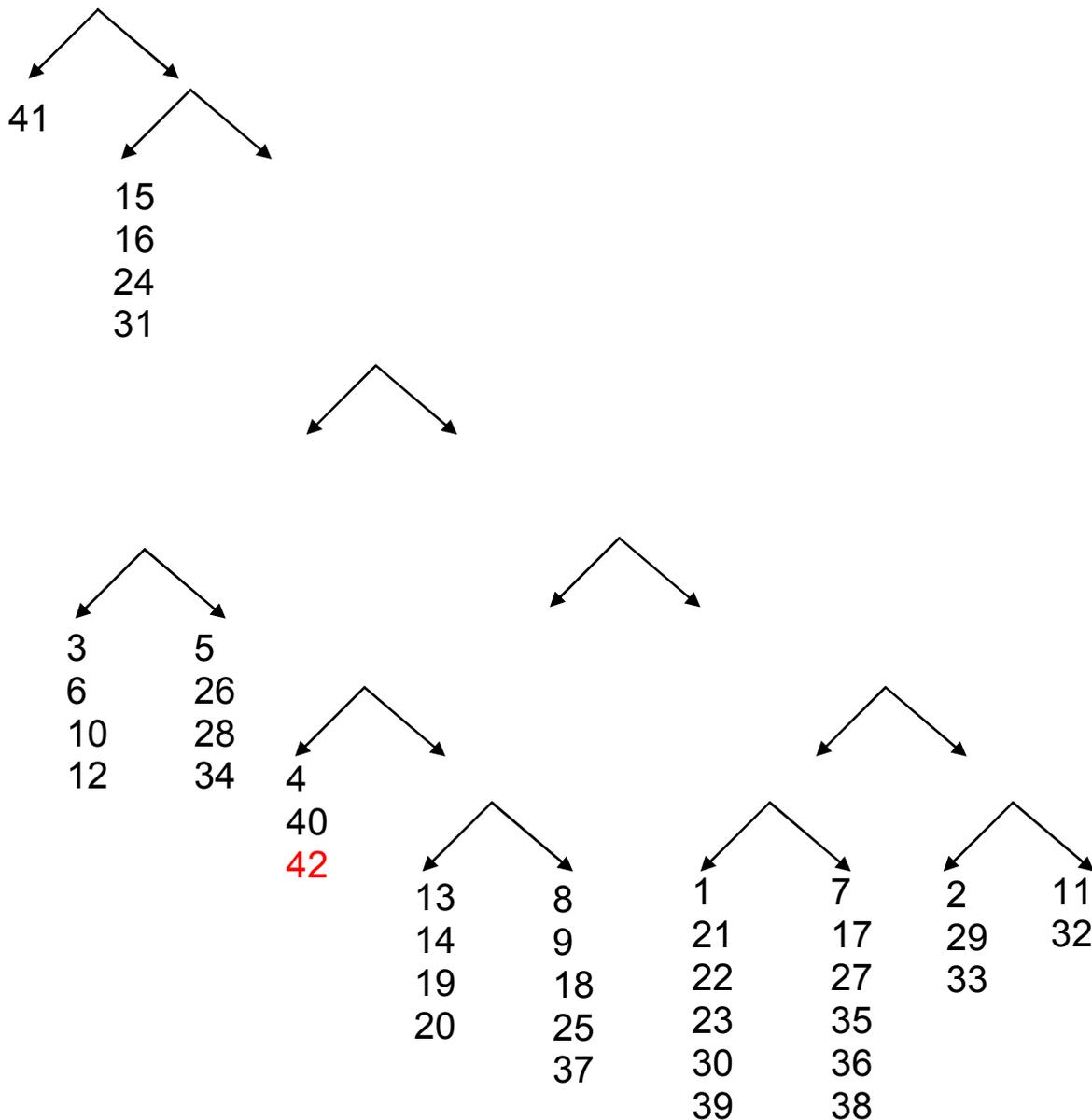


Figure 2: Classification of the samples in groups using Twinspan. Numbers 1 till 41(in black) are the different hedgerow identities and number 42 is the bamboo field (in red).

Group 1 contains only one hedge (number 41), notable for the fact that the land owner had used this part to plant a number of trees that are definitely absent elsewhere on his land. Group number 2 contains four hedgerows (numbers 15, 16, 24 and 31). Group 3 consists of four identities (3, 6, 10 and 12). Group number 4 has as well four hedges (5, 26, 28 and 34). Group 5 contains hedgerows number

4 and 40 and the bamboo field (number 42). Hedges 13, 14, 19 and 20 make up group 6. Group number 7 contains five hedgerows (numbers 8, 9, 18, 25 and 37). Group 8 consists of six identities (1, 21, 22, 23, 30 and 39). Group number 9 has as well six hedges (7, 17, 27, 35, 36 and 38). Group 10 contains only three hedgerows (numbers 2, 29 and 33). Hedges 11 and 32 are part of group 11.

The principal indicator species for every sample are listed in table 3.

Species-area relation

On average there were 23 species recorded in every hedgerow, with a minimum of 9 and a maximum of 39. Along the sides of the bamboo field 39 plant species were found. So in absolute numbers the bamboo plantation is doing quite good. Yet it is necessary to make a correction for the area in which the plants were situated. For this purpose a species-area relation was constructed using the data collected in the hedges. The standard equation used was $S = cA^z$. In this case c was 2,366 and z was 0,381 which gave us $S = 2,366 A^{0,381}$ with an R^2 of 0,5401 (Figure 3). According to this equation the bamboo field should contain 37 species, which is exceeded by the 39 species found along the borders of the bamboo field. The bamboo field is situated in the upper part of figure 3 (encircled in black).

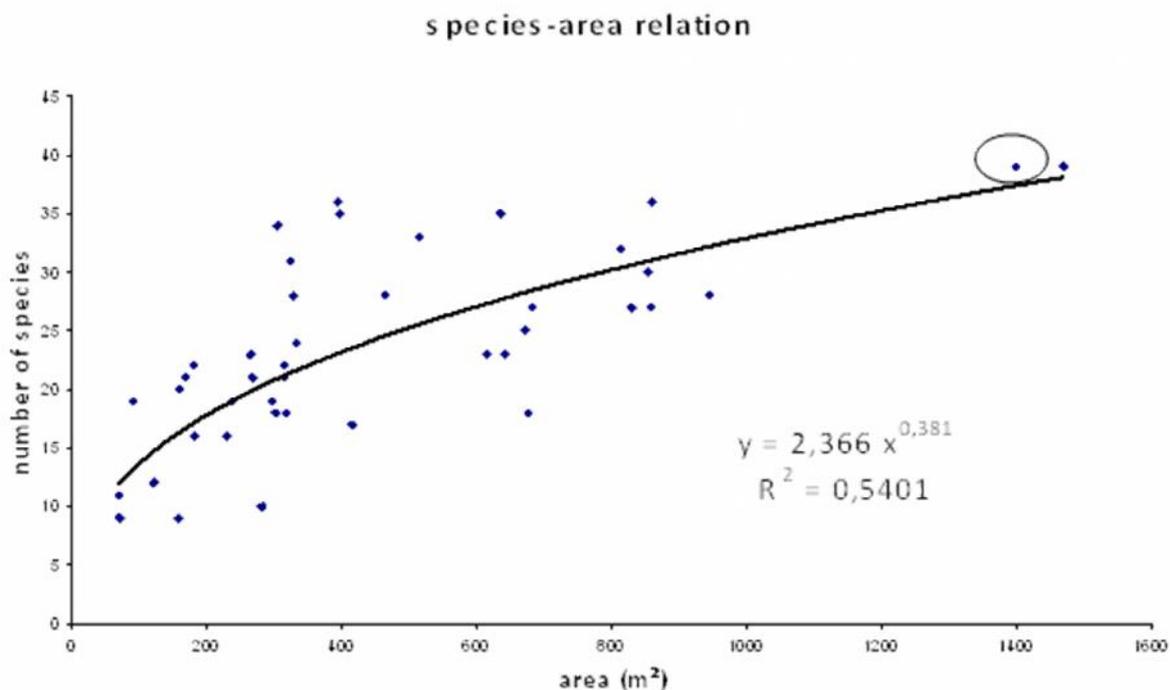


Figure 3: Species-area relation. The bamboo field is encircled.

Sørensen similarity index

To compare the bamboo field with the 41 different hedgerows, 41 Sørensen similarity indices were calculated, ranging from 0,13 till 0,58 with an average of $0,41 \pm 0,10$. In figure 4 these indices were plotted against the distance of the different groups in the Twinspan-analysis. As could be expected similarity decreases when groups are less related to each other.

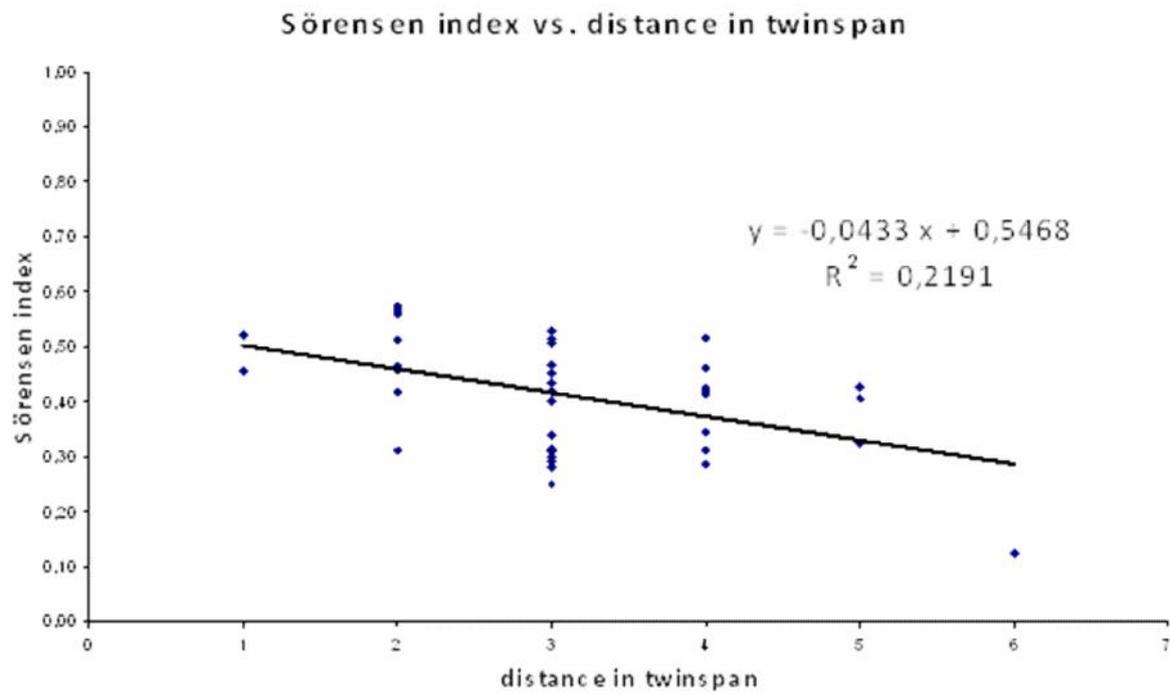


Figure 4: Sørensen similarity index versus distance of groups in Twinspace.

Discussion

Experimental strategy

As main method in this paper, we used the Twinspan classification method. This method is essentially a divisive polythetic classification algorithm, that operates on the basis of the presence or absence of a number of principal indicator species, and not necessarily on the basis of species abundances. Twinspan has been used elsewhere for similar purposes (Abd El-Ghani et al. 2011).

The choice for Twinspan resides in the fact that the data presented here are part of an even larger set, spanning all the hedgerows to be found in 1 km² of the Irish countryside village of Ballyboughal. This set means to provide a full overview of all the rare species in the neighbourhood, as well as their possible migrating pathways through the landscape; details of the full analysis, which is still ongoing, will be published elsewhere. As it was the main focus to make a complete overview of all species, and time was running short, we opted to focus on noticing only the presence and not also the abundance of the (in total) more than 200 species. The presence of the bamboo field within our study area, however, prompted us to try and give an answer to the question regarding the effect of such an exotic plantation, even though one might come up with a number of remarks on the side. For one – the data set as it is now, does not allow for a full biodiversity study (using, for example, Shannon-Wiener indices, which require the knowledge of species abundances). Nevertheless, even by using only species richness, we believe that we can show two things: (1) the fact that the vegetation around the bamboo field sports indicator species that can be seen in many other sample zones (hedgerows) and (2) that the species richness is sufficiently high and comparable with what can be expected in the region.

Is bamboo invasive or not ?

As demonstrated in this study, the vegetation around the local biomass production site in Ballyboughal does not differ significantly from the other hedgerow populations in the area. Hence, there is hope that biomass cultivation and local biodiversity protection might not oppose each other.

One possible way of becoming an invasive species, is through effective seed dispersal. Most bamboo species flower infrequently. In fact, many bamboos only flower at intervals as long as 65 or 120 years. These taxa exhibit mass flowering (or gregarious flowering), with all plants in a particular species flowering worldwide over several years. In these species, all plants of the same stock flower at the same time, regardless of differences in geographic locations or climatic conditions, and afterwards the bamboo dies. The lack of environmental impact on the time of flowering indicates the presence of some sort of “alarm clock” in each cell of the plant which signals the diversion of all energy to flower production and the cessation of vegetative growth (Soderstrom and Calderon 1979). This mechanism, as well as the evolutionary cause behind it, is still largely a mystery. However, considering whether bamboo is just exotic or also invasive in Europe, the infrequent mass flowering is at least a piece of evidence against the invasive character of the plant. Invasiveness is often coupled to an efficient and quick dispersion strategy, which cannot be executed with rhizome spreading alone.

Moreover, bamboo is not the only plant group for which a similar effect has been described. To quote in this respect a recent study on poplar (*Populus* sp.) : “A comparison among 21 poplar plots, 0.1-13 ha large and adjacent arable fields, indicates that small poplar plantations may increase floristic diversity on a landscape scale, mainly by providing a different type of habitat that may favour shade-tolerant and draught-sensitive species. This is reflected by a relatively low number of species shared by both types of habitat. Poplar plantations also show greater floristic heterogeneity compared to arable fields.” (Weih et al. 2003, Karačić 2005). Archaux et al. (2010) demonstrated the same for a

poplar plantation in France, indicating that the management techniques that are applied to the field have a major impact on the herbs that are conserved in and about the plantation. Similar opportunities have been described for the local fauna around biomass plantations (Verch 1986, Christian 1997, Bergström and Guillet 2002, Schulz et al. 2009).

As these studies indicate, a small field of biomass crop between fields and grasslands with other uses should not become a threat for the local flora; even more so – because of the longer periods between harvests (or at least the recommended practices that the fields are never harvested in their entirety), biomass fields such as the bamboo plantation in Ballyboughal might well serve as a refuge and a seed bank for different local species (and certainly more so than the average maize or wheat field). Assessment of a series of management strategies for different biocrops might even favour this effect. One could for example surmise that small plots, mixed into the types of land use that are common for the area in which the plantation(s) are being organised, together with additional attention to the creation of enough landscape elements that allow plant and animal migration, might then actually benefit local biodiversity. Given the expected increase in biomass plantations in Europe, it would be wise to direct an additional research effort towards increasing the ecological yield of these plantations (in terms of an enhanced biodiversity), in parallel with the efforts that wish to enhance the economic yield.

Conclusion

Clustering the edge of a bamboo field together with a number of hedgerows fails to demonstrate great differences in species composition between the vegetation around the field and in the hedges. Species richness is not affected along the borders of the bamboo field, as shown by the species-area-curve. Neither of these two methods could therefore indicate a negative influence of bamboo on the indigenous plant species, at least for this property in Ireland. We therefore raise the hypothesis that bamboo biomass plantations need not have negative influence on the rural flora, given well adjusted management of the plantations.

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References

- Abd El-Ghani M., Bornkamm R., El Sawaf N., Turky H (2011) Plant species distribution and spatial habitat heterogeneity in the landscape of urbanizing desert ecosystems in Egypt. *Urban Ecosystems* 14, 585-616
- Archaux F., Chevalier R. and Berthelot A. (2010). Towards practices favourable to plant diversity in hybrid poplar plantations. *For. Ecol. Manage.* 259, 2410-2417.
- El Bassam N., Meier D., Gerdes C. and Korte A.M. (1999) Modern biofuels from bamboo: Bio-oil, charcoal and gas! *Temperate Bamboo Quarterly* IV(1), 12-18.
- Bergström R. and Guillet C. (2002) Summer browsing by large herbivores in short-rotation willow plantations. *Biomass and Bioenergy* 23, 27-32.
- Bystriakova N., Kapos V., Lysenko I. and Stapleton C.M.A. (2003) "Distribution and conservation status of forest bamboo biodiversity in the Asia-Pacific Region". *Biodiversity and Conservation* 12 (9), 1833-1841. doi:10.1023/A:1024139813651.

- Christian D.P. (1997) Wintertime use of hybrid poplar plantations by deer and medium-sized mammals in the Midwestern U.S. *Biomass & Bioenergy* 12, 35-40.
- Commission of the European Communities (2006) Green paper: A European strategy for sustainable, competitive and secure energy. SEC(2006) 317.
- Closset-Kopp D., Saguez R. and Decocq G. (2011) Differential growth patterns and fitness may explain contrasted performances of the invasive *Prunus serotina* in its exotic range. *Biological Invasions* 13, 1341-1355.
- Ebeling S.K., Stöcklin J., Hensen I. and Auge H. (2011) Multiple common garden experiments suggest lack of local adaptation in an invasive ornamental plant. *Journal of Plant Ecology* 4, 209-220.
- Farrelly D. (1984) *The Book of Bamboo*. Sierra Club Books. ISBN 087156825X.
- Gerber E., Schaffner U., Gassman A., Hinz H.L., Seier M. and Müller-Schärer H. (2011) Prospects for biological control of *Ambrosia artemisiifolia* in Europe: learning from the past. *Weed Research* 51, 559-573.
- Gratani L., Crescente M.F., Varone L., Fabrini G. and Digiulio E. (2008) Growth pattern and photosynthetic activity of different bamboo species growing in the Botanical Garden of Rome. *Flora* 203, 77-84.
- Hill M.O. and Šmilauer P. (2005) *TWINSPAN for Windows version 2.3*. Centre for Ecology and Hydrology & University of South Bohemia, Huntingdon & Ceske Budejovice.
- Huxley A., Griffiths M. and Levy M. (1992) *New RHS Dictionary of Gardening*. Macmillan New RHS Dictionary of Gardening. ISBN 0-333-47494-5.
- Immel F., Renaut J. and Masfaraud J.F. (2011) Physiological response and differential leaf proteome pattern in the European invasive Asteraceae *Solidago canadensis* colonizing a former cokery soil. *Journal of Proteomics* 75, 1129-1143.
- Karačić A. (2005) *Production and Ecological Aspects of Short Rotation Poplars in Sweden*, Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala, ISBN 91-576-7012-9.
- Mennema J. (1994) *Heimans, Heinsius en Thijssse's geïllustreerde flora van Nederland*. W. Versluys, Amsterdam, 23th edition
- Potters G., Brems A., Valcke R., Dewil R., d' Haese L., Samson R and Gielis J. (2009) Energy crops in Western Europe: is bamboo an acceptable alternative? 8th World Bamboo Congress proceedings 3, 22-34.
- Potters G., Schutte F., Van Goethem D., De Nollin S., Samson R. and Gielis J. *Bamboo as a crop in Western Europe - A SWOT analysis*. *Acta Horticultura*, in press.
- Rose F. and O'Reilly C. (2006) *The Wild Flower Key (Revised Edition) - How to identify wild plants, trees and shrubs in Britain and Ireland*, Warne, 576 p.
- Schulz U., Brauner O. and Gruss H. (2009) Animal diversity on short-rotation coppices - A review. *Landbauforschung Volkenrode* 59, 171-182.
- Scurlock J.M.O., Dayton D.C. and Hames B. (2000) Bamboo: an overlooked biomass resource? *Biomass and Bioenergy* 19, 229-244.
- Shaw R.H., Tanner R., Djeddour D. and Cortat G. (2011) Classical biological control of *Fallopia japonica* in the United Kingdom - lessons for Europe. *weed Research* 51, 552-558.
- Soderstrom T.R. and Calderon C.E. (1979) "A Commentary on the Bamboos (Poaceae: Bambusoideae)." *Biotropica* 11 (3), 161-172. doi:10.2307/2388036. JSTOR 2388036.
- Stace C. (2010) *New Flora of the British Isles*, Cambridge University Press, 1266 p.
- Stoeglehner G. and Narodoslawsky M. (2009) How sustainable are biofuels? Answers and further questions arising from an ecological footprint perspective. *Bioresource Technol.* 100, 3825-3830.

- Streeter D., Hart_Davies C., Hardcastle A., Cole F. and Harper L. (2010) Collins Flower Guide (Britain and Ireland), Collins, 704 p.
- Van Goethem D., Ooms A., Bogaerts L., Jespers E., Klimina I., Samson R., O'Connor D., O'Connell P., Denollin S. and Potters G. Bamboo growth under Irish skies: pushing the limits of a sturdy plant, *Acta Hort.*, in press.
- Verch R.L. (1986) Non-game breeding bird activity in an intensively cultured *Populus* plantation, U.S. Department of Agriculture Forest Service, North Central Forest Experiment Station, St. Paul, MN.
- Weih M., Karacic A., Munkert H., Verwijst T. and Diekmann M. (2003) Influence of Young poplar stands on floristic diversity in agricultural landscapes (Sweden). *Basic and Applied Ecology* 4, 149-156.
- "*Arundinaria gigantea* (Walt.) Muhl. giant cane". PLANTS Database. USDA. <http://plants.usda.gov/java/profile?symbol=ARGI>.

Tables

Table 1: List of all samples, with number of species and dimensions per sample.

Numbers 1 till 41: hedgerows, number 42: bamboo field.

sample	number of species	area (m ²)
1	23	642
2	9	70
3	39	1470
4	30	854
5	24	333
6	17	416
7	23	616
8	35	396
9	27	858
10	28	945
11	32	813
12	19	91
13	35	635
14	34	305
15	22	180
16	22	314,5
17	33	516
18	28	464,75
19	36	860
20	36	393,75
21	18	676
22	31	324
23	16	229,5
24	25	672
25	28	327,5
26	27	682,5
27	11	70
28	27	830
29	20	160
30	18	318
31	10	282
32	21	168
33	21	315
34	12	121,5
35	23	264
36	16	182,5
37	19	237,5
38	19	295,75
39	21	267,75
40	18	302
41	9	157,5
42	39	1400

Table 2: Summary of the data collected on hedgerows.

	number of species	area (m ²)
average	23	440
minimum	9	70
maximum	39	1470

**Table 3: List of principal indicator species per sample.
Numbers 1 till 41: hedgerows, number 42: bamboo field.**

group	sample	indicator species					
1	41	<i>Quercus rubra</i>	<i>Prunus avium</i>				
2	15	<i>Cirsium arvense</i>	<i>Equisetum arvense</i>	<i>Lotus spp.</i>			
	16	<i>Heracleum sphondylium</i>	<i>Equisetum arvense</i>	<i>Lotus spp.</i>			
	24	<i>Heracleum sphondylium</i>	<i>Convolvulus sepium</i>	<i>Urtica spp</i>	<i>Trifolium repens</i>		
	31	<i>Heracleum sphondylium</i>	<i>Cirsium vulgare</i>				
3	5	<i>Cirsium arvense</i>	<i>Veronica chamaedrys</i>	<i>Crataegus spp.</i>			
	26	<i>Cirsium arvense</i>	<i>Acer pseudoplatanus</i>	<i>Crataegus spp.</i>	<i>Vicia cracca</i>		
	28	<i>Cirsium arvense</i>	<i>Galium verum</i>	<i>Crataegus spp.</i>	<i>potentilla reptans</i>		
	34	<i>Cirsium arvense</i>	<i>Crataegus spp.</i>				
4	3	<i>Cirsium arvense</i>	<i>Ulex europaeus</i>	<i>Corylus avellana</i>	<i>Crataegus spp.</i>	<i>Lathyrus pratensis</i>	
	6	<i>Cirsium arvense</i>	<i>Corylus avellana</i>	<i>Sorbus intermedia</i>			
	10	<i>Heracleum sphondylium</i>	<i>Corylus avellana</i>	<i>Crataegus spp.</i>	<i>Sorbus aucuparia</i>		
	12	<i>Cirsium arvense</i>	<i>Corylus avellana</i>	<i>Acer platanoides</i>			
5	4	<i>Cirsium arvense</i>	<i>Anthriscus caucalis</i>	<i>Galium aparine</i>	<i>Geranium robertianum</i>		
	40	<i>Cirsium arvense</i>	<i>Galium aparine</i>	<i>Salix alba</i>			
	42	<i>Cirsium arvense</i>	<i>Fraxinus excelsior</i>	<i>Galium aparine</i>	<i>Taraxacum officinale</i>	<i>Plantago lanceolata</i>	
6	13	<i>Cirsium arvense</i>	<i>Acer pseudoplatanus</i>	<i>Ranunculus repens</i>	<i>Vicia cracca</i>		
	14	<i>Cirsium arvense</i>	<i>Acer pseudoplatanus</i>	<i>Rumex crispus</i>	<i>Taraxacum officinale</i>	<i>Athyrium femina</i>	<i>flix-</i>
	19	<i>Cirsium arvense</i>	<i>Geum arbanum</i>	<i>Ranunculus repens</i>	<i>Trifolium pratense</i>	<i>Athyrium femina</i>	<i>flix-</i>
	20	<i>Cirsium arvense</i>	<i>Geum arbanum</i>	<i>Ranunculus repens</i>	<i>Trifolium pratense</i>	<i>Athyrium femina</i>	<i>flix-</i>

7	8	<i>Cirsium arvense</i>	<i>Stellaria graminea</i>	<i>Crataegus spp.</i>	<i>Ranunculus acris</i>	<i>Athyrium femina</i>	<i>felix-</i>
	9	<i>Cirsium arvense</i>	<i>Stellaria graminea</i>	<i>Urtica spp</i>	<i>Athyrium femina</i>	<i>felix-</i>	
	18	<i>Cirsium arvense</i>	<i>Stellaria graminea</i>	<i>Athyrium femina</i>	<i>felix-</i>		
	25	<i>Cirsium arvense</i>	<i>Veronica chamaedrys</i>	<i>Bellis perennis</i>	<i>Athyrium femina</i>	<i>felix-</i>	
	37	<i>Cirsium arvense</i>	<i>Tilia spp</i>	<i>Athyrium femina</i>	<i>felix-</i>		
8	7	<i>Cirsium arvense</i>	<i>Acer pseudoplatanus</i>	<i>Ranunculus acris</i>			
	17	<i>Cirsium arvense</i>	<i>Ulex europaeus</i>	<i>Ranunculus repens</i>	<i>Ranunculus acris</i>	<i>Aegopodium podagraria</i>	
	27	<i>Cirsium arvense</i>	<i>Ranunculus acris</i>				
	35	<i>Cirsium arvense</i>	<i>Stellaria graminea</i>	<i>Ranunculus acris</i>			
	36	<i>Cirsium arvense</i>	<i>Vicia sepium</i>				
	38	<i>Cirsium arvense</i>	<i>Vicia sepium</i>	<i>Ranunculus acris</i>			
9	1	<i>Hedera helix</i>	<i>Ranunculus acris</i>				
	21	<i>Cirsium arvense</i>	<i>Hedera helix</i>	<i>Asplenium scolopendrium</i>			
	22	<i>Cirsium arvense</i>	<i>Ulex europaeus</i>	<i>Hedera helix</i>	<i>Ranunculus acris</i>		
	23	<i>Cirsium arvense</i>	<i>Hedera helix</i>				
	30	<i>Cirsium arvense</i>	<i>Hedera helix</i>	<i>Cirsium vulgare</i>			
	39	<i>Cirsium arvense</i>	<i>Hedera helix</i>	<i>Ranunculus acris</i>			
10	2	<i>Cirsium arvense</i>	<i>Prunus spinosa</i>				
	29	<i>Cirsium arvense</i>	<i>Medicago lupulina</i>	<i>Prunus spinosa</i>			
	33	<i>Cirsium arvense</i>	<i>Hedera helix</i>	<i>Prunus spinosa</i>			
11	11	<i>Cirsium arvense</i>	<i>Ulex europaeus</i>	<i>Epilobium palustre</i>	<i>Lathyrus pratensis</i>		
	32	<i>Cirsium arvense</i>	<i>Medicago lupulina</i>	<i>Lathyrus pratensis</i>			

Aluminum Tolerance in Moso Bamboo (*Phyllostachys pubescens*)

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Abstract

Moso bamboo (*Phyllostachys pubescens*) is widely distributed in the acid soil region of Southern China, exists region with a high toxicity risk for aluminum. To evaluate the Al tolerance of Moso bamboo, seed germination and root elongation in Moso were compared with the same processes in two rice cultivars, and physical and physiological damages were examined under various levels of Al stress. Results showed that Moso bamboo seed germination was inhibited when Al concentration increased to 500 μM , and the median lethal concentration was 2,000 μM . In comparison, the rice seed germination was not inhibited even at a concentration of 2,000 μM Al. Aluminum accumulated mainly in the cell wall of root apices, and entered into protoplasts as treating time prolonged and/or Al concentration increased, which resulted in apoptosis. The bamboo root epidermis degraded significantly in the presence of 2,000 μM Al. In conclusion, Moso bamboo is only weakly tolerant for Al.

Keywords

Aluminum tolerance; *Phyllostachys pubescens*; Seed germination; Seedling; Apoptotic body

Introduction

It has been recognized worldwide that the utilization of an enormous amount of fossil fuel has created various adverse effects on the environment including climate change and acid rain, which can lead to soil acidification (Demirbas, 2006). In China, recent studies showed that soil pH declined significantly ($p < 0.001$) from the 1980s to the 2000s in the major Chinese crop-production areas mainly due to the heavy chemical fertilization (Guo et al., 2010). Aluminum, the richest metal in soils, is solubilized as a phytotoxic form once the pH decreases below 5.0, mainly as Al^{3+} from non-phytotoxic silicate or oxide forms which restricts plant growth (Tahara et al., 2008).

Since 1980s, the bamboo industry has been greatly developed not only in China, but also around the world. The total area of bamboo plantation in China is about 5 million ha and native Moso bamboo (*Phyllostachys pubescens*) plantation area exceeded 3 million ha (Jiang, 2002), making Moso bamboo the most important species for culm and shoot production, and for the bamboo industry. With the development of bamboo industry, bamboo plantation management has intensified greatly with large inputs of chemical fertilizers and other resources, which resulted in lowered soil pH (Xu et al., 2008) and an increase of active Al (Gui et al., 2008). However, little is known about Al tolerance of Moso bamboo except for some progress on shoot quality (Hong et al., 2008). Many studies have been done on rice, which belongs to the Poaceae family together with bamboo, revealing that rice can increase pH value in the rhizosphere by root and consequently reduce the uptake of Al (Ganesan et al., 1993), and exudates organic acid, such as malic acid and citric acid, to tolerate Al stress (Satoru et al., 2000).

This study was carried out to investigate the Al tolerance of Moso bamboo by determination of seed germination and seedling growth with two rice cultivars (Al sensitive one and Al tolerant one) as control, Al distribution and its damage on seedlings.

Materials and Methods

Materials

In the present study, Moso bamboo and rice seeds were used. Moso bamboo seeds were collected from Guangxi province, China, and preserved in a refrigerator at 4°C after drying. The seed purity rate, 1,000 grain weight and water content were 91.08%, 28.35 g and 12.1%, respectively. Two rice cultivars, Al sensitive IR1552 and tolerant Azucena, were used as the controls (Ma, 2007). The rice seeds were provided by China National Rice Research Institute, Hangzhou, China.

Plant Material and Al Treatment

The bamboo and rice seeds were put in 0.2% potassium permanganate solution for 30 min sterilization, and then soaked in deionized water for 24 h after rinsing three times with deionized water. The cleaned seeds were put on two-layer filter paper soaked with incubation solution in Petri dishes for incubation at $25 \pm 2^\circ\text{C}$ and 95% relative humidity in a growth chamber. The incubation solution was 0, 50, 200, 500 and 2,000 μM $AlCl_3$ solution, respectively, and pH was adjusted to 4.5 by 0.1M HCl. During the incubation, the filter paper and incubation solution were replaced every 2 days. Each treatment contained 50 seeds with three replicates.

Measurement of Seed Germination and Seedling Root Elongation

The germinated seed was measured and determined once the radical length reached the seed length. In the experiment, the number of germinated seeds was counted and recorded every day. During the incubation, the bamboo and rice seed germination were culminated on the 4th and 6th day, respectively, and the germination process finished after 14 days.

Fourteen-day old seedlings of bamboo and rice with 3 to 5 cm long roots, which germinated in the absence of Al, were used to investigate the effects of Al toxicity on seedling root elongation. The culture condition and treatment were the same as described above. Each treatment contained ten seedlings with three replications. The root length was measured every day and this experiment was carried out for 3 days.

Calculation of Seed Germination Rate and Germination Potential

The seed germination rate (G) and germination potential (Gv) were used to indicate the influences of Al stress on the bamboo and rice seed germination. The seed germination rate and germination potential were calculated as follows:

$$G = \text{Germinated seed number} / \text{Total tested seed number} \times 100 \quad (1)$$

$$Gv = \text{Germinated seed number during first 6 days} / \text{Total tested seed number} \times 100 \quad (2)$$

Fluorescence Gallium and DAPI Staining of Roots

Fluorescence gallium and DAPI staining methods were described as Silva et al. (2000). In brief, root tip sections were examined within 4 to 6 h after preparation. Images were collected with a TCS-SP confocal system with an inverted microscope DMIRBE (SM 510 META, ZEISS, Germany) and either a 203/0.60 numerical aperture or a 403/1.25 numerical aperture oil objective, therefore affording a theoretical lateral resolution of at least 800 nm. Lasers used were the Coherent UV with excitation lines of 351 and 361 nm to visualize DAPI stained nuclei and a Uniphase argon laser line at 100% in the Acousto-Optical tuneable filter for the visualization of the Al-lumogallion complex. The argon laser was left in the “parked” setting (lowest possible output), and the photomultiplier used to collect the fluorescence was set between 405 and 488 nm. Emitted fluorescence was collected at wavelengths from 500 to 550 nm. DIC images were collected concurrent with the fluorescence images using a transmitted light detector and argon laser illumination.

Statistical Analysis

All statistical analysis was carried out with DPS (Data Processing System) 9.50 software. The significant difference was assessed by Tukey's test.

Results

Seed Germination Rate and Germination Potential Inhibition

One of the very early symptoms of Al toxicity was the inhibition of seed germination, a characteristic that can be assessed by the seed germination rate and germination potential. As shown in Fig. 1A, the Moso bamboo seed germination rate ranged from 53% to 60% while the Al concentration increased from 0 to 500 μM , however, it decreased greatly to 33% when Al concentration increased to 2,000 μM , indicating that the high concentration of Al had a significant impact on Moso bamboo seed germination rate. In contrast to Moso bamboo, the two rice cultivars showed no significant effects from Al stress even at a concentration of 2,000 μM .

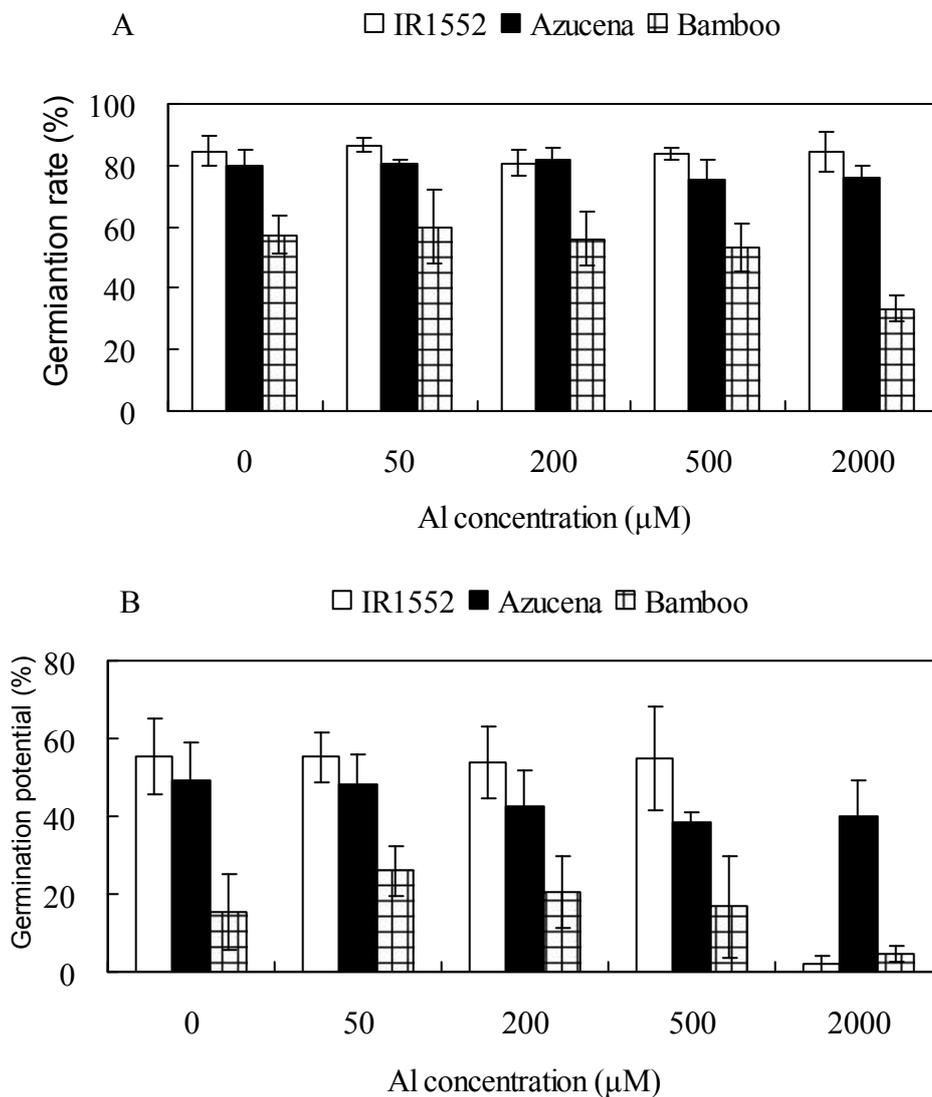


Fig. 1 Effects of Al stress on the germination rate and germination potential of Moso bamboo

Germination potential of Moso bamboo and the two rice cultivars showed different responses to Al stress (Fig. 1B). For Moso bamboo, the germination potential increased from 15% (control) to 26% (50 mM Al) and then decreased to 21% (200 mM Al) and 17% (500 mM Al), showing no significant difference. When Al concentration increased to 2,000 μM , the bamboo germination potential

decreased dramatically to 5% ($p < 0.005$). For the sensitive cultivar IR1552, it was kept steady at around 55% with Al less than 500 μM , however, it was decreased significantly to 2% ($p < 0.001$) when Al concentration increased to 2,000 μM . The germination potential of the tolerant cultivar Azucena tended to decrease from 49% to 40% with the increase of Al concentration, but no significant difference was shown.

The relationship between the seed germination and Al concentration was analyzed. The seed germination rate and germination potential of Moso bamboo correlated significantly with Al concentration ($p < 0.005$), with a correlation coefficient of -0.864 and -0.993 , but such a relationship was not observed in the tolerant rice cultivar Azucena. For the sensitive rice cultivar IR1552, only the germination potential correlated significantly ($p < 0.001$) with Al concentration with a correlation coefficient as -0.974 , suggesting that Moso bamboo was more sensitive to Al stress than the sensitive rice cultivar.

Seedling Root Growth Inhibition

The root growth of Moso bamboo and rice seedlings were all significantly inhibited when Al concentration exceeded 200 μM (Fig. 2). When at low Al concentration of 50 μM , the root growth of Azucena was significantly promoted compared with the control on the 1st day ($p < 0.005$) and 2nd day ($p < 0.001$), suggesting a beneficial effect of low Al concentration on Azucena growth. As treating time prolonged, the Moso bamboo seedling root growth decreased steadily, while at the 3rd day the two rice cultivars showed no difference from that of the 2nd day, suggesting that Moso bamboo was less tolerant to Al stress than rice (Fig. 2).

Physical Damage and Al Distribution in the Seedling Root Apices

Roots of Moso bamboo seedlings grown in the absence of Al were white and delicate. With the increase of Al concentration, roots turned yellow, podgy and tough, and physical damages, such as severe epidermal degradation, were apparent when Al concentration reached 2,000 μM (Fig. 3A, B).

According to the confocal scanning microscopy result, Al was observed mainly in the 1–2 mm terminal part, i.e., meristematic zone and elongation zone, of the root apices when treated with 2,000 μM of Al for 48 h. In the mature zone, Al decreased significantly and mainly distributed around the vascular bundles (Fig. 3C).

Apoptosis in the Root Apices

Apoptosis is the process of programmed cell death (PCD), during which biochemical events lead to characteristic cell changes, including blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation, and death. Apoptotic bodies are cell fragments produced during apoptosis. Al first accumulated in the cell wall of root apices of Moso bamboo seedlings, and then penetrated into protoplasts as treating time prolonged and/or Al concentration increased (figures not shown). When Al was observed to be present only in the cell wall, no apoptotic bodies could be found (Fig. 4A). When Al was present in some of the protoplasts, no obvious apoptotic body could be found (Fig. 4B). When Al was widely present in protoplasts, a number of apoptotic bodies could be found (Fig. 4C). From these results, it can be inferred that apoptotic bodies occurred only when Al entered protoplasts.

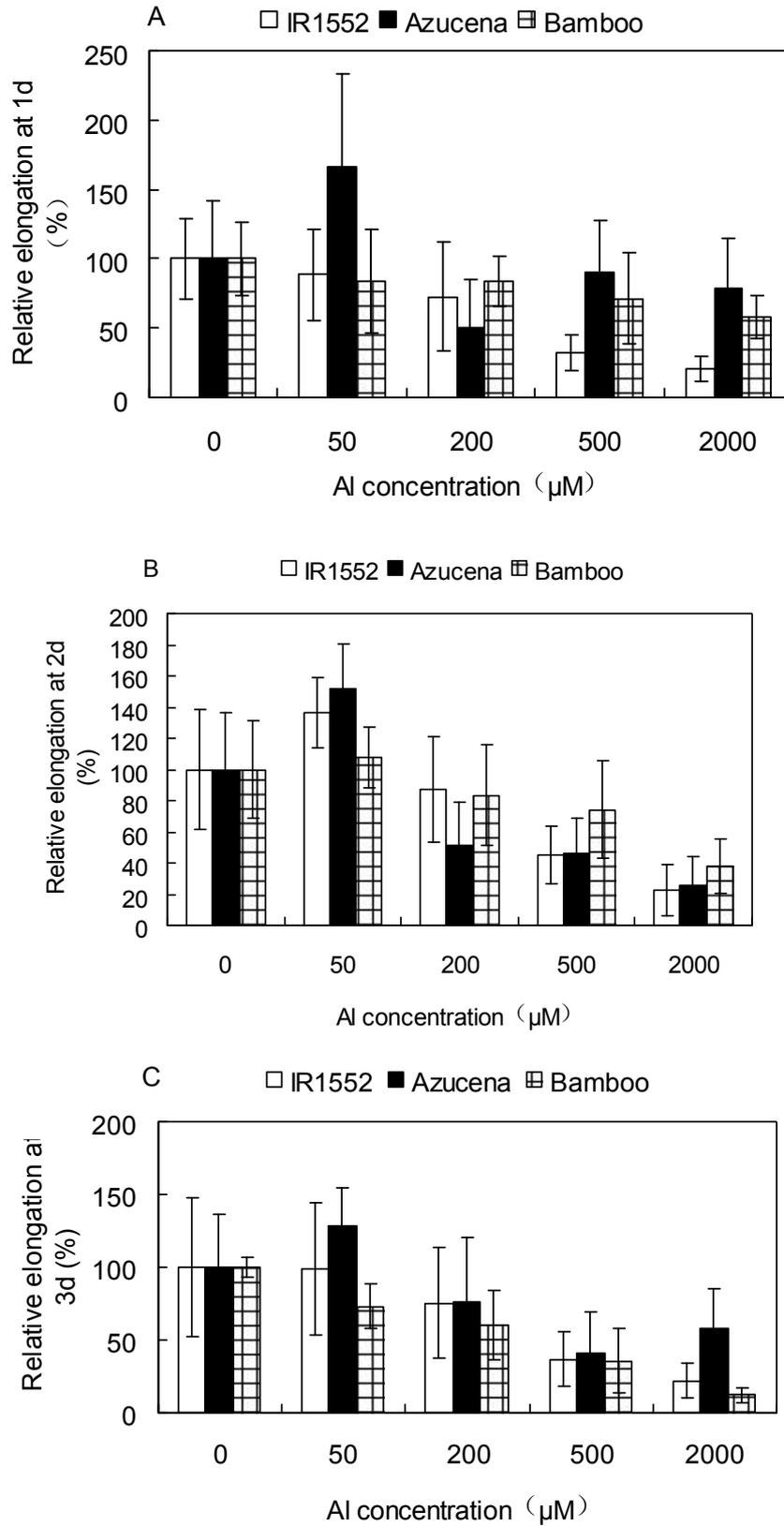


Fig. 2 Effects of Al stress on the root relative growth of Moso bamboo in 1-3 d

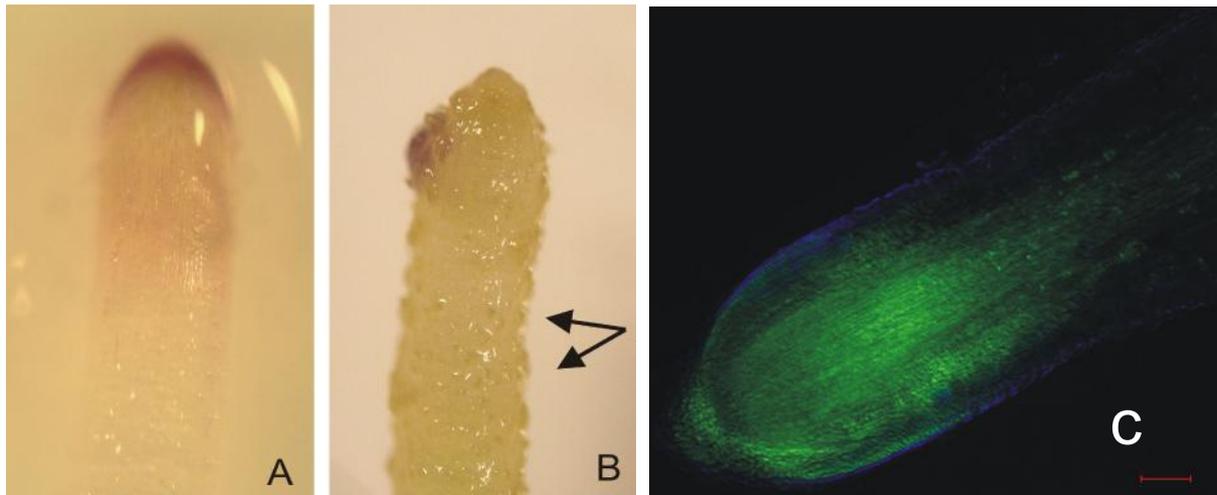


Fig. 3 Al stress on root growth and its distribution in root apices of Moso bamboo

A: Root apices of 14-day-old seedlings in the absence of Al. B: Root apices of 14-day-old seedlings in the presence of 2,000 μM Al. Arrows point to epidermal degradation. C: Confocal scanning microscopy image of longitude section of root apices treated with 200 μM Al for 48 h. Scale size is 100 μm .

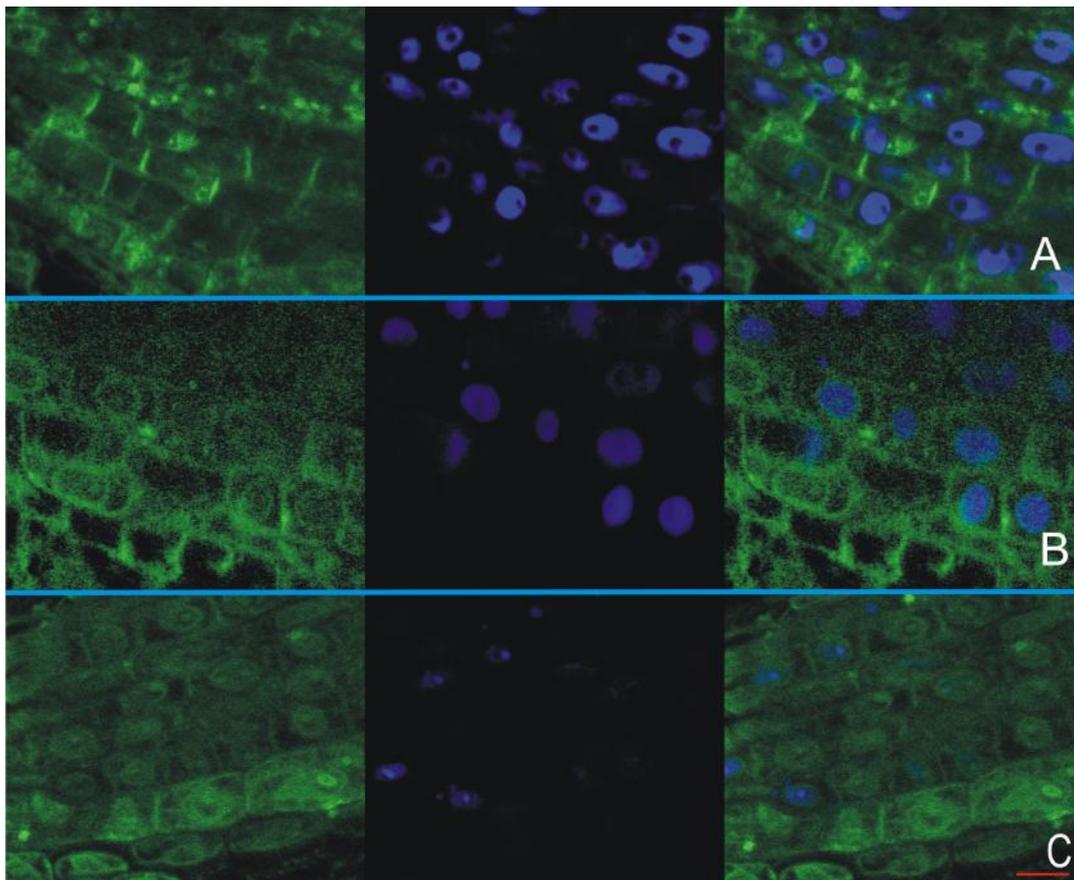


Fig. 4. Confocal scanning microscopy images of longitude section of root apices treated with Al solution
 Left: lumogallion stain. Middle: DAPI stain. Right: overlay of lumogallion and DAPI stain. A: seedlings treated with 500 μM for 24 h, and no presence of Al in protoplasts and apoptosis were observed. B: seedlings treated with 500 μM for 24 h, and Al presented in protoplasts and no apoptosis were observed. C: seedling treated with 2,000 μM for 48 h, and Al presented in protoplasts and apoptosis were observed. Scale size is 10 μm .

Discussion and Conclusion

Al Tolerance of Moso Bamboo

The effect of Al stress on Moso bamboo seed germination and seedling root elongation has not been investigated. In our study, Moso bamboo seed germination and root growth were significantly inhibited when Al concentration exceeded 2,000 μM and 200 μM , respectively, suggesting that Moso bamboo seed was more tolerant than the sensitive rice cultivar. The difference in Al tolerance in rice is governed by high heritability including both additive and dominance effects, with a preponderance of additive effects. Both general combining ability and specific combining ability are important in the genetics of Al tolerance, though the former is more relevant than the latter (Wu et al., 1997). As reported, the effects of Al on seed germination were different among plant species: 0.5 μM Al could inhibit the rape seed germination (Liu et al., 2009), while 1 mM Al had no significant effect on the germination of *Herba Leonuri* (Chen et al., 2006). In some cases, Al of low concentrations could stimulate seed germination, e.g. Al of 0.37–3.7 μM could increase wheat seed germination rate (Li et al., 2004). With regards to growth inhibition, root growth of soybean was inhibited at 5 μM Al (Silva et al., 2001), while only 30% of the plant growth of loblolly pine was inhibited even at a concentration of 580 μM Al (Moyer-Henry et al., 2005).

The exchangeable Al concentration in the red soil region depends on soil type, management mode, environment and so on. As reported, in the typical red soil region, soil exchangeable Al ranges from 1.0 to 162 mmol/kg and the content mainly depends on soil type. Usually, the exchangeable Al in various soil parent materials is present in the order of: shale>quaternary red soil>sandstone>limestone (Lv et al., 2008). Apparently, most of the content of soil exchangeable Al was higher than that in the experiment used (0.05–2.0 mM). Hence, the growth of Moso bamboo would be influenced greatly by the acidified soil. The low soil pH combined with Al toxicity are responsible for the low crop productivity of the red soil in Southern China.

Al Distribution and Its Damage

Aluminum mostly accumulated in the cell wall of loblolly pine under Al stress (Moyer-Henry et al., 2005), and the amounts of Al accumulated in cell wall accounted for 85–99.9% Al in plant roots (Ma, 2007). Though Heim et al. (1999) found out that most Al was stored in the non-plastid in plant roots, Silva et al. (2000) found that Al was fixed on the plastid and nucleus in the non-differentiated cell of soybean in a culture under a low Al concentration. In our study, Al was observed to be mainly accumulated in the meristematic zone and elongation zone of the root apices, and a decreased amount was observed in the mature zone, mainly distributed around the vascular bundles. It has been reported that Al causes substantial physical and physiological damages in that it can harden root apices (Gunsé et al., 1997), degrade epidermis (Kopittke et al., 2008), inhibit DNA synthesis (Kochian et al., 2005) and destroy membrane stability (Ahn et al., 2002). Obviously, the accumulation of Al in the root tips directly resulted in the apoptotic body occurrence and root elongation block. Aluminum stress induced the decrease in bamboo seed germination and seedling root damage was closely related to Al concentration.

Conclusions

In conclusion, the results of this study suggested that Moso bamboo seed is sensitive to Al stress, even more so than the sensitive rice cultivar. The germination rate and potential of Moso bamboo seed were significantly inhibited when Al concentration was more than 2 mM, and the bamboo seedling elongation was influenced greatly as well. Both physical and physiological damages were observed as Al concentration increased, and apoptosis may have been mainly caused by Al present in the protoplasts of bamboo seedlings, as supported by microscopy evidence. Therefore, more attention should be paid to Moso bamboo plantation in the acid soil region.

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References

- Ahn, S. J.; Sivaguru, M.; Chung, G. C.; Rengel, Z.; Matsumoto, H. 2002. Aluminium-induced growth inhibition is associated with impaired efflux and influx of H⁺ across the plasma membrane in root apices of squash (*Cucurbita pepo*). *Journal of Experiment Botany*, 53, 959–1966.
- Chen, J. H.; Ye, L. J.; Liu, P. 2006. The effect of aluminum on germination of motherwort seeds and roots growth of motherwort herb. *Seed*, 1, 11–13.
- Demirbas, A. 2006. Hazardous emissions, global climate change and environmental precautions. *Energy Sources, Part B*, 1, 75–84.
- Ganesan, K.; Sankaranarayanan, C.; Balakumar, T. 1993. Physiological basis of differential aluminum tolerance in rice genotypes. *Commun. Soil Sci. Plant Anal*, 24, 2179–2191.
- Gui, R. Y.; Li, G. D.; Fang, W.; Zhuang, S. Y. 2008. Iron and aluminum forms of the soil in Mosobamboo stands under the extensive and integrated culture management. *Bamboo Journal*, 25, 26–31.
- Gunsé, B.; Poschenrieder, C.; Barceló, J. 1997. Water transport properties of roots and root cortical cells in proton- and Al-stressed maize varieties. *Plant Physiol*, 113, 595–602.
- Guo, J. H.; Liu, X. J.; Zhang, Y.; Shen, J. L.; Han, W. X.; Zhang, W. F.; Christie, P.; Goulding, K. W. T.; Vitousek, P. M.; Zhang, F. S. 2010. Significant acidification in major Chinese croplands. *Science*, 327, 1008–1010.
- Heim, A.; Luster, J.; Brunner, I.; Frey, B.; Frossard, E. 1999. Effects of aluminum treatment on Norway spruce roots: aluminum binding forms, element distribution, and release of organic substances. *Plant Soil*, 216, 103–115.
- Hong, W.; Lin, C. Y.; Wu, C. Z.; Song, P.; Hong, T.; Fan, H. L.; Chen, C. 2008. Effects of different aluminum toxicity on nutritional quality of bamboo shoots in moso bamboo. *Journal of Bamboo Research*, 2, 13–18.
- Jiang, Z. H. 2002. *Bamboo and rattan in the world*. Shenyang: Liaoning Science and Technology Publishing House, 2002, 9.
- Kochian, L. V.; Pineros, M. A.; Hoekenga, O. A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant and Soil*, 274, 175–195.
- Kopittke, P. M.; Blamey, F. P. C.; Menzies, N. W. 2008. Toxicities of soluble Al, Cu, and La include ruptures to rhizodermal and root cortical cells of cowpea. *Plant Soil*, 303, 217–227.
- Li, C. S.; Liu, P.G.; Xu, D.; He, W. B.; Zhu, J. 2004. Effect of acid-Al on the germination of soaked buckwheat seeds. *Seed*, 12, 11–13.
- Liu, Q.; Long, W. W.; Hu, C.; Fang, Y. S. 2009. Effects of Aluminium Stress on *Brassica campestris* L. Seeds Germination and Seedlings Growth. *Seed*, 7, 5–10.

- Lv, H. Z.; Wang, K. R.; Xie, X. L.; Chen, A. L. 2008. Dynamic Effects of Rice Straw on Different Forms of Active Aluminum in Acidic Red Soil. *Chinese J. Soil Sci*, 39, 321–324.
- Ma, F. J. 2007. Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants. *Int Rev Cytol*, 264, 225–252.
- Moyer-Henry, K.; Silva, I.; Macfall, J.; Johannes, E.; Allen, N.; Goldfarb, B.; Rufty, T. 2005. Accumulation and localization of aluminium in root tips of loblolly pine seedlings and the associated ectomycorrhiza *Pisolithus tinctorius*. *Plant, Cell and Environment*, 28, 111–120.
- Satoru, I.; Tadao, W.; Ryouichi, S.; Paul, O. M. 2000. Comparison of the amount of citric and malic acids in Al media of seven plant species and two cultivars each in five plant species. *Soil Science and Plant Nutrition*, 46(3), 751–758.
- Silva, I. R.; Smyth, T. J.; Dana, F. M.; Thomas, E. C.; Nina, S. A.; Thomas, W. R. 2000. Aluminum accumulation at nuclei of cells in the root tip. Fluorescence Detection Using Lumogallion and Confocal Laser Scanning Microscopy. *Plant Physiology*, 123, 543–552.
- Silva, I. R.; Raper, C. D.; Carter, T. E.; Rufty, T. W. 2001. Differential aluminum tolerance in soybean: An evaluation of the role of organic acids. *Plant Physiol*, 2, 200–210.
- Tahara, K.; Yamanoshita, T.; Norisada, M.; Hasegawa, I.; Kashima, H.; Sasaki, S.; Kojima, K. 2008. Aluminum distribution and reactive oxygen species accumulation in root tips of two *Melaleuca* trees differing in aluminum resistance. *Plant Soil*, 307, 167–178.
- Wu, P.; Zhao, B.; Yan, J.; Luo, A.; Wu, Y.; Senadihra, D. 1997. Genetic control of seedling tolerance to aluminum toxicity in rice. *Euphytica*, 97, 289–293.
- Xu, X. W., Cui, H. P.; Wu, J. S.; Jin, A. W. 2008. Evaluation of ecology impact of bamboo forest with model fertilizing. *J. Zhejiang For. Sci. & Tech*, 1, 38–42.

Modelling Seasonal Variations of Chlorophyll Fluorescence in Bamboo Leaves.

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Abstract

In the search of renewable energy resources and bio-based materials, bamboo has been proposed as a high yield biomass product that can be used as a phytoremediator on polluted soils. For this reason, a yield indicating growth model needs a health index parameter to monitor the performance of the plants. Therefore, chlorophyll fluorescence was measured on the leaves of *Phyllostachys humilis* in Ballyboughal, Co. Dublin, Ireland. Measurements were attained on the leaves of each node of the plant and taken on a seasonal basis. The parameter most frequently used in chlorophyll fluorescence is Fv/Fm, also known as TRo/ABS (ratio of number of photons trapped and number of photons absorbed). A seasonal dip, as well as a larger variation, of this parameter in spring compared to the rest of the year was observed. Over the year, the upper leaves of the plant perform better than the bottom leaves. These findings were integrated in a mathematical model, which aims at predicting and explaining plant performance at other sites.

Keywords

Phyllostachys, chlorophyll fluorescence, seasonal variations, Europe

Introduction

One of the priority challenges for humanity the coming decades is the mitigation of global warming by the use of alternative energy resources. The aim of the European Union is to diminish greenhouse gas emissions by 20% in 2020 compared to emission levels of 1990, and to increase the use of renewable energy sources up to 20% of total energy consumption (<http://ec.europa.eu/eu2020>). As a result, the potentials of fast-growing plant species like poplar (*Populus* spp.) (Mann and Tolbert 2000; Laureysens et al. 2005a;b), willow (*Salix* spp.) (Adegibi et al. 2001; Vervaeke et al. 2003; Keoleian and Volk 2005) and *Miscanthus* (Lewandowski et al. 2000; Clifton-Brown et al. 2004) for biomass production are nowadays intensively studied. Although some of them are also extremely fast-growing, bamboo species have largely been neglected in the search to renewable energy sources (El Bassam 1998; Scurlock et al. 2000; Potters et al. 2009). On top of the potential as biomass producer, bamboo can also be used for renewable bio-based materials such as wood, composites, vessels, chemicals and energy (Schutte et al. 2011 submitted) and for the removal of pollutants - such as heavy metals - out of the soil (Potters et al. 2009).

As plant growth and development is closely related with the physiological process of photosynthesis (Goldschmidt 1999; Vu 1999), it is important to understand how the environment affects photosynthesis. In combination with other techniques such as photosynthetic gas exchange, fluorescence spectroscopy can be used to study regulation and control parameters of photosynthesis in leaves (Ribeiro et al. 2009) and to assess the possible damages of different stress signals (Papageorgiou and Govindjee 2004).

The shape of the fluorescence transient of any sample is determined by the physiological state of the sample at that moment and the physical and chemical environmental conditions around the sample (Strasser et al. 2000), such as light intensity (Tsimilli-Michael et al. 1995; Srivastava and Strasser 1996; Krüger et al. 1997), temperature (Guisé et al. 1995; Srivastava et al. 1997; Strasser 1997), drought (Van Rensberg et al. 1996) or chemical influences (Ouzounidou et al. 1997). This implicates that although light is the driving force of photosynthesis, it is also a stress factor, causing photoinhibition. Photoinhibition is a process taking place where photosynthesis receives excess excitation energy. This is called the excitation pressure hypothesis. At low temperatures, this may occur under quite low light levels (Huner et al. 1996; Huner et al. 1998; Öquist and Huner 1999).

The site of inhibition of photosynthesis is photosystem II (Kyle 1987) and photoinhibition characteristically causes a loss of variable chlorophyll fluorescence (Baker and Horton 1987). One of the most frequently used parameters in chlorophyll fluorescence is the ratio between variable and maximum fluorescence (F_v/F_m), which is also described as the yield of the number of trapped photons versus the number of absorbed photons (TR_o/ABS) (Srivastava et al. 1999). Across a wide range of higher plants species, this parameter has an optimal value of 0.83. When exposed to abiotic and biotic stresses in the light, F_v/F_m in plants will decrease (Baker 2008). This parameter is therefore frequently used as a stress detector under environmental stress conditions, such as light (Lichtenthaler and Burkart 1999) and temperature (Öquist and Huner 1991).

Seasonal changes in chlorophyll fluorescence have been reported in different plants, with depressions depending on plant species; in winter in Mediterranean shrub species (Ain-Lhout et al. 2004; Karatavas and Manetas 1999), during cold winter days in conifer species (Adams and Demmig-Adams 1994; 1995; Adams et al. 1994) or in spring and early summer in Mediterranean grassland species (Figueroa et al. 1997), when ambient temperatures were increasing rapidly and rainfall was low. Aside from seasonal changes, chlorophyll fluorescence can also vary between sun-exposed and shaded leaves (Strand and Öquist 1985; Strand and Lundmark 1987; Ottander and Öquist 1991; Sveshnikov et al. 2006; Porcar-Castell et al.; 2008a; b). Also midday depression is common in higher plant species like

in Mediterranean grassland species (Fernandez et al. 1998) and in other bamboo species (Kumar et al. 2002).

The objective of this study was to compare different models to describe seasonal, diurnal and height-dependent variation of Fv/Fm, and the interaction between them, in the leaves of *Phyllostachys humilis*, a bamboo species with a high biomass production (Van Goethem et al. 2011 submitted).

Materials and methods

In April 2005, a 5 m x 80 m strip of *Phyllostachys humilis* was planted on a clay soil in Ballyboughal, co, Dublin, Ireland (N 53° 31' 41" E 6°15'31"). Plants were produced via *in vitro* techniques and delivered by Oprins Plant NV (Rijkevorsel, Belgium) and were planted after hardening during one growth season in a 2 L pot. At the moment of planting, the average height was about 50 cm. During the first two years after planting, the plants were regularly watered during summer and Simazine© was used to suppress upcoming weeds. No more watering was necessary from the third year onwards, and neither was spraying with herbicides.

Field data were collected from different culms each season, during the summer (day 210-216) and autumn (day 286-297) of 2009; during spring (day 119-135), summer (day 197-202), autumn (day 274-288) of 2010, two periods in the winter of 2011 (day 13-25 and day 53-56); and during spring (day 96-105) and autumn (day 284-302) of 2011 (Fig.1). At least three leaves on each node of the culm were measured in random order with the Handy Pea (Hansatech instruments Ltd., England, Norfolk) after dark adaptation with leaf clips for 30 minutes. Biolyzer HP 3.0 (Fluoromatics Software) was used to calculate Fv/Fm as an indication of the performance of the leaves. The data were analysed in the statistical program R (R development core team 2011) using a linear and a nonlinear mixed model (Pinheiro and Bates 2011) with leaf nested in node nested in culm.

In the linear model, Fv/Fm was approximated using the harmonic function;

$$Fv/Fm = \text{Sine}(\text{DOY}) \times H$$

where Sine(DOY) is the combination function of $\sin = \sin(2 \cdot \pi \cdot \text{DOY} / 365.25)$ and $\cos = \cos(2 \cdot \pi \cdot \text{DOY} / 365.25)$ with DOY = day of the year, H = number of the node of the measured leaf started at the top of the culm.

The effect of daytime was verified by using Tukey HSD test on three moments during daytime: morning, midday and afternoon; with morning measurements taken between 8am and 10am, midday measurements between 12pm and 14pm, and afternoon measurements between 16pm and 18pm.

For the nonlinear model, the function:

$$Fv/Fm = \frac{[a(\text{DOY}-d)^2]}{[b+(\text{DOY}-d)^2]} + c$$

was used, with DOY = day of the year, parameter a is the maximum value minus the minimum value, b is the width of the dip, c is the minimum value and d is the DOY where the minimum was reached. We test the effect of H (node number) and daytime (morning, midday and afternoon) and the two-way interaction of them on this model.

In both models, variance functions allowing for different standard deviations (for season as well as for number of the node) were used as described by Pinheiro & Bates (2000). They were both optimized based on the Akaike Information criterion (AIC) (Pinheiro and Bates 2000).

Results and discussion

In both models, variance functions were used because of the difference in variability between seasons. The variability in F_v/F_m was small in summer, autumn and winter. Towards the end of the winter the variability in F_v/F_m increased, reaching maximum values in spring (Fig. 1, Fig. 3). Also the variation of F_v/F_m in node number differs and is higher in the top leaves (Fig. 2).

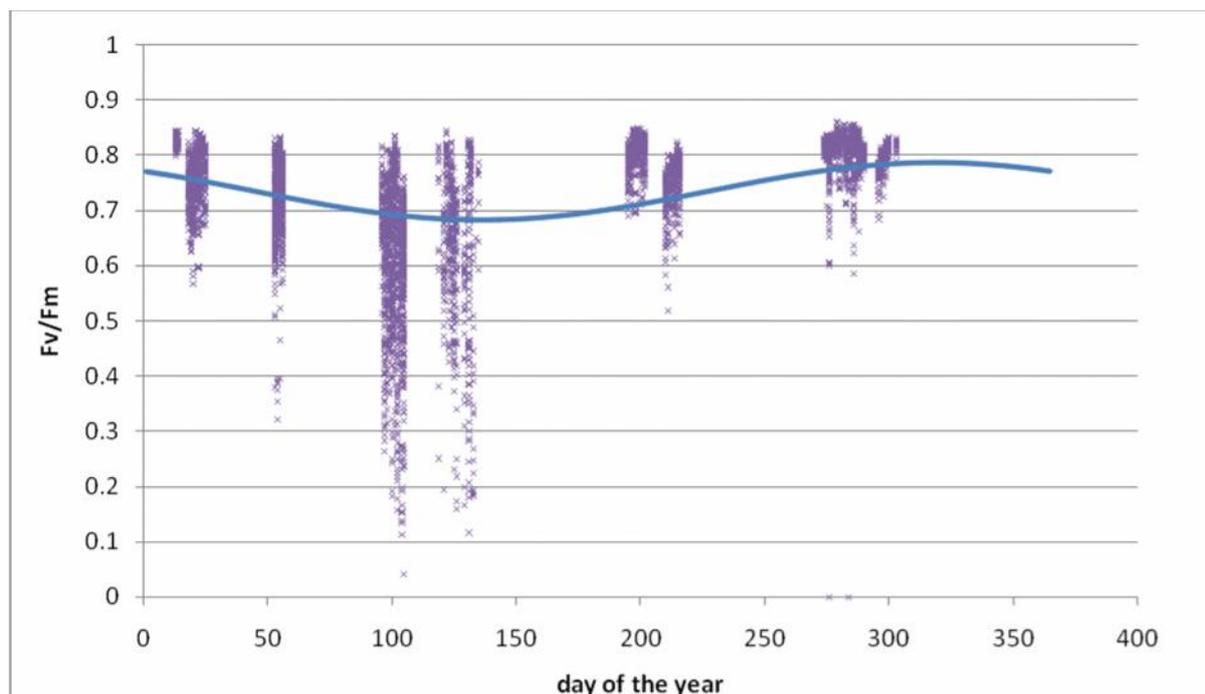


Figure 1: F_v/F_m in function of day of the year. The small crosses represent the measured data of 2009-2011, the line is the fit of the harmonic function of the 5th node ($H=5$).

Linear model

The results of the linear harmonic function with interactions between H and $\text{Sine}(\text{DOY})$ are shown in the anova table (Table 1). Both the effect of node number and $\text{Sine}(\text{DOY})$ were significant, as well as the interaction between them.

Table 1: Results for analysis of variance with the linear mixed model. $\text{Sine}(\text{DOY}) = \text{combination function of } \sin = \sin(2 * \pi * \text{DOY} / 365.25) \text{ and } \cos = \cos(2 * \pi * \text{DOY} / 365.25) \text{ with } \text{DOY} = \text{day of the year, } H = \text{number of the node of the measured leaf started at the top of the culm, NumDF} = \text{numerator degrees of freedom; denDF} = \text{denominator degrees of freedom.}$

Effects	numDF	denDF	F-value	p-value
H	1	721	208.56	<0.001
$\text{Sine}(\text{DOY})$	2	4296	443.08	<0.001
$H:\text{Sine}(\text{DOY})$	2	4296	9.18	<0.001

The effects of the different parameters on the F_v/F_m parameter are presented in table 2 and shown in figure 1. The efficiency of PSII is better in autumn, and shows a depression in spring. This spring dip can also be found in Mediterranean grassland species (Figuroa et al. 1997).

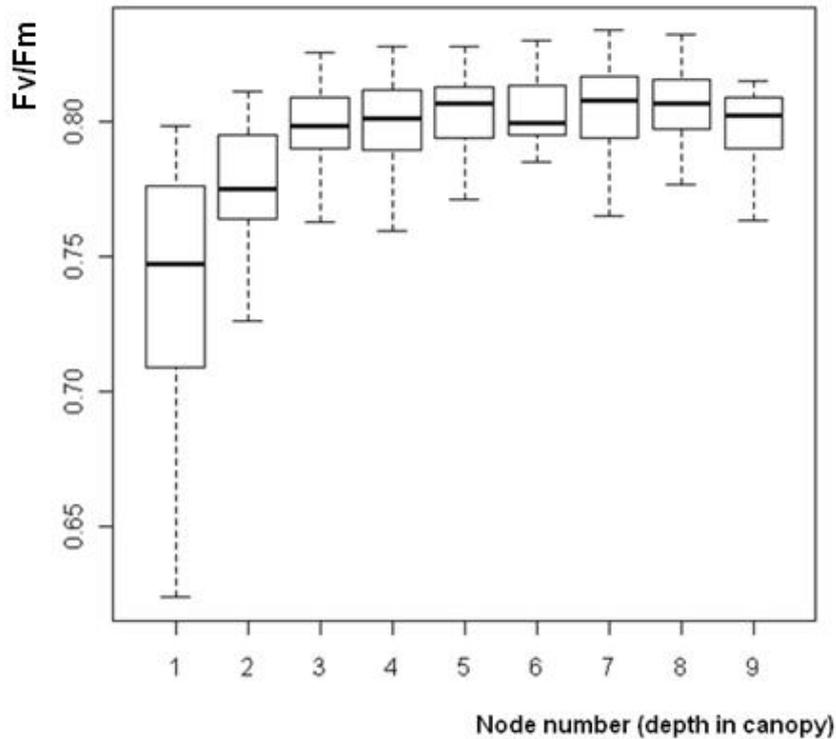


Figure 2: Effect of leaf implantation (node number 1 represent the highest node in the culm) on the variation of F_v/F_m . Data collected in autumn 2009.

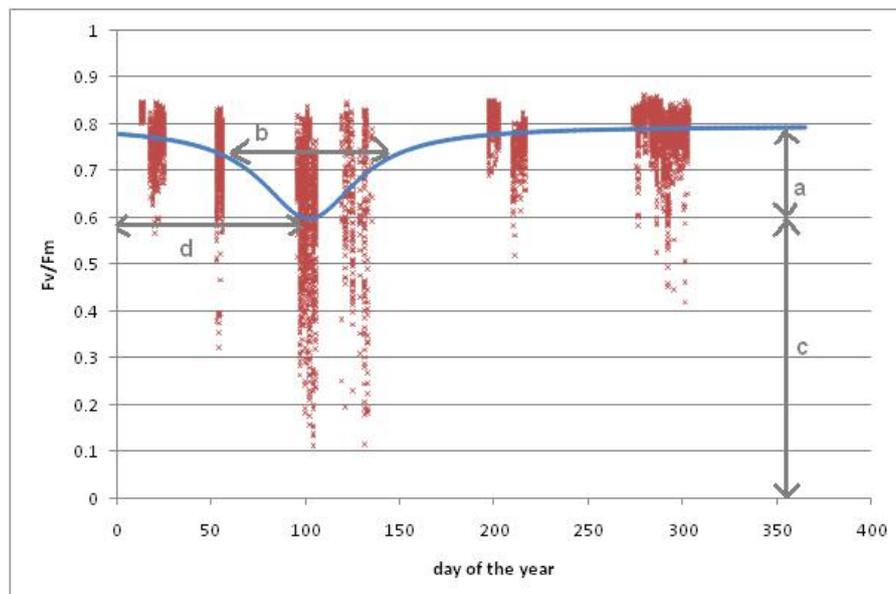


Figure 3: F_v/F_m in function of day of the year. The small crosses represent the measured data of 2009-2011, the line is the fit of the nonlinear function of the 5th node ($H=5$) at midday. Parameter a is the difference between the maximum value and the minimum value of F_v/F_m , parameter b represents the wide of the dip, parameter c represents the minimum value of F_v/F_m and parameter d represents the time where the dip has reached his minimum value.

Table 2: Estimated values, standard error and p-value of the harmonic model. Sine(DOY) = combination function of $\sin= \sin(2*\pi *DOY/365.25)$ and $\cos= \cos(2*\pi *DOY/365.25)$ with DOY = day of the year, H = number of the node of the measured leaf started at the top of the culm, SE= Standard error, DF= degrees of freedom.

Effect	Value ($\times 10^{-2}$)	SE ($\times 10^{-2}$)	DF	t-value	p-value
H	0.666	0.0429	721	15.52	<0.001
Sine(DOY)sin	-4.67	0.511	4296	-9.14	<0.001
Sine(DOY)cos	4.05	0.453	4296	8.94	<0.001
H:Sine(DOY)sin	0.187	0.0483	4296	3.87	<0.001
H:Sine(DOY)cos	-0.86	0.0529	4296	-1.62	0.10

The positive value of H suggests that the lower leaves are performing less than the leaves more deeply in the canopy. Although the lower leaves are always performing better, the extent of the node effect differs between season, with a larger effect in spring. This effect is also present in overwintering conifers, where the Fv/Fm value is not only lower in colder periods compared to summer (Ottander et al. 1995; Ensminger et al. 2004), but also more exposed needles had a lower Fv/Fm compared with the more shaded (Strand and Öquist 1985; Strand and Lundmark 1987; Ottander and Öquist 1991; Svishnikov et al 2006; Porcar-Castell et al.; 2008a; b). Also in the evergreen shrub *Mahonia repens*, the sun-exposed leaves have lower photosynthetic capacities during winter, whereas shade leaves did not (Logan et al 1998). This is consistent with the excitation pressure hypothesis, that under lower temperatures, even lower light levels can emit excessive excitation energy. (Huner et al. 1996; Huner et al. 1998; Öquist and Huner 1999).

We verify if there is a midday depression present in *P. humilis*, by applying a Tukey HSD test. As shown in table 3, no significant effect is present between the different moments of the day.

Table 3: Tukey HSD test between the different periods of the day. Diff= mean difference; Lwr= lower bound; Upr= upper bound; Adj p-value= adjusted p-value.

	Diff ($\times 10^{-3}$)	Lwr ($\times 10^{-3}$)	Upr ($\times 10^{-3}$)	Adj p-value
morning-afternoon	4.73	-0.98	10.446	0.13
midday-afternoon	0.20	-5.52	5.913	0.99
morning-midday	4.53	-1.15	10.219	0.15

Nonlinear model

The results of the nonlinear model are shown in table 4. We see that the node number only has effect on parameter a and daytime has a significant effect on the parameters a, b, and d. No significant effect was found on parameter c. The reason that in this model the effect of daytime is significant, suggests that this model has a higher power than the linear model. If we plot this model (Fig.3), we see that the fit is better than obtained with the linear model.

In table 5, the estimated values show that in the nonlinear model, the top leaves are performing less than the leaves more deeply in the canopy (the effect of H on parameter a is a positive value, and this parameter represents the maximum height of the Fv/Fm value (Fig. 3)). The difference with the linear model is that in this model, the difference between the bottom leaves and the top leaves is larger in all the other seasons than spring, whereas in the linear model, the effect was larger in spring time. This

might be due to the effect of the Sine function: because the sine function cannot reach low values in spring time - since it then would have to have even higher values in autumn than predicted already, this effect is compensated with having smaller Fv/Fm values for the top leaves only in spring. This also suggests that the nonlinear model is better than the linear harmonic model.

Table 4: Anova table of the nonlinear mixed model. H= node number and DT= daytime. NumDF = numerator degrees of freedom; denDF = denominator degrees of freedom.

Parameter	Effect	numDF	denDF	F-value	p-value
a	(Intercept)	1	6851	57568.18	<0.001
	H	1	6851	10.22	0.001
	DT	2	6851	36.39	<0.001
b	(Intercept)	1	6851	3912.78	<0.001
	DT	2	6851	225.52	<0.001
c	(Intercept)	1	6851	10843.96	<0.001
d	(Intercept)	1	6851	1723.03	<0.001
	DT	2	6851	12.5	<0.001

Also shown in table 5 is the estimated value for the daytime effect. In the afternoon, maximum values are higher than in the morning or midday, but no significant difference between morning and midday. Morning measurements are earlier reaching the dip than midday or afternoon measurements (parameter d), and the dip is less wide (parameter b), with no significant differences between midday and afternoon.

Table 5: Estimated values, standard error and p-value of the nonlinear mixed model. H= node number and DT= daytime, with DTO= morning, DTM= midday and DTA = afternoon, SE= Standard error.

	A		b		c		d	
	Value	SE	Value	SE	Value	SE	Value	SE
H	3.1 x10 ⁻³	0.72 x10 ⁻³	NS	NS	NS	NS	NS	NS
DTO	0.184 ^a	0.00704	665.0 ^a	71.5	0.598 ^a	0.006	92.0 ^a	2.50
DTM	0.182 ^a	0.00712	955.0 ^b	116.0	0.598 ^a	0.006	101.8 ^b	2.94
DTA	0.188 ^b	0.0071	1184.8 ^b	119.9	0.598 ^a	0.006	100.6 ^b	2.70

Values followed by the same letter within a column are not significantly different from each other at P <0.05. NS= not significant.

Conclusions

Although harmonic functions are often used to model temporal data (<http://scs.math.yorku.ca> 2011), the analysis from both models suggests that the nonlinear function is a better representative for describing PSII efficiency in the leaves of *Phyllostachys humilis*. With both models, we can see that there is a significant effect of season on chlorophyll fluorescence, with a seasonal dip in spring, recover in summer and high values in autumn and winter.

There has been found a significant interaction between season and node number, with different interpretations depending on the model used. In the linear model the node number effect is larger in spring, while in the nonlinear model, the effect is larger in the other seasons. Since in the sine function one season can effect the other, less low values will be predicted in spring, and this is compensated by predicting lower values in the top leaves in this period. In general, node number has a significant effect, with the top leaves having higher Fv/Fm values than the bottom leaves. This can be explained by the decreasing light intensity in the canopy.

Only in the nonlinear model, we find a significant daytime effect, with morning values having a less wide spring dip that is present a week earlier in the year, and afternoon values having lower Fv/Fm values in summer, autumn and winter compared to the morning and midday values. That an significant daytime effect is found in the nonlinear model, but not in the linear model, confirms that the nonlinear model is a better model than the linear one.

In future uses, this model can be used to model variation for other bamboo species or even other plants species, when the same trend can be observed.

References

- Adams, W.W., III, Demmig-Adams, B., 1994. Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. – *Physiol. Plant.* 92: 451-458.
- Adams, W.W., III, Demmig-Adams, B., Verhoeven, A.S., Baker, D.H., 1994. Photoinhibition during winter stress: Involvement of sustained xanthophyll cycle-dependent energy dissipation. – *Aust. J. Plant Physiol.* 22: 261-276.
- Adams, W.W., III, Demmig-Adams, B., 1995. The xanthophyll cycle and sustained thermal energy dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter. – *Plant, Cell Environ.* 18: 117-127.
- Adegibi H.G., Volk T.A., White E.H., Abrahamson L.P., Briggs R.D., Bickelhaupt D.H., 2001. Biomass and nutrient removal by willow clones in experimental bioenergy plantations in New York State, *Biomass and Bioenergy* 20, 399-411.
- Ain-Lhout F., Diaz Barradas M.C., Zunzunegui M., Rodriguez H., Garcia Novo F., Vargas M.A., 2004. Seasonal differences in photochemical efficiency and chlorophyll and carotenoid contents in six Mediterranean shrub species under field conditions. *Photosynthetica* 42 (3): 399-407.
- Baker N.R., Horton P., 1987. Chlorophyll fluorescence quenching during photoinhibition. In *Photoinhibition* (eds. D.J. Kyle, C.B. Osmond & C.J. Arntzen), pp.145-168. Elsevier, Amsterdam.
- Baker, N.R., 2008. Chlorophyll fluorescence: A Probe of Photosynthesis In Vivo. *Ann. Rev. Plant Biol.* Vol. 59, pp.89-113.
- Clifton-Brown J.C., Stampfl P.F., Jones M.B., 2004. *Miscanthus* biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions, *Global Change Biology* 10, 509-518.
- El Bassam, N. 1998. *Energy Plant Species: Their use and impact on environment and development.* James and James, London.
- Ensminger, I., Sveshnikov, D., Campbell, D.A., Funk, C., Jansson, S., Lloyd, J., Shibistova, O., Öquist, G. 2004. Intermittent low temperatures constrain spring recovery of photosynthesis in boreal Scots pine forests. *Global Change Biology* 1: 995-1008.
- Fernandez-Baco, L., Figueroa, M.F., Luque, T., Davy, A.J., 1998. Diurnal and seasonal variations in chlorophyll a fluorescence in two Mediterranean-grassland species under field conditions. *Photosynthetica* Vol.35, No. 4 pp.535-544.
- Figueroa M.E., Fernández-Baco L., Luque T., Davy A.J., 1997. Chlorophyll fluorescence, stress and survival in populations of Mediterranean grassland species. *Journal of Vegetation Science* 8: 881-888.
- Goldschmidt E.E., 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. *HortScience* 34, 1020-1024.
- Guissé, B., Srivastava, A. and Strasser, R.J. 1995. The polyphasic rise of the chlorophyll a fluorescence (O-K-J-I-P) in heat stressed leaves. *Arch. Sci. Genève* 48: 147-160.
- Huner, N.P.A., Maxwell, D.P., Gray, G.R., Savitch, L.V., Krol, M., Ivanov, A.G., Falk, S. 1996. Sensing environmental temperature change through imbalances between energy supply and energy consumption: Redox state of the photosystem II. *Physiologia Plantarum* 98: 358-364.
- Huner N.P.A., Öquist, G. & Sarhan, F. 1998. Energy balance and acclimation to light and cold. *Trends in Plant Science* 3: 224-230.
- Karavatas, S., Manetas, Y.: Seasonal patterns of photosystem II photochemical efficiency in evergreen sclerophylls and drought semi-deciduous shrubs under Mediterranean field conditions. – *Photosynthetica* 36: 41-49, 1999.

- Keoleian, G.A., Volk, T.A., 2005. Renewable Energy from Willow Biomass Crops: Life Cycle Energy, Environmental and Economic Performance. *Critical Reviews in Plant Sciences* 24,385-406.
- Krüger, G.H.J., Tsimilli-Michael, M. and Strasser, R.J. 1997. Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. *Physiol. Plant.* 101: 265–277.
- Kumar, R., Mohinder, P., Teotia, U.V.S., 2002. Diurnal changes in chlorophyll fluorescence in four species of bamboo. *J. Bamboo and Rattan*, Vol.1, No.4, pp.341-349.
- Kyle D.J. 1987. The biochemical basis for photoinhibition of photosynthesis II. In *Photoinhibition* (eds. D.J. Kyle, C.B. Osmond & C.J. Arntzen), pp. 197-226. Elsevier, Amsterdam.
- Laureysens I., De Temmerman L., Hastir T., Van Gysel M., Ceulemans R., 2005a. Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture. II. Vertical distribution and phytoextraction potential. *Environmental Pollution* 133, 541-551.
- Laureysens I., Pellis A., Willems J., Ceulemans R., 2005b. Growth and production of a short rotation coppice culture of poplar. III. Second rotation results. *Biomass and Bioenergy* 29, 0-21.
- Lewandowski I., Clifton-Brown J.C., Scurlock J.M.O., Huisman W., 2000. Miscanthus: European experience with a novel energy crop, *Biomass and Bioenergy* 19, 209-227.
- Lichtenthaler H.K., Brukart S. 1999. Photosynthesis and high light stress. *Bulg. J. Plant Physiol.* 25, Vol. 3-4, pp. 3-16.
- Logan B.A., Grace S.C., Adams W.W. III., Demig-Adams, B., 1998; Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. *Oecologia* 116, 9-17)
- Mann L., Tolbert V., 2000. Soil Sustainability in Renewable Biomass Plantings. *AMBIO: A Journal of the Human Environment* 29, 492–498.
- Öquist G., Huner N.P.A., 1991. Effects of cold acclimation on the susceptibility of photosynthesis to photoinhibition in Scots pine and in winter and spring cereals: a fluorescence analysis. *Functional Ecology* 5, 91-100.
- Ottander, C., Campbell, D. & Öquist, G. 1995. Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. *Planta* 197: 176-183.
- Ottander C., Öquist, G. 1991. Recovery of photosynthesis in winter-stressed Scots pine. *Plant, Cell and Environment* 14: 345-349.
- Ouzounidou, G., Moustakas, M. and Strasser, R.J. 1997. Sites of action of copper in the photosynthetic apparatus of maize leaves: Kinetic analysis of chlorophyll fluorescence, oxygen evolution, absorption changes and thermal dissipation as monitored by photoacoustic signals. *Aust. J. Plant Physiol.* 24: 81–90.
- Papageorgiou G.C., Govindjee, 2004. *Chlorophyll a Fluorescence: a Signature of Photosynthesis*. Springer.
- Pinheiro, J.C., Bates, D.M., 2000. *Mixed-effects models in S and S-plus*. Springer.
- Pinheiro, J., Bates, D., 2011. Saikat DebRoy, Deepayan Sarkar and the R Development Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-100.
- Porcar-Castell A, Juurola E, Nikinmaa E, Berninger F, Ensminger I, Hari P, 2008a. Seasonal acclimation of photosystem II in *Pinus sylvestris*. I. Estimating the rate constants of sustained thermal energy dissipation and photochemistry. *Tree Physiology* 28: 1475-1482.
- Porcar-Castell A, Juurola E, Ensminger I, Berninger F, Hari P, Nikinmaa E, 2008b. Seasonal acclimation of photosystem II in *Pinus sylvestris*. II. Using the rate constants of sustained thermal energy dissipation and photochemistry to study the effect of the light environment. *Tree physiology* 28: 1483-1491.

- Potters, G., Schutte, F., Van Goethem, D., Denollin, S., Samson, R. and Gielis, J. Bamboo as a crop in Western Europe – A SWOT analysis. *Acta Hort.*, submitted.
- Potters, G., Brems, A., Valcke, R., Dewil, R., Haese, d', L., Samson, R., Gielis, J., 2009. Energy crops in Western Europe: is bamboo an acceptable alternative? 8th World Bamboo Congress proceedings: vol. 3, pp.22-24.
- R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/http://www.R-project.org/>.
- Ribeiro R.V., Machado E.C., Santos M.G., Oliveira R.F. 2009. Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees. *Environmental and Experimental Botany* 66, 203-211.
- Schutte, F., Gielis, J. and Potters, G. IKEBANA - Trying to get Europe addicted to bamboo, *Acta Hort.*, submitted.
- Scurlock, J.M.O., Dayton, D.C. and Hames, B. 2000. Bamboo: An Overlooked Biomass Resource? ORNL/TM-1999/264. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- Srivastava, A. and Strasser, A. 1996. Stress and stress management of land plants during a regular day. *J. Plant Physiol.* 148: 445–455.
- Srivastava A, Strasser RJ, Govindjee, 1999. Greening of peas: parallel measurements of 77 K emission spectra, O-J-I-P chlorophyll *a* fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. *Photosynthetica* Vol 37p pp.365-392.
- Strand, M. & Lundmark, T. 1987. Effects of low night temperatures and light on chlorophyll fluorescence of field grown seedlings of Scots pine (*Pinus sylvestris* L.). *Tree Physiology* 3: 211-224.
- Strand, M. & Öquist, G. 1985. Inhibition of photosynthesis by freezing temperatures and high light levels in cold acclimated seedlings of Scots pine (*Pinus sylvestris*). –II. Effects on chlorophyll fluorescence at room temperature and 77K. *Physiologia Plantarum* 65: 117-123
- Strasser, B.J. 1997. Donor side capacity of photosystem II probed by chlorophyll *a* fluorescence transients. *Photosynth. Res.* 52: 147–155.
- Strasser R.J., Srivastava, Tsimilli-Michael M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U and Mohanty P (eds) *Probing Photosynthesis: Mechanism, Regulation and Adaptation*, Chapter 25, pp 443--480. Taylor and Francis, London, UK
- Sveshnikov, D., Ensminger, I., Ivanov, A.G., Campbell, D., Lloyd, J., Funk, C., Huner, N.P.A. & Öquist, G. 2006. Excitation energy partitioning and quenching during cold acclimation in Scots pine. *Tree Physiology* 26: 325-336.
- Tsimilli-Michael M., Krüger G.H.J., Strasser R.J., 1995. Suboptimality as driving force for adaptation: A study about the correlation of excitation light intensity and the dynamic fluorescence emission in plants. In Mathis, P. (ed.) *Photosynthesis: From Light to Biosphere*, Vol 5: 981-984. Kluwer Academic, The Netherlands.
- Van Goethem D., Ooms A., Bogaerts L., Jaspers E., Klimina I., Samson R., O'Connor D., O'Connell P., Denollin S., Potters G. Bamboo Growth under Irish Skies: Pushing the Limits of a Sturdy Plant, *Acta Hort.*, submitted.
- Van Rensberg I., Krüger G.H.J., Eggenberg P., Strasser R.J., 1996. Can screening criteria for drought resistance in *Nicotiana tabacum* L. be derived from the polyphasic rise of the chlorophyll *a* fluorescence transient (OJIP)? *South African J.Bot.* 62: 337-341.
- Vervaeke P., Luysaert S., Mertens J., Meers E., Tack F.M.G., Lust N., 2003. Phytoremediation prospects of willow stands on contaminated sediment: a field trial, *Environmental Pollution* 126, 275-282.

Vu J.C.V. 1999. Photosynthetic responses of citrus to environmental changes. In: Pessaraki, M. (ed.) Handbook of Plant and Crop Stress. Marcel Dekker, New York, pp. 947-961.
<http://scs.math.yorku.ca>, 2011.

Session 3. Fibres

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Interfacial Adhesion of Bamboo Fibre Composites: surface treatment with chitosan

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Abstract

In this study, systematic experimental results describing the dynamic wetting properties of bamboo fibres were analyzed by applying the molecular-kinetic theory of wetting. Results suggest that the bamboo fibre surface represents a well defined system for wetting analysis. In this way, meaningful information on interfacial interactions can be obtained with the aim to conduct a study of the adhesion in terms of surface free energies of bamboo fibres with different thermoplastics. In order to improve the compatibility for bamboo fibre, chitosan coating was applied. The effect of this treatment was evaluated by using contact angle measurements following the Wilhelmy technique. The surface free energy components were calculated following the acid-base theory. These values were then used to calculate the theoretical work of adhesion. The wetting behaviour of various thermoplastic matrices (polyvinylidene fluoride, polypropylene and grafted maleic anhydride polypropylene) was characterized. Surface topography was examined by AFM and surface chemical components were identified using XPS. Additionally, unidirectional bamboo fibre composites were prepared in order to obtain a direct measure of the effect of fibre modification on their adhesion by performing 3-point bending tests.

The results indicate that the high concentration of lignin on the surface of bamboo fibres is responsible for their wetting properties. It was possible to obtain experimental wetting data on bamboo fibres with reasonable accuracy, allowing meaningful information on interfacial interactions to be deduced. In this way, surface components of bamboo fibres and thermoplastic matrices were matched, resulting in an improvement of the physical adhesion of bamboo fibre composites, as verified by micromechanical test results. Moreover, the correlation between the theoretical work of adhesion and practical adhesion shows a direct relationship. In this manner, it was shown that theoretical work of adhesion obtained by wetting analyses can predict the adhesion strength, particularly for thermoplastic matrices.

Keywords

fibre-matrix adhesion, wetting, natural fibre composites, bamboo fibre

1. Introduction

The use of bamboo fibres as reinforcement fibre in (polymer) composite materials has attracted interest due to specific mechanical properties which are comparable to glass fibres. Low cost, environmental friendliness and natural abundance make such fibres possible substitutes to synthetic reinforcing fibre materials, especially for polymer matrix composites. To achieve good wetting and adhesion of the bamboo fibre with different polymers, the fibre surface needs to be characterized. However, natural fibres have several complex characteristics such as liquid sorption, different cross sections along the fibre length, chemical heterogeneity, which make obtaining meaningful data from wetting measurements particularly challenging. Therefore, the interpretation of their wetting behaviour as quasi-equilibrium phenomena can be invalid.

The use of thermoplastics as matrices for natural fibre composites represents an approach with low environmental effects. However, the hydrophilic nature of natural fibres reduces their potential as reinforcing agents due to low interfacial interactions with hydrophobic thermoplastic matrices, such as polyethylene and polypropylene. In the case of bamboo technical fibres, the surface is covered with lignin instead of cellulose or hemi-cellulose like most other natural fibres (Fuentes et al. 2011). While lignin has a less hydrophilic nature, chemical modifications should still be considered to optimize the interface. Other problems arise when using thermoplastic matrices, as the lack of occurrence of covalent bonding and low viscosity of melted thermoplastics, making maximization of the physical adhesion indispensable to obtain better composites.

Bonding between the reinforcing fibre and the matrix has a significant effect on the properties of the composite since stress transfer and load distribution efficiency at the interface is determined by the degree of adhesion between the components. Using the experimental data obtained from wetting measurements, fibres and matrices can be examined and matched in terms of their surface energy components; predicting and verifying their compatibility allows more suitable combinations and therefore better composites to be made.

In this study, a novel procedure based on an autoclave treatment is presented, allowing stable and reproducible advancing contact angles to be measured. The wetting behaviour of bamboo fibres and thermoplastic matrices (polyvinylidene fluoride PVDF, polypropylene PP and grafted maleic anhydride polypropylene MAPP) is characterized through the Wilhelmy technique, the molecular-kinetic theory of wetting is used to interpret the contact angle experimental data, the fibre surface is characterized by AFM and XPS. Surface energy components of bamboo fibres and thermoplastic matrices are estimated by using the acid-base approach. Furthermore, the surface of the bamboo is altered in order to change the surface energy components using a chitosan coating treatment. Additionally, unidirectional bamboo fibre composites are prepared in order to obtain a direct measure of the effect of physical adhesion by performing 3-point bending tests (transverse and longitudinally).

2. Results and discussion

2.1. Wetting behaviour

In the case of natural fibres, a direct measurement of the contact angle is problematic, and so their wetting behaviour is difficult to study. The Wilhelmy technique represents a reliable method to study the wetting behaviour of bamboo fibres at different immersion speeds (Fuentes et al. 2011). We could prove that by using autoclave treatment we could minimize surface waviness, roughness and liquid penetration, without changing surface chemistry. Thus the bamboo fibre surface represented a well defined system and so its wetting behaviour can be studied and a meaningful interpretation of wetting

data is ensured (Fig. 1).

For this to be the case, contact angles of bamboo fibres must show an expected dependence to immersion velocity. The molecular-kinetic theory (Blake 2006; Bertrand et al. 2009) provides a reasonable fit to the data, confirming the expected immersion velocity dependency, reproducibility and stability of the advancing contact angle in a bamboo-water system; indicating that the measured contact angle is the true advancing contact angle (Fig. 2).

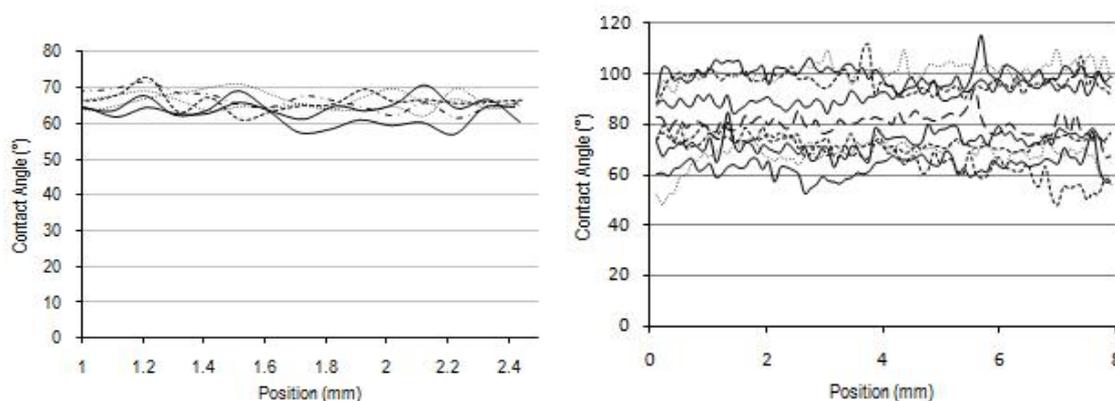


Fig.1. Advancing contact angle for autoclaved (left) and non-autoclaved (right) bamboo fibres in water. The results indicate a reduction of hysteresis caused mainly by fibre surface irregularities reduction at different length scales due to the autoclave treatment (Fuentes et al. 2011).

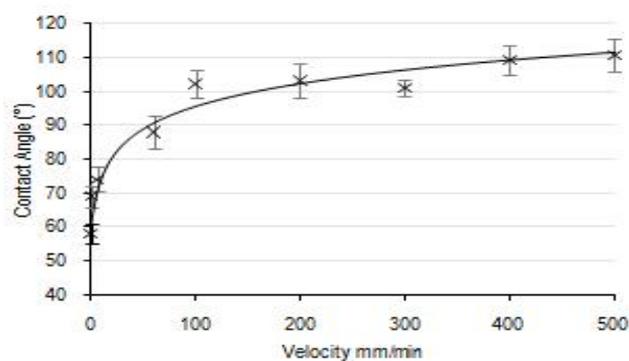


Fig.2. Dynamic contact angle as a function of wetting velocity for water on bamboo technical fibre, according to the molecular kinetic theory. The theoretical curve through the data was obtained by nonlinear regression of experimental data; the extrapolated static angle is obtained.

2.2. Surface chemical composition

Figure 3 shows the results regarding surface chemical constituents of both autoclave-treated and non-treated technical bamboo fibres obtained from the decomposition of the high resolution carbon 1s spectrum for each fibre. Lignin content on the fibre surface was analyzed by determining the oxygen-to-carbon atomic ratio, and the relative concentration of the C1 component. The results clearly indicate that technical bamboo surface constituents are close to our references for lignin, indicating that bamboo technical fibres may be homogeneously covered with lignin and possibly some other molecules, but not with cellulose. On autoclave treatment, the surface chemistry does not change.

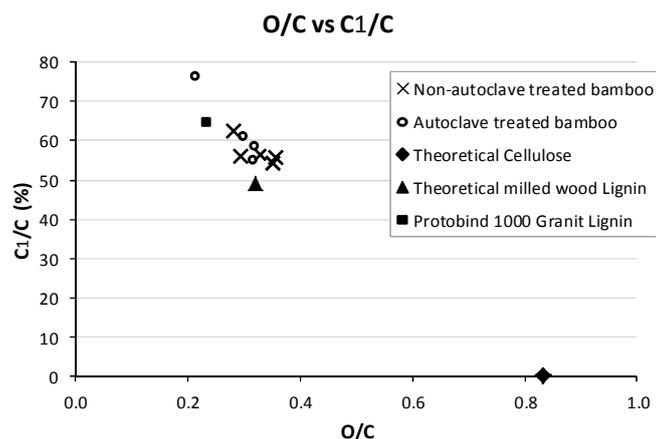


Fig.3. C1/C ratios versus O/C ratios for chemical groups at the surface of bamboo fibres (Fuentes et al. 2011). Theoretical values for cellulose and lignin according to Shchukarev et al. (2002).

2.3 Surface energy components and work of adhesion

Once the advancing (static) and equilibrium contact angles of the liquids on the solids have been obtained (see table 1), the surface tension components can be determined. According to the acid-base theory, the total surface tension of the bamboo fibres was estimated to be 38,8 mN/m, with an acid and base contribution of 0,3 mN/m and 10 mN/m respectively (see table 2). Accordingly, the fibre's surface is relatively hydrophobic and a Lewis base, this means that a strong acidic Lewis matrix could give the best adhesion performance. So for optimal physical adhesion, the surface energy of the substrate should be as high as possible, while the two phases have the same Lifshitz-van der Waals components and the acidic component of the substrate is equal to the basic component of the liquid and vice versa (Connor et al. 1997; Della Volpe et al. 2004).

TABLE 1. The advancing contact angles of water, ethylene glycol (EG), and methylene iodide (MI) on untreated and chitosan treated bamboo fibres and the equilibrium contact angle of these liquids on thermoplastics films according to Andrieu et al. (1994).

Material	Water	EG	MI
Bamboo	60.3 ± 2.3	42.8 ± 0.8	47.9 ± 1.1
Chitosan	98.0 ± 4.1	64.1 ± 5.8	67.3 ± 4.2
PP	86.0 ± 1.1	49.4 ± 1.7	57.7 ± 1.0
MAPP	82.8 ± 0.7	65.3 ± 0.9	61.1 ± 0.6
PVDF	66.2 ± 0.3	36.0 ± 0.4	32.8 ± 0.3

The measured contact angles and the there from calculated surface tension components⁹ can be seen in table 1 and 2. As expected, PVDF possesses higher acidity among the three examined matrices due to the different electro-negativities of carbon, fluorine, and hydrogen. Accordingly, the total surface energy of PVDF is higher than that of PP and MAPP, with a higher polar fraction (Lee et al. 2008).

TABLE 2. Surface tension components of the treated and untreated bamboo fibres and thermoplastics.

⁹ SurfTen 4.3, a program for the calculation of acid-base solid surface free energy components by Claudio Della Volpe

Material	γ^{tot} (mJ/m ²)	γ^{LW} (mJ/m ²)	γ^{ab} (mJ/m ²)	γ^+ (mJ/m ²)	γ^- (mJ/m ²)
Bamboo	38.82 ± 0.76	35.44 ± 0.06	3.37 ± 0.57	0.28 ± 0.06	10.13 ± 1.25
Bam-chitosan	25.13 ± 1.42	24.39 ± 1.38	0.74 ± 0.44	0.53 ± 0.26	0.26 ± 0.18
PP	30.87 ± 0.52	29.90 ± 0.47	0.97 ± 0.20	0.12 ± 0.05	1.97 ± 0.31
MAPP	29.07 ± 0.37	28.52 ± 0.31	0.56 ± 0.20	0.02 ± 0.01	3.72 ± 0.32
PVDF	34.64 ± 0.53	31.16 ± 0.47	3.48 ± 0.23	0.93 ± 0.10	3.26 ± 0.23

For the case of PP and MAPP, we find a deviation in the magnitude of the polar surface energy component, which may be zero since pure PP is a nonpolar polymer. This effect could be related to aging processes or surface contamination. The work of physical adhesion is calculated for the different materials by using equation 1 with bamboo as the substrate.

$$W_a = \gamma_l + \gamma_s - \gamma_{sl} \quad (\text{Eq. 1})$$

where γ_l is the liquid surface tension, γ_s is the solid surface energy and γ_{sl} is the interfacial energy. The following values for W_a were obtained: 75 mJ/m² for PVDF, 67 mJ/m² for MAPP and 69 mJ/m² for PP. Looking at these results one would expect really good results when using PVDF and more or less the same results for PP and MAPP. It has to be noted that these values only relate to intramolecular forces and do not relate to covalent bonding.

Figure 4 and 5 show the transversal and longitudinal properties of MAPP, PP and PVDF bamboo composites made with the same procedure either at 175 °C or 200 °C and with a volume fraction of 40%. The calculated values for the work of adhesion correlate reasonably well with the obtained results for the transversal properties, shown in figure 4. As predicted by the calculated work of adhesion our results for PVDF are better than the others (once the PVDF is properly molten at 200°C). Proving that the work of adhesion has really improved.

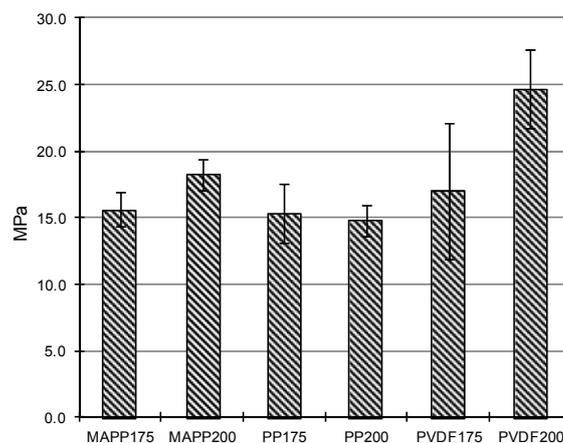


Fig.4. Transversal properties of MAPP, PP and PVDF bamboo composites at different processing temperatures

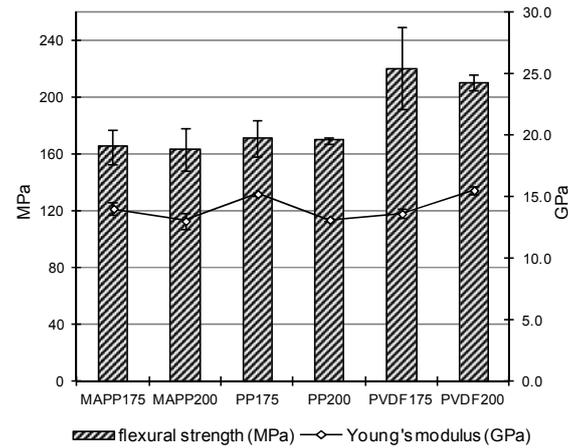


Fig.5. Longitudinal properties of MAPP, PP and PVDF bamboo composites at different processing temperatures.

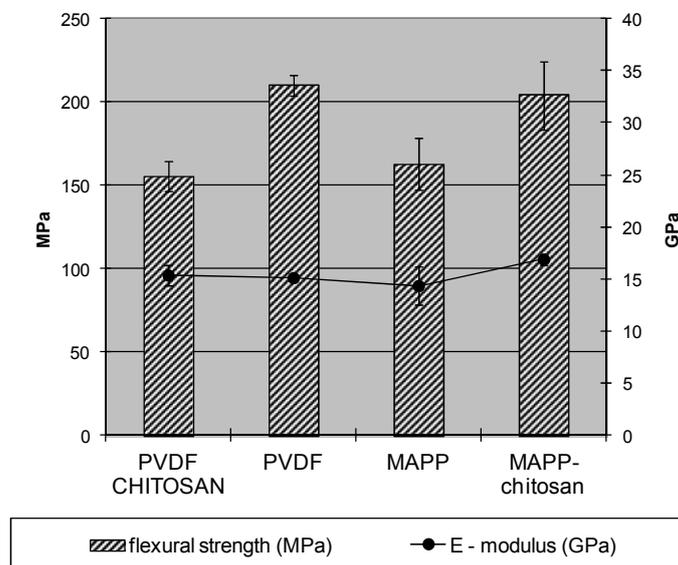


Fig.6. Longitudinal properties of chitosan coated and uncoated, MAPP and PVDF bamboo composites.

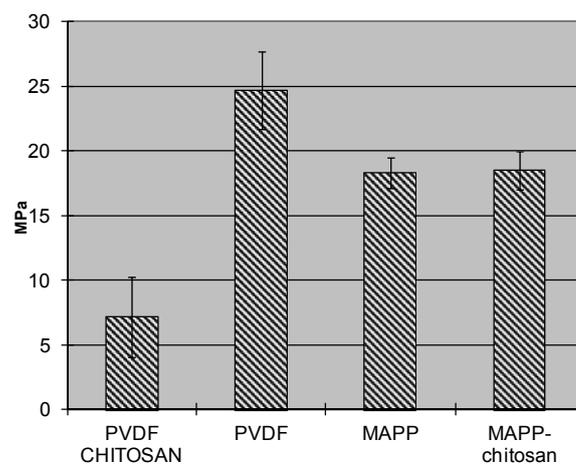


Fig.7. Transversal strength of chitosan coated and uncoated MAPP and PVDF bamboo composites.

2.4 Bamboo fibre surface treatments

Bamboo fibres and prepregs were coated with chitosan using an aqueous chitosan-CO₂ solution (Sakai

et al. 2002).

The main idea of this treatment is to cover the surface with chitosan, increasing the amount of available hydroxyl-groups on the surface. In this way, the properties of MAPP-bamboo composites might be increased, since lignin has fewer hydroxyl-groups at the surface, and MAPP has the capability to form covalent bonds with hydroxyl groups through esterification.

The obtained results in tables 1 and 2 for MAPP are very similar to the results obtained for PP. The small differences in values are created by the presence of the maleic anhydride in MAPP and aging processes (Aranberri et al. 2003).

The change is relatively small because we worked with a 1wt% MAPP. Calculating the work of adhesion with chitosan as the substrate (56 mJ/m^2), we see that this has reduced for the chitosan coated fibre. So theoretically we would expect lower mechanical properties with chitosan coated bamboo, if we just consider physical interactions. However, chitosan may increase the presence of hydroxyl groups at the surface and may promote chemical bonding, increasing the mechanical properties of the composite. Looking at figures 6 and 7 we only see this improvement in the longitudinal direction, suggesting possible chemical bonding. We have an increase of 22% for the longitudinal strength while the transversal remains the same. The later shows that the transversal test does not confirm the improvement of adhesion strength, possibly because of lower transverse strength of the chitosan coating. Further tests need to be carried out to evaluate this hypothesis.

The calculated work of adhesion for PVDF with chitosan coated bamboo (59 mN/m) is much smaller than for PVDF with untreated bamboo (75 mN/m). So theoretically one would expect a composite made with bamboo and PVDF to be much stronger than a composite made with chitosan coated bamboo and PVDF, since there are no chemical bonding mechanisms. The results of the three point bending correspond with the theory and are shown in figures 6 and 7.

The composite with the uncoated fibre is about 28% stronger in the longitudinal direction and about 300% better in the transversal. Showing that the adhesion is better in the uncoated fibre. Both composites show about the same longitudinal stiffness, which is around 80% of the theoretical value of 19,2 GPa according to the rule of mixtures, indicating similar levels of wetting.

3. Conclusions

The high concentration of lignin on the surface of technical bamboo fibres, as concluded from XPS results, seems to be responsible for their wetting properties, whereas fluctuations during wetting experiments between bamboo fibres may be due more to surface topography than to any other type of non-equilibrium phenomena. Autoclave treatment was used to smoothen the lignin surface layer.

The wetting behaviour of bamboo fibres appears to conform well to the predictions of the molecular-kinetic theory. Therefore, it was possible to obtain experimental wetting data on bamboo fibres with reasonable accuracy, allowing meaningful information on interfacial interactions to be deduced. In this way, surface components of bamboo fibres and thermoplastic matrices were matched, resulting in the improvement of the physical adhesion of bamboo fibre composites revealed by 3-point bending test results. Accordingly, the properties of PVDF lend themselves well for the preparation of bamboo composites with high interface strength.

References

- Andrieu C., C. Sykes and F. Brochard. Average Spreading Parameter on Heterogeneous Surfaces : *Langmuir*, 1994, Vols. 10, 2077-2080.
- Aranberri-Askargorta I., T. Lampke, A. Bismarck. Wetting behavior of flax fibers as reinforcement for polypropylene : *Journal of Colloid and Interface Science* , 2003, Vols. 263, 580–589.
- Bertrand E., T.D. Blake, J.D. Coninck, Influence of solid–liquid interactions on dynamic wetting: a molecular dynamics study, *J. of Physics: Condensed Matter*. 2009, 21, 464124.
- Blake T.D., The physics of moving wetting lines, *J. of Colloid and Interface Science*. 2006, 299, 1-13.
- Della Volpe C., D. Maniglio, M. Brugnara, S. Siboni, M. Morra, The solid surface free energy calculation I. In defense of the multicomponent approach. *J. of Colloid and Interface Science*. 2004, 271, 434-453.
- Fuentes C.A., L.Q.N. Tran C. Dupont-Gillain, W. Vanderlinden, S. De Feyter, A.W. Van Vuure, I. Verpoest, Wetting behaviour and surface properties of technical bamboo fibres, *Colloids Surf. A*. 2011, 380, 89-99.
- Lee, J-S Park S., T.R. Lee. The Wettability of Fluoropolymer Surfaces: Influence of surface dipoles : *Langmuir*, 2008, Vols. 24, 4817-4826.
- M.Connor, J. Bidaux, J. Manson, A criterion for optimum adhesion applied to fibre reinforced composites. *Journal of materials science* 1997, 32, 5059-5067.
- Sakai Y., K. Hayano, H. Yoshika, T. Fujieda, K. Saito, H. Yoshioka, Chitosan-coating of cellulosic materials using an aqueous chitosan-Co₂ solution. *Polymer Journal*, 2002, 34, 144-148.
- Shchukarev A., B. Sundberg, E. Mellerowicz, P. Persson, XPS study of living tree, *Surface and Interface Analysis*. 2002, 34, 284-288.

Gluability Variation of *Dendrocalamus asper* for Bamboo Composites

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Abstract

The aim of this research was to determine the buffer capacity, wettability and adhesive penetration of an Asian bamboo, *Dendrocalamus asper* Backer. These properties were analyzed in order to prove its suitability to be promoted as raw material for the manufacture of structural composite products especially Plybamboo (PYB), Laminated Bamboo Lumber (LBL), Oriented Strand Board (OSB), Parallel Strand Lumber (PSL) and Oriented Strand Lumber (OSL). The buffer capacity were determined in three location along the culm length but the wettability and adhesive penetration were evaluated in the effect of three locations and three of bamboo culm surface. *D. asper* is more stable toward alkaline rather than acid. There are slightly differences in the values taken from the different locations in the culm. Moreover, *D. asper* has similar wettability and adhesive penetration to other bamboo and wood species that are used in composite products. Nevertheless, there were significant differences from the culm location and surface. From a practical point of view, this is not desirable behavior because the variation in wettability and adhesive penetration may cause problems in adhesive bonding when it is used as raw material in wood composite with conventional commercial resin.

Keywords

Gluability, Buffer capacity, Wettability, Adhesive penetration, *Dendrocalamus asper*

Introduction

Bamboo is a non-wood lignocellulosic material which has been widely used as a material for construction, furniture manufacture and daily household uses. In the late 20th century, bamboo has received increasing attention as the substitute raw material for use in the wood composite manufacture such as Plybamboo (PYB), Laminated Bamboo Lumber (LBL), Oriented Strand Board (OSB), Parallel Strand Lumber (PSL) and Oriented Strand Lumber (OSL). However, bamboo is heterogeneous and anisotropic material. Moreover, there are several different characteristics between bamboo and wood. Consequently, the additional adjustment of manufacturing processes may be required, such as the adhesive system. The adhesive is not only a significant cost factor in wood composite production but also it is the key factor for some of the product properties, future development of bamboo-based composites will require an analysis of the gluability of bamboo. This property is influenced by bamboo surface properties, such as buffering capacity, wettability and adhesive penetration etc (Blomquist et al. 1983).

In wood composites, the buffer capacity influences the curing rate of resin and adhesion between wood and adhesive. The buffer capacity is the quantitative measure of the resistance of wood to pH change on addition of hydroxide ions. Maloney (1993) suggested that the wood which requires a larger amount of acid or base to adjust the pH to the level required for optimum adhesive cure is considered as a high buffering capacity species. Several studies (Johns and Niazi 1980; Van Niekerk and Pizzi 1994; Zanetti and Pizzi 2003) have reported that the wood-composite properties depend on buffer capacity of raw material. All together, the surface wettability determines the rate of adhesive penetration into the wood surface. Wettability is the wetting degree of a liquid drop on a solid surface to form a contact angle (θ), as seen in the figure 1. This angle can be used to measure the wetting of a solid surface (Blomquist et al. 1983; Marra 1992; Frihart 2005). As the tendency of a drop to spread out over a flat, solid surface increases, the contact angle decreases. Thus, the contact angle provides an inverse measure of wettability. The sessile drop method is measured by a contact angle meter using an optical subsystem to capture the profile of a pure liquid on a solid substrate. The adhesive penetration is the ability of the adhesive to move into the coarse capillary structure of wood or wood based composite. The bonding between an adhesive and the wood substrate can be formed when the adhesive can sufficient interpenetrate into wood components and then solidify to develop to an interlocking mechanism (Marra 1992; Frihart 2005). The adhesive penetration into the wood depends on the permeability, porosity, roughness, wettability, temperature, pressure and time (Barbu 2000; Marra 1992; Hare and Kutscha 1974; Tarkow and Southerland 1964). The determination of adhesive penetration into wood can be easily investigated by visible light, fluorescence and scanning electron microscopy.

The objectives of this study are (1) to determine the buffer capacity in each location of *Dendrocalamus asper* culms, (2) to investigate the surface wettability through contact angle measurement and (3) to measure the effective penetration of liquid adhesive into bamboo in different locations and surfaces of the culms.

Materials and Methods

Materials

In this study, the three years old of *D. asper* culms were collected from plantations located in Nakorn Sri Thammarat, south of Thailand. Bamboo culms were harvested from plantation and transported immediately to the lab. These bamboos had an average culm length of 18 m. The culm diameter at the bottom was about 11.5 cm, while the top culm diameter was about 2 cm. The average culm wall thickness was 1.6 cm. The average specific gravity at 12% moisture content was 0.75. The culms were divided into three parts (bottom, middle and top) each of 6 m lengths.

Measurement of Buffer Capacity

The method of buffer capacity measurement was processed according to Maloney and Borden Chemical Inc. (Maloney 1993). The bamboo chips were ground into small particles and screened into the range of 0.25 mm (a 60[#] mesh screen) and 0.45 mm (a 40[#] mesh screen). 30 g of dry bamboo powder were soaked in 400 ml of distilled water at 20°C for 30 minutes. The mixture was stirred during the soaking. Then, it was filtered with a filter paper using the vacuum. 150 g of the extracted solution was determined for the pH value by pH meter (Docu-pH⁺ meter Sartorius) which was calibrated with standardized buffer solutions before each titration. The initial pH of the solution was recorded, and it was then titrated to a pH of 3.5 or 10 with 0.01N H₂SO₄ or 0.01N NaOH solution, respectively. The pH was recorded after each addition (ml) of acid or base. The buffer capacity was determined from the titration curve which represents pH versus volume (ml) of acid or based needed to change the pH to 3.5 or 10. Three replications were done for each condition. Comparisons between location (bottom, middle and top parts) were done.

Determination of Wettability

The study of bamboo wettability was investigated by contact angle measurement as previously studies (Bodig 1962; Freeman and Wangaard 1960; Mara 1992; Wellons 1980). A contact angle meter (Kyowa DM 300 with Famas software) was used to observe the contact angle. Bamboo specimen was cut into the size of 20 x 40 mm x culm wall thickness and was then removed the outer and inner layers. All specimens were conditioned at 20°C and 65% RH until the constant weight was reached. Each bamboo surface was sanded by 220-grid sandpaper immediately before 2 mg of distilled water was dropped onto the surface of the specimens. The angle made between the droplet and the bamboo surface, as present in figure 1, was measured after 2 seconds. Comparisons between location in bamboo culm (bottom, middle and top parts) and surface (inner, outer and culm wall surfaces) were done. Figure 2 illustrates three surface of bamboo culm.

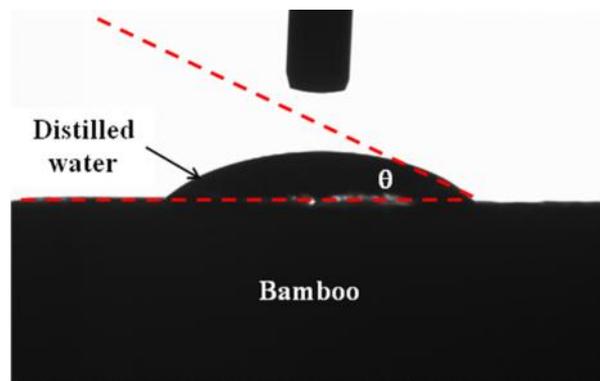


Figure 1 Image of contact angle (θ) of the water sessile drop on a bamboo surface

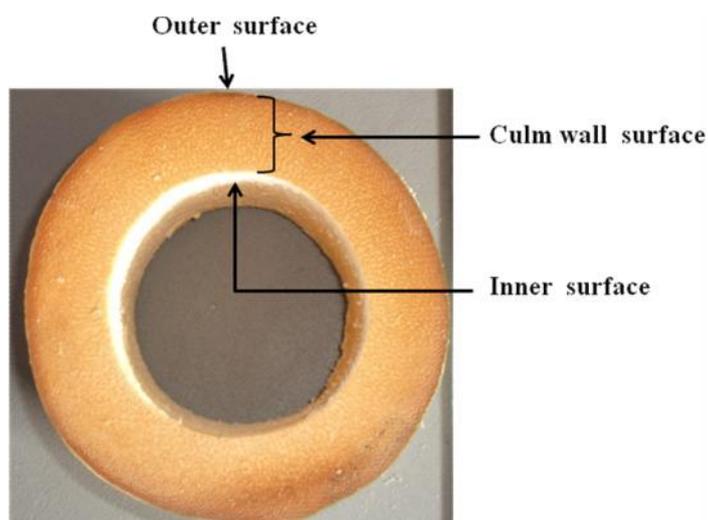


Figure 2 Inner, outer and culm wall surfaces of bamboo culm.

Adhesive Penetration Measurement

The procedure of adhesive penetration measurement was adapted from the measurement of liquid adhesive penetration into *Dendrocalamus strictus* by Ahmad (2000). As well, bamboo specimen was cut into the size of 20 x 40 mm x culm wall thickness and was then removed the outer and inner layer. All specimens had been placed in a conditioning chamber at a temperature of $20 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 2\%$ to the final average moisture content of 12% at the time of glue spreading. Table 1 presents the working properties of urea-formaldehyde adhesive used in this study. It was obtained from Dynea, Thailand. UF adhesive was blended according to the specification of its supplier. It was then spread on one surface of the bamboo specimen with a hand brush. Spread rate was approximately 200 g/m^2 of a single surface. The specimens were allowed an additional 10 minute open-assembly and then heated in a convection oven at 103°C for 12 hours. The specimens with the cured adhesive layer were cross-cut through the glue line, and then observed using a stereo microscope (Olympus SZ-CTV). The images of the adhesive layer on bamboo surface were captured using a camera (Olympus DP 12), as present in figure 3. The images were analyzed the average penetration, the average distance of penetration of the three most distant adhesive objects detected, by ImagePro Plus software (Version 5.0.2.9). Comparisons between location (bottom, middle and top parts) and surface (inner, outer and culm wall surfaces) were done as well.

Table 1 The working properties of urea-formaldehyde adhesive used in this study

Properties	Results
Appearance	White-colored liquid
Viscosity at 20°C (cps)	208
Solid content 3 hrs at 105°C (%)	65.20
pH at 20°C	8.86
Density at 20°C	1.27

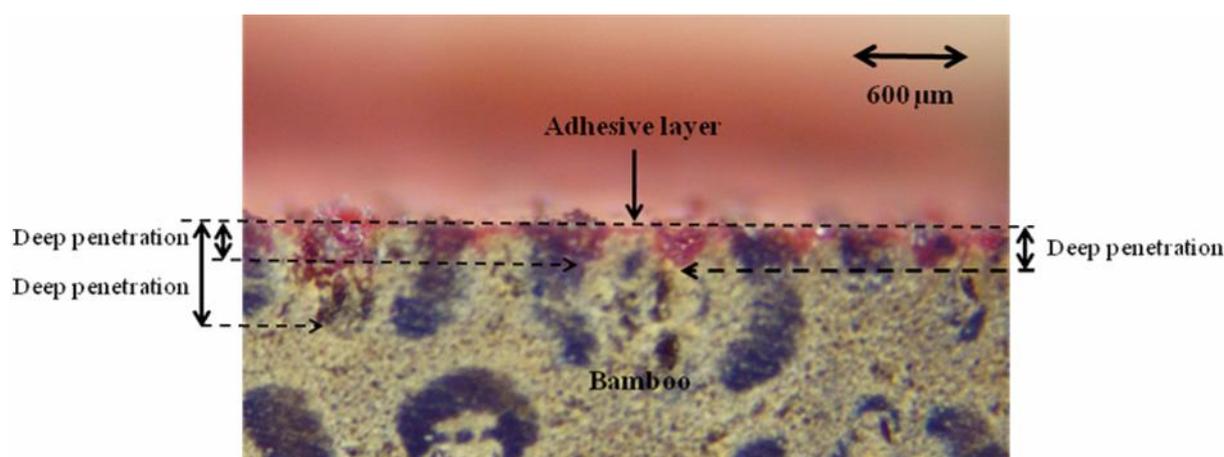


Figure 3 Illustration of deep of urea formaldehyde adhesive penetration onto bamboo surface.

Results and Discussion

Buffer Capacity

Figure 4 shows the pH changes of *D. asper* at different culm location during the addition of 0.01N H₂SO₄ (a) and 0.01N NaOH (b) to the required final pH value at 3.5 and 10, respectively. The quantity of 0.01 N H₂SO₄ used to change the pH value from 4.8 to 3.5 is 39.33 ml, but the quantity of 0.01 NaOH used to change the pH value from 4.8 to 10 is 117.67 ml. These results suggest that *D. asper* slightly resists in acid but it much more constant in alkaline. It might be conclude that the buffer capacity of *D. asper* is greater towards alkaline than that of acid. This appears to be an important parameter when *D. asper* is bonded with adhesives which are sensitive to pH. Such an acid-based adhesive (i.e., UF) normally used with the acid-based catalyst, the pH value of the adhesive layer on bamboo surface would quickly decrease to the optimum level of curing rate, which normally occurs between pH of 3 to 4. In contrast, it would be a problem when an alkaline-base adhesive (i.e., PF) used with base-based catalyst is applied. Because *D. asper* is stable in alkaline condition, a larger amount of base catalyst would be required to increase the pH to the optimum level which is required for the optimum cure, approximately 10 to 12. This may cause problems for its use as raw material in wood composite with conventional commercial PF adhesive. Some strategies, such as the use of special adhesive or adjusted hot-pressing parameters, might be applied to improve resin curing and hence improve product properties too.

Both of buffering capacity differs in each location in the culm, as illustrated in figure 4. Notable, the bottom part of the culm shows highest buffer capacity value. One possible explanation might be rest on the variation of chemical composition of bamboo. The chemical properties of raw material especially water extracts and inorganic matters have effect on its buffer capacity. This hypothesis can be confirmed by finding of Passialis et al. (2008). They found out that hot-water extracts and inorganic elements, which evidently are present in the bark of wood, have significantly effect on the buffer capacity of wood.

Figure 4 also presents the initial pH value of three different locations along the culm. The results suggest that the average of pH value of *D. asper* is 4.8, which is on the acid side. It is desirable that the pH value of *D. asper* is not different from common wood species (Fengel and Wegener 1984). Moreover, it has no variation in pH value at the different locations on the culm. This observation is consistent with Malanit et al. (2009). The same technology and practices might be applied to this bamboo specie when being used as an alternative raw material in composites manufacture.

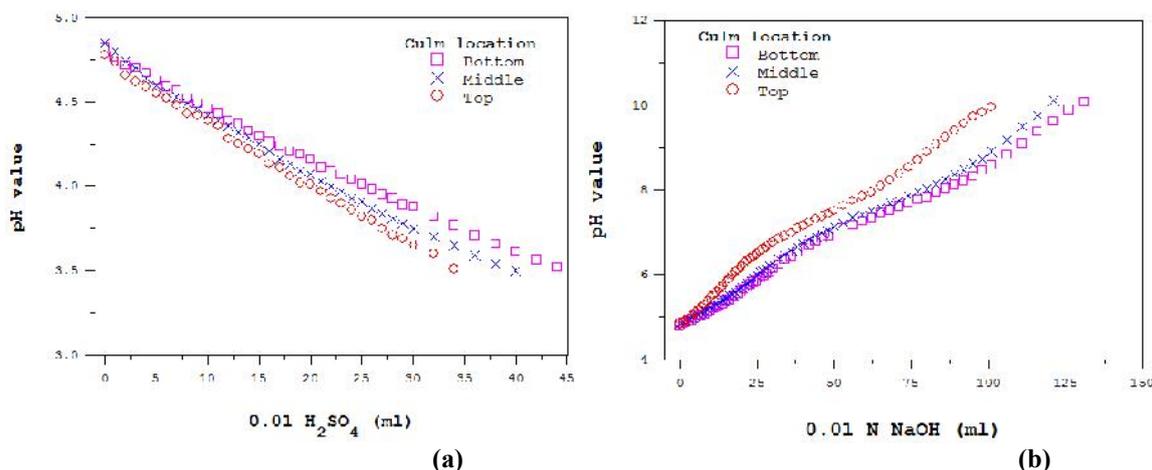


Figure 4 The pH changes of *D. asper*, in relation to the culm location, during the acid (a) and based (b) addition.

Contact Angle and Wettability

Table 3 presents the contact angle using distilled water at different culm location and both surfaces. The average contact angle of *D. asper* is 35.44° which is slightly lower than that of common wood species such as Aspen, Yellow-poplar and White Oak which have the contact angle of 38° , 51° and 50° , respectively (Freeman and Wangaard 1960). The good wetting occurs when the small contact angle appears and the liquid can spread or flow spontaneously across the solid surface.

Table 4 presents the analysis of variance performed on the different location and surface. The analysis indicated that there is significant difference ($P < 0.05$) in contact angles between culm location. The average value of contact angle slightly increases from the bottom to the top part. The increment of bamboo specific gravity from the bottom to the top part of the culm might be explained this phenomenon. Malanit et al. (2008) studies the specific gravity variation of *D. asper* along the culm length. The specific gravity of *D. asper* at 12 percent moisture content is in the range of 0.55 to 0.90. The value significantly increases from bottom to the top of the culm. Additionally, the contact angle within each surface of the bamboo culm was found to be significantly different ($P < 0.05$), as illustrated in table 4. The contact angle of outer surface is greater than that of the inner surface. The larger angle at the outer surface implied that the outer surface is more difficult to be wetted than the inner surface. One possible explanation might rest on the difference of vessel size between outer and inner part of the bamboo culm. Liese (1988) reported that vessels are larger at the inner part of the culm and become small toward the outer part. Moreover, the outer surface of bamboo culm is covered by wax that makes it hard for the adhesive to wet and penetrate to the cellular structure. The results suggested that the average value of contact angle of culm wall surface is quit similar to that of outer surface. However, the culm wall surface shows the higher standard deviation than the outer surface. The high standard deviation indicates that the data are spread out over a large range of these values. It could also be explained by the SG variation between the inner and the outer part of the culm, as mention before.

Blomquist et al. (1983) and Marra (1992) stated that the material with a higher contact angle has poor surface wettability resulted in poor bonding because of greater tendency for starved joints. It can be confirmed by previous studies. Nugroho and Ando (2001) reported that the internal bond strength of LBL tended to decrease when bamboo plates were laid on outer-outer layer type. They explained that the outer surface of bamboo culm contains chemicals such as wax and silica, which has the seriously

effect on the bonding strength of the glue line. Additionally, the outer surface of bamboo is harder than the inner layer, which can seriously affect the wettability and the penetration of resin.

Table 3 Average value of contact angle and adhesive penetration on the surface of *D. asper*, separated by culm location and surface

Culm location	Culm surface	Contact angle (Degree)	Average penetration (μm)
Bottom	Inner surface	28.68 (2.52) ¹	131.25 (34.52)
	Outer surface	38.29 (1.94)	96.27 (22.02)
	Culm wall surface	39.73 (6.11)	103.91 (49.45)
Middle	Inner surface	27.87 (2.47)	125.25 (29.61)
	Outer surface	39.88 (2.07)	80.42 (26.60)
	Culm wall surface	40.06 (4.66)	94.53 (49.29)
Top	Inner surface	27.42 (2.67)	114.06 (37.47)
	Outer surface	37.50 (1.90)	71.67 (26.13)
	Culm wall surface	36.51 (4.89)	87.50 (49.66)

Note ¹ Number in parenthesis is associated to standard deviation

Table 4 The variance analysis for contact angle and average penetration of *D. asper*.

Source of variation	F-value	
	Contact angle (n=27) ¹	Average penetration (n=72)
Culm location	5.45 ^{**}	5.09 ^{**}
Culm surface	108.69 ^{**}	19.05 ^{**}
Interaction	1.70 ^{NS}	0.13 ^{NS}

Note ¹ n is the number of replications per treatment combination

^{**} indicates significance at the 1% level of probability.

^{NS} indicates not significant.

Furthermore, Anwar et al. (2005) reported that the shear strength property of Plybamboo is very low due to the high wettability of outer surface of bamboo culm. They recommended that the adhesive used for wood cannot be used for bamboo without modify the formulation. It must be modified for the optimizing bonding process, such as increasing of the filler amount in the glue mixture, using the liquid which has the low surface tension as the solvent in glue mixture, or adding the surfactant into the glue mixture.

Adhesive Penetration

Table 3 presents the average of adhesive penetration on different location and surface of *D. asper*, while table 4 shows the analysis of variance of the average penetration of the adhesives in *D. asper*. The result showed that there was significant difference in the average penetration between the three culm locations. The results also indicate that there are significant differences of average penetration of the three surfaces of bamboo culm.

According to Blomquist et al. (1983) and Marra (1992), the adhesive can penetrate into the surface of coarse capillary materials such as wood. It should be noted that the adhesive penetration is dependent upon the surface characteristics of wood i.e., degree of roughness, wettability and size of the wood's cell. Liese (1998) explained that the total culm tissue of bamboo consists of 50% parenchyma, 40% fibers and 10% conducting cells. The percentage distribution shows a specific pattern within the culm, both horizontally and vertically. The parenchyma and conducting cells are more frequent in the inner third of the wall, while the percentage of fiber is higher in the outer part. In the vertical direction, the fiber amount increases from bottom to top with the decreasing parenchyma content. Therefore, specific gravity of bamboo increases along the culm from bottom to the top. All together, it increases from the inner to the outer layer of the culm. Interestingly, there is no significant difference of the average penetration between the outer and culm wall surface. Because of the lack of ray cell in culm wall surface or radial direction in bamboo, the adhesive can equally penetrate in both directions. Additionally, the penetration into the culm wall surface shows the greater standard deviation value than the outer surface. With the difference specific gravity, adhesive will penetrate the surface in different degree. For the inner surface, an adhesive will over-penetrate into the bamboo and don't remain available on the surface for bonding, while the adhesive will under-penetrate into the outer surface of bamboo which may give poor bonding. Thus, the viscosity and composition of adhesive used in bamboo composites must be modified for the difference application. An adhesive which is applied on the outer surface tends to be much low in viscosity for better penetrating of bamboo, while the adhesive used onto the inner surface must be high in viscosity for leave more on the surface.

Conclusions

The gluability variation of *D. asper* such buffer capacity, wettability and adhesive penetration have been analyzed. The following conclusions can be drawn from this part of the study:

1. Buffer capacity of *D. asper* is more stable toward alkaline rather than acid. There are slightly differences in the values along the culm location.
2. *D. asper* has high wettability compared to other commercial wood species. In addition, wettability decreases from the bottom to the top part of the culm. Furthermore, wettability of bamboo culm outer surface is found to be lower than inner part. However, the wettability of the outer and culm wall surface is slightly different.
3. The adhesive penetration of *D. asper* at different culm location and surface is significant different from one other. The penetration also decreases from the bottom to the top part of the culm. Moreover, the penetration on culm inner surface is greater than that of outer and the culm wall surface.

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References

- Ahmad, M. 2000. Analysis of Culcutta bamboo for structural composite materials. Doctoral thesis, Virginia Polytechnic Institute and State University, USA.
- Anwar, U.M.K.; Paridah, M.T.; Hamdan, H.; Abd Latif, M.; Zaidon, A. (2005). Adhesion and bonding properties of plybamboo manufactured from *Gigantochloa scortechinii*. American Journal of Applied Sciences (Special Issue), 53-58.
- Barbu, M.C; Pruckner, M; Resch, H. 2000. On the wettability of medium density fiberboard. In Proceeding of the 4th European Panel Products Symposium, Bangor, UK. pp.14-21.
- Blomquist, R.F.; Christiansen, A.W.; Gillespie, R.H.; Myers, G.E. 1983. Adhesive bonding of wood and other structural materials. The Pennsylvania State University, USA.
- Bodig, J. 1962. Wettability related to gluability of five Philippine mahoganies. Forest Product Journal, 12(6), 265-270.
- Fengel, D.; Wegener, G. 1984. Wood: chemistry, ultrastructure, reaction. Walter de Gruyter. Berlin and New York.
- Freeman, H.G.; Wangaard, F.F. 1960. Effect of wettability of wood on glue-line behavior of two urea resins. Forest Product Journal, 10(6), 311-315.
- Frihart, C.R. 2005. Wood adhesion and adhesives. In Rowell, R.M. Handbook of wood chemistry and wood composites. CRC Press, 2000 N.W. Corporate Blvd., Boca Raton, Florida, USA. pp. 215-278.
- Hare, D.A.; Kutscha, N.P. 1974. Microscopy of eastern spruce plywood gluelines. Wood Science and Technology, 6(3), 294-304.
- Johns, W.E.; Niazi, K.A. 1980. Effect of pH and buffering capacity of wood on the gelation time of urea-formaldehyde resin. Wood and Fiber Science, 12(4), 255-263.
- Liese, W. 1998. The anatomy of bamboo culms. International Network for Bamboo and Rattan, Beijing, People's Republic of China.
- Malanit, P.; Barbu, M.C.; Liese, W; Frühwald, A. 2008. Macroscopic aspects and physical properties of *Dendrocalamus asper* Backer for composite panels. Journal of Bamboo and Rattan, 7(3&4), 151-163.
- Malanit, P.; Barbu, M.C; Frühwald, A. 2009. The gluability and bonding quality of an Asian bamboo (*Dendrocalamus asper*) for the production of composite lumber. Journal of Tropical Forest Science, 21(4), 361-368.
- Maloney, T.M. 1993. Modern particleboard and dry-process fiberboard manufacturing (updated edition). Miller Freeman, San Francisco.
- Marra, A.A. 1992. Technology of wood bonding, Van Nostrand Reinhold, New York, USA.
- Nugroho, N.; Ando, N. (2001). Development of structural composite products made from bamboo II: fundamental properties of laminated bamboo lumber. Journal of Wood Science, 47, 237-242.
- Passialis, C; Voulgaridis, E; Adamopoulos, S; Matsoukam M. 2008. Extractives, acidity, buffering capacity, ash and inorganic elements of black locust wood and bark of different clones and origin. Holz als Roh- und Werkstoff, 66, 395-400.
- Tarkow, H.; Southerland, C. 1964. Interaction of wood with polymeric material. I. Nature of the Adsorbing Surface. Forest Product Journal, 14(4), 184-186.
- Van Niekerk, J.; Pizzi, A. 1994. Characteristic industrial technology for exterior Eucalyptus particleboard. Holz RohWerkstoff, 52, 109-112.
- Wellons, J.D. 1980. Wettability and gluability of douglas-fir veneer. Forest Product Journal, 30(7), 53-55.
- Zanetti, M.; Pizzi, A. 2003. Upgrading of MUF resins by buffering additives-part 2: hexamine sulphate mechanisms and alternate buffers. Journal of Applied Polymer Science, 90(1), 215-226.

Novel Approaches to Process Bamboo Plants into UV-blocking Fibres

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Abstract

Currently viscose production methods are primarily used to process bamboo into commercial textile fibres. However, viscose methods use large quantities of chemicals and hence the process is not considered as environmentally friendly. The process also fails to retain bamboo's inherent unique properties such as ultraviolet (UV) screening and antibacterial functions. Hence, it is necessary to design an effective and more eco-friendly manufacturing method that would also retain the unique properties of raw bamboo plant into the fibres. In this research, bamboo was processed using new methods involving thermo mechanical treatments such as ultra-sonication, shaker milling and boiling with continuous stirring. Sodium hydroxide, hydrogen peroxide, enzyme and water were used separately in this process and their effects on fibre processing were compared. The morphology and UV shielding ability were analysed before and after processing. It was demonstrated that bamboo can be processed into fibres using only water and ball milling without the aid of any hazardous chemicals. The combination of mild acid hydrolysis and ultrasonic treatment with hydrogen peroxide was effective in the fibre separation and provided better appearance of fibres.

Keywords

Bamboo, fibres, UV absorbance, ultra-sonication, shaker milling.

1. Introduction

The textile industry is considered as one of the worst polluters due mainly to the use of non-eco-friendly raw materials and chemicals during fibre production. Even cotton, the most common natural fibre, has been identified as one of the most non “green” crops because of the use of high amounts of irrigation water and pesticides (Afrin, Tsuzuki & Wang 2009). The price of cotton is also increasing dramatically. Therefore, it is necessary to look for new renewable raw materials for textile fibre production. Bamboo has been identified as a more eco-friendly crop due to its fast growth rate, excellent carbon sequestration activity, needs for little water and no pesticides to grow (Austin, Ueda & Levy 1970; Liese 2009). However, the current commercial manufacturing process of bamboo fibres uses large quantities of harmful chemicals and hence it is questionable if bamboo textiles are considered as “green” products. Therefore, there is a strong need for developing a new method to produce textile fibres from raw bamboo plants in a more eco-friendly manner.

In our recent research (Afrin et al. 2011; Afrin, Tsuzuki & Wang 2011), the origin of the novel properties of bamboo such as UV screening and antibacterial functions was identified as the aromatic chemical components of lignin. However, in the current commercial manufacturing methods, raw bamboo plants are dissolved in chemicals to collect the cellulose rich substance and the functional chemical component activity of lignin is lost during the process, so that the resulting fibres cannot retain the unique properties of raw bamboo (Afrin, Tsuzuki & Wang 2011).

Ultra violet (UV) protection is vital to prevent premature ageing and in extreme case, skin cancer (ARPANSA 2003). However, typical summer choice, like cotton fibres give poor protection against the harmful rays of sun due to the absence of functional chemical components (Hoffmann et al. 2001; Afrin, Tsuzuki & Wang 2011).

In this study, new manufacturing methods were investigated to process raw bamboo plants into fibres while retaining the lignin which contributes to the UV screening property of raw bamboo in the final fibres, with special emphasis on the environmental impact of the manufacturing method from the viewpoint of processing chemicals and by-products.

Bamboo belongs to a grass family *Poaceae* (also known as the *Gramineae*), subfamily *Bambusoideae*, tribe *Bambuseae* (Hidalgo-Lopez 2003) with 75 genera and 1250 species (Liese 1998) ranging from tiny species (a few centimetre in height) to giant species (40 metres tall) (Hidalgo-Lopez 2003; Ueda 1960). It contains bast or ligno-cellulosic fibres and considered as natural nanocomposites (Afrin, Tsuzuki & Wang 2010) where cellulose nanofibrils are embedded in the matrix of lignin and hemicelluloses (Rao & Rao 2005). The stem of bamboo has only basic tissues and vascular ramifications but no xylem and phloem (Okumura et al. 2011). The vascular bundles are embedded in the parenchyma ground tissue located in the culm. The vascular bundles are composed of two metaxylem vessels, protoxylem, phloem with sieve tubes and companion cells fibres. Usually a culm consists of 52 mass % parenchyma, 40 mass % fibres and 8 mass% conducting tissue (Liese 1998). However, the amount of the fibres are 60-70 wt% of total bamboo stem (Liese 2003). The fibres are multi-layered with variable length. The lignification of fibre cell walls occurs even prior to the end of internode elongation (Gritsch & Murphy 2005). Due to the presence of a high amount of lignin (approximately 28 wt% using the Chinese Standard GB5889-86) (Afrin et al. 2011) in the middle lamella (Parameswaran & Liese 1976) and high mechanical strength (Jain, Kumar & Jindal 1992) due

to the alteration in the orientation of cellulose microfibrils (Gritsch & Murphy 2005), it is difficult to separate the fibres from each other.

To overcome this problem, in this study, mechanical and thermo-chemical approaches such as ultrasonication, shaker milling and boiling with continuous stirring were investigated. Ultrasonic treatment has been recently identified as an eco-friendly method in textile fibre processing (Li et al. 2011). Mild solvents such as enzymes and water were also used to aid the fibre processing.

2. Experimental

2.1 Materials

Six years old, dried matured bamboo (*Phyllostachys pubescens*) plant samples were purchased from Earthcare Farm at Crystal Waters Permaculture Village of Queensland, Australia. Reagent grade sodium hydroxide (NaOH), 35% hydrogen peroxide (H₂O₂) and sulphuric acid (H₂SO₄) from Sigma-Aldrich, Australia were used. Cellulase enzymes (GC 220) were purchased from Enzyme Solutions Pty Ltd, Australia. Cotton balls were bought from a local supermarket in Geelong, Australia, to use as a benchmark for the studies of UV absorption characteristics against bamboo and processed fibres or films. These cotton balls seems to be bleached but were not coated with any chemicals.

2.2 Machines and Investigations

The bamboo stems were split on the point of nodes and the internodes were removed. The nodes were milled into powder (around ~500 µm in diameter) in a Hafco hammer milling machine (Super Power BM-52VF, Australia). A Herless vertical band saw (Australia) fitted with a 14 TPI blade was used to split the bamboo culms into the pieces around 5 cm in length. The morphology of bamboo powder and fibres was studied using a scanning electron microscope (SEM) (Neoscope, JCM-5000). For SEM imaging, gold coating was applied on the samples with an Emitech sputter coater. UV-Vis spectroscopy was performed in reflectance mode using a Varian Cary 3 instrument equipped with an integrating sphere (Labsphere DRA-CA-30) and the reading were converted to absorbance value mathematically. An ultrasonic bath (Decon F5300b) was used for ultrasonic treatment. Shaker milling was done in an 8000M Mixer /Mill (Spex, USA). An Eppendorf centrifuge (model 5430R) was used for washing the fibres and particles at 6000 rpm at 21°C. Zirconox® balls of 0.4-0.6 mm in diameter and 70 mL polystyrene sterile containers were used for shaker milling.

2.3 Methods

2.3.1 Ultrasonic Treatment

Figure 1 shows the schematic diagram of ultrasonic processing of hammer milled crushed bamboo powder. The bamboo nodes were crushed into powder to give high surface areas for fast reaction. Three separate chemicals, namely, NaOH (10%), enzyme (10%) and H₂O₂ (10%) were used independently. The concentration of these chemicals is relatively low to minimize the environmental impact of the process. In particular, H₂O₂ breaks down to water and oxygen after the reaction as per Equation 1 so that it is considered as environmentally more benign than NaOH, carbon disulphide (CS₂) and other chemicals commonly used in the fibre processing plants:



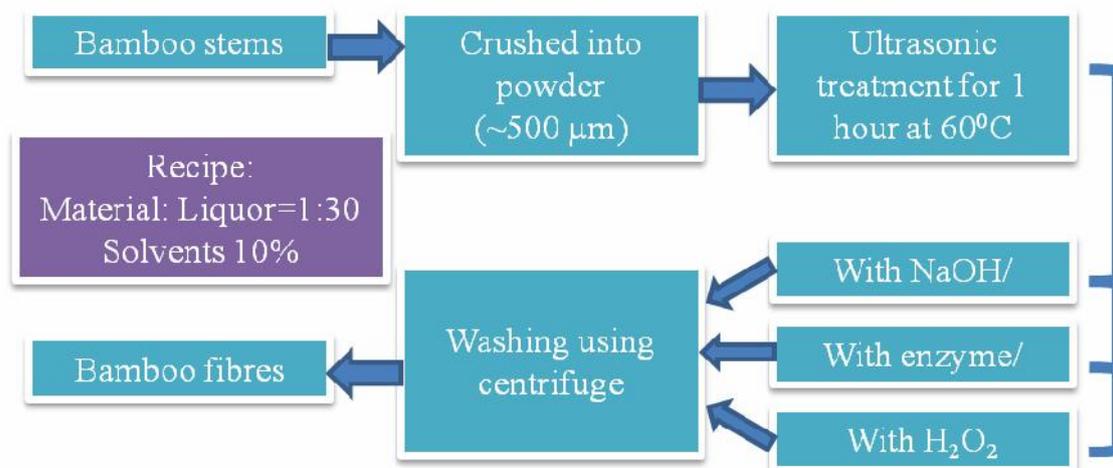


Figure 1: Production process of bamboo fibres using ultrasonic treatment.

2.3.2 Shaker Milling Treatment

For ultrasonic processing with enzyme, the bamboo powders in deionised water were treated with the cellulose enzyme for 18 hours at conditioned lab at 17-18⁰C at pH 5 prior to ultrasonic treatment. Very mild odour has been noticed in the bamboo enzyme mixture after 18 hours which indicates minor bacterial contamination. Then the processed powder was filtered and washed with de-ionized water using a centrifuge and dried in oven at 40⁰C for 18 hours.

Ball milling was used to assist the fibre processing by applying mechanical forces. Figure 2 shows the flow chart of the process. The ball milling was conducted in four different solutions, namely, deionised water, NaOH solution (10%), H₂O₂ solution (10%) and in the presence of enzyme (10%). 0.5 g of bamboo powder was used in each batch. The milled powder was dried in oven at 40⁰C overnight.

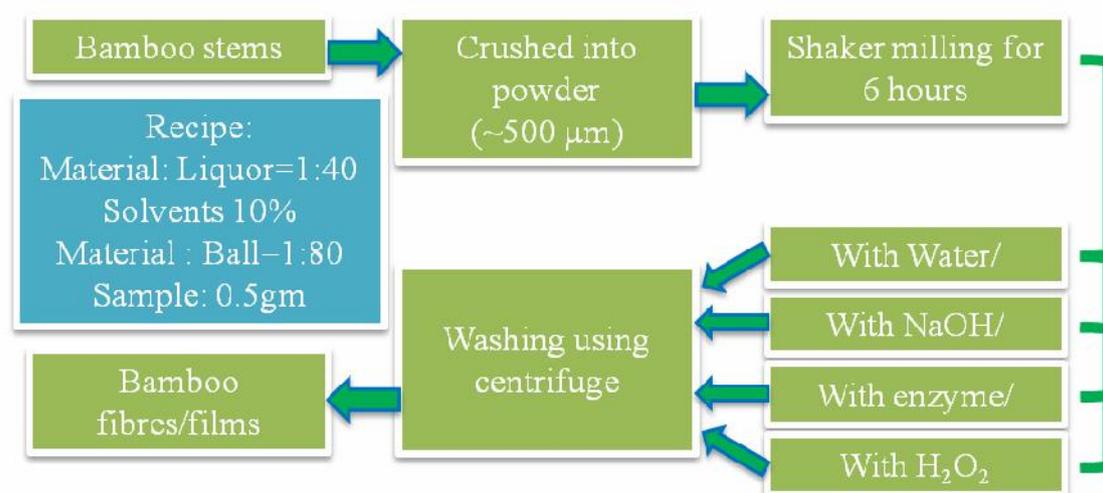


Figure 2: Production process of bamboo fibres using shaker milling.

2.3.3 Combination of Acid Hydrolysis and Ultrasonic Treatment

In this technique (Figure 3), bamboo was not crushed into powder but used as small solid blocks of 50 mm x 12 mm x 5 mm in order to obtain long fibres. The hard woody cortex and epidermis were removed from the specimen before processing. H_2SO_4 (1.5g/L) was used for pre-treating the blocks and then an ultrasonic treatment was carried out in a hydrogen peroxide aqueous solution (10%) for 2 hours at 60°C . After ultrasonic treatment, the bamboo specimen was kept in the same hydrogen peroxide solution while stirred with a magnetic stirrer at room temperature for 12 hours, then the fibres were collected, washed and dried.

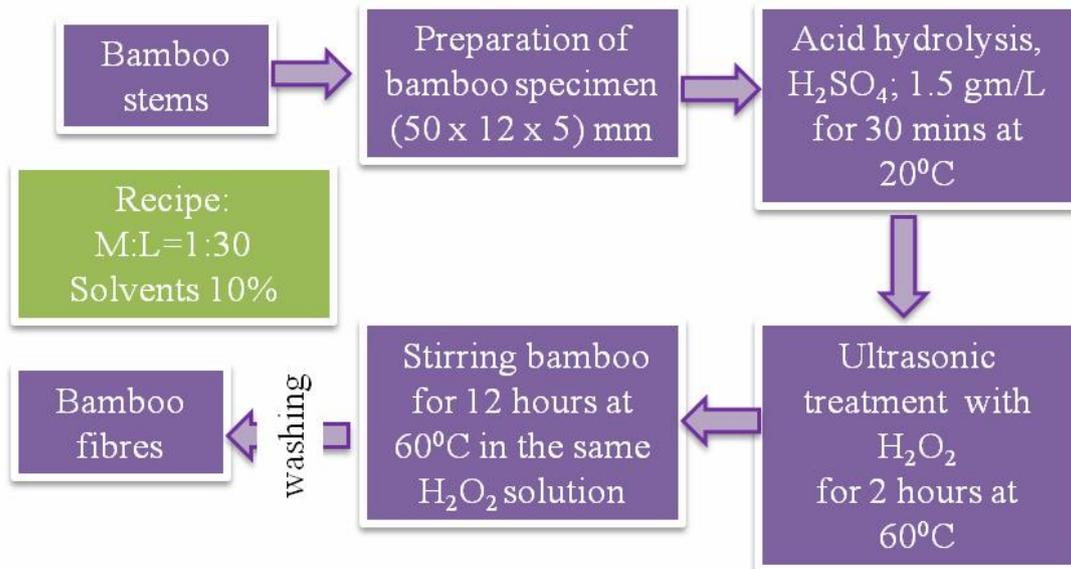


Figure 3: Production process of bamboo fibres using combination of acid hydrolysis and ultrasonic treatment.

3. Results and discussions

3.1 Fibres Appearance

3.1.1 Ultrasonic Treatment

Figure 4 compares the SEM micrographs of the processed fibres after ultrasonic treatment with NaOH (Fig 4b), H₂O₂ (Fig 4c) and enzyme (Fig 4d) with the untreated bamboo powder (Fig 4a). After NaOH treatment, particles with irregular shapes were observed along with some fibres. H₂O₂ treatment produced only fibres without particles. Enzyme treatment also produced fibres but they were highly agglomerated compared to the other two treatments.

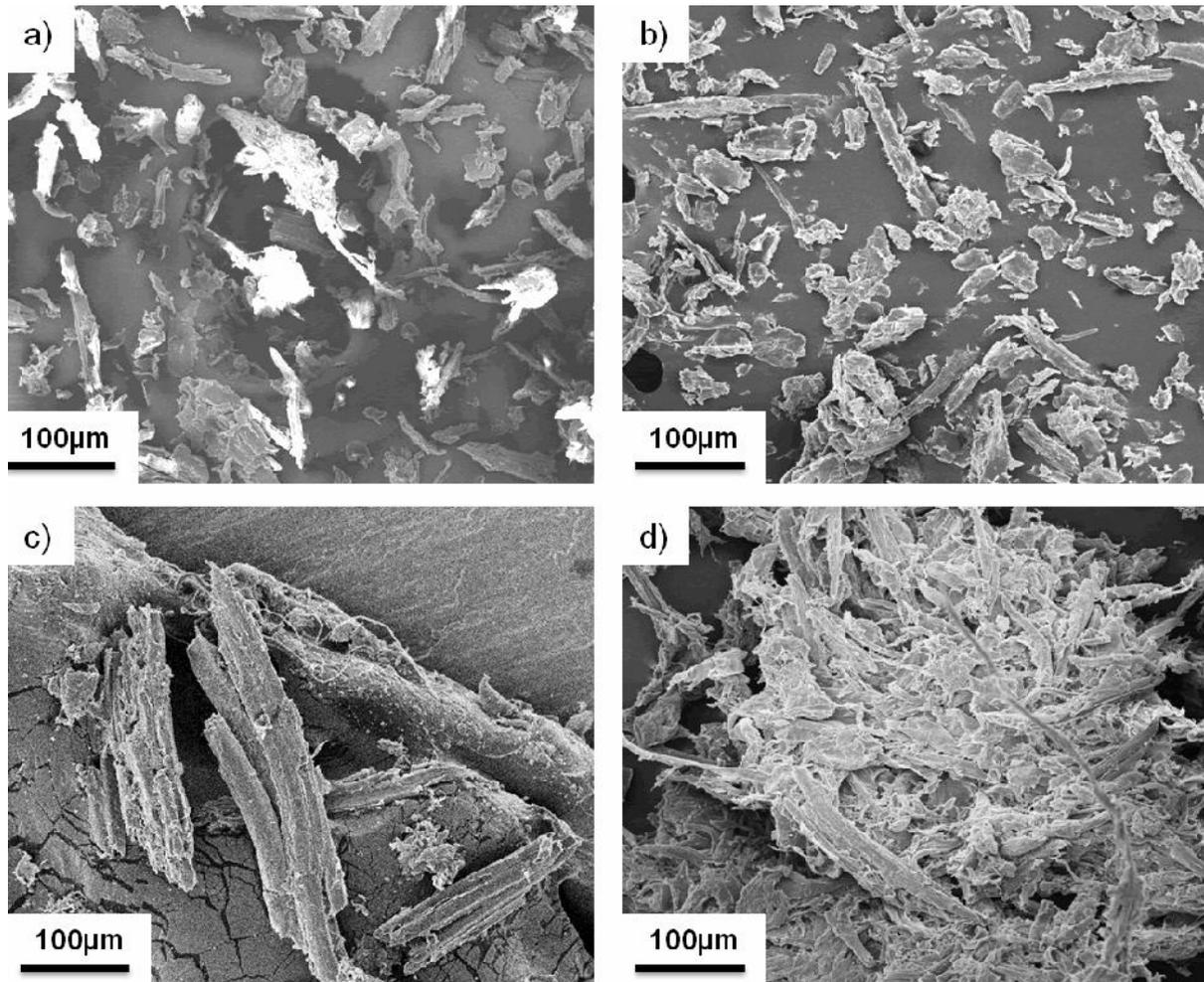


Figure 4: SEM images of bamboo samples a) before ultrasonic treatment b) after ultrasonic treatment with NaOH c) after ultrasonic treatment with H₂O₂ d) after ultrasonic treatment with enzyme.

3.1.2 Shaker Milling Treatment

Figure 5 shows the samples after shaker milling with water, NaOH (10%), H₂O₂ (10%) and enzyme (10%). Shaker milling for 6 hours with water produced fibrous structures of around 12 µm in diameter (Figure 5a). The treatment with NaOH (Figure 5b) and H₂O₂ (Figure 5c) resulted in the formation of continuous films consisting of small particles after drying. Enzyme treatment led to the formation of fibrous structures to some extent but the fibres were not separated from each other (Figure 5d).

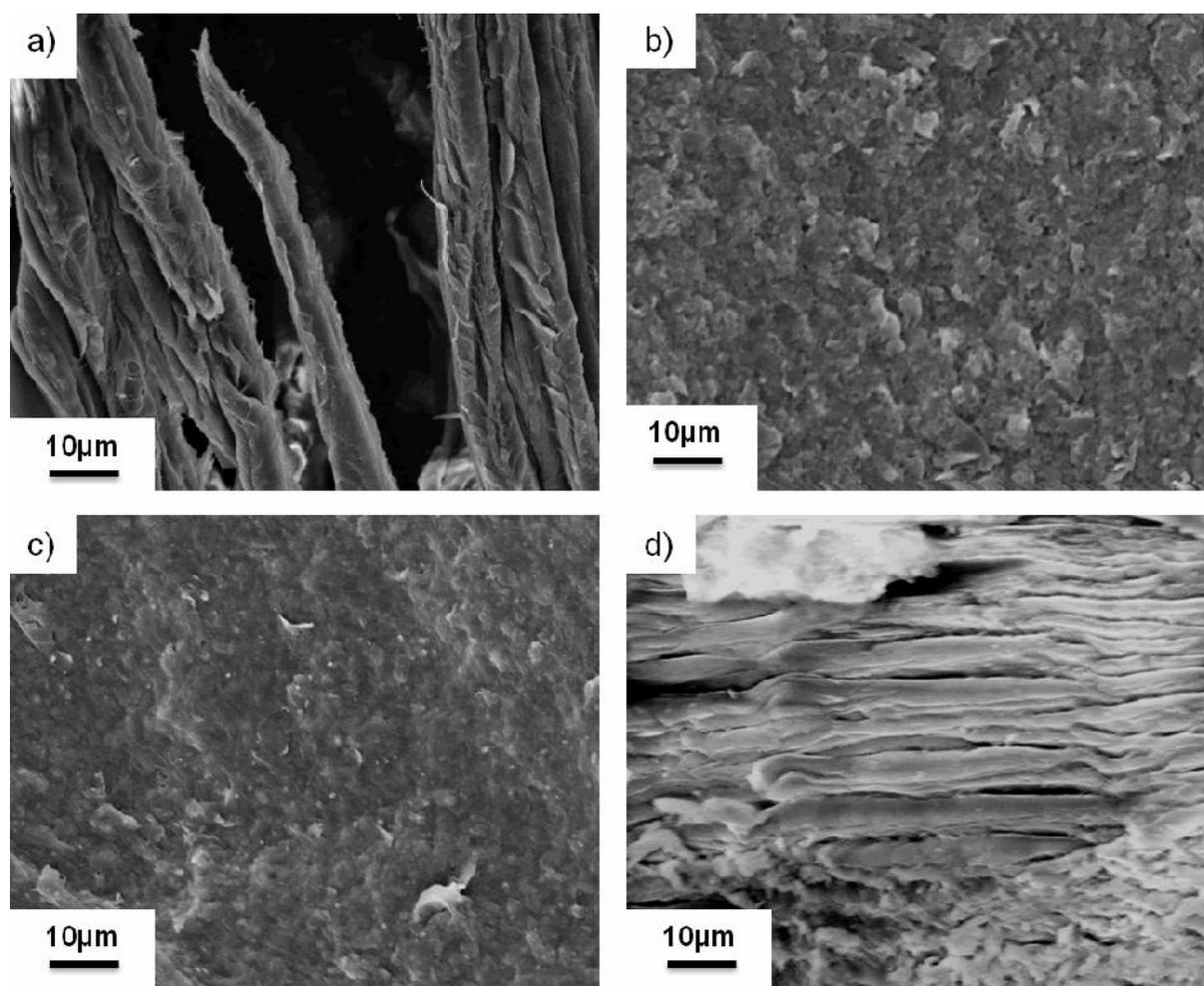


Figure 5: SEM images of bamboo samples after shaker milling with a) water b) NaOH c) H₂O₂ and d) enzyme.

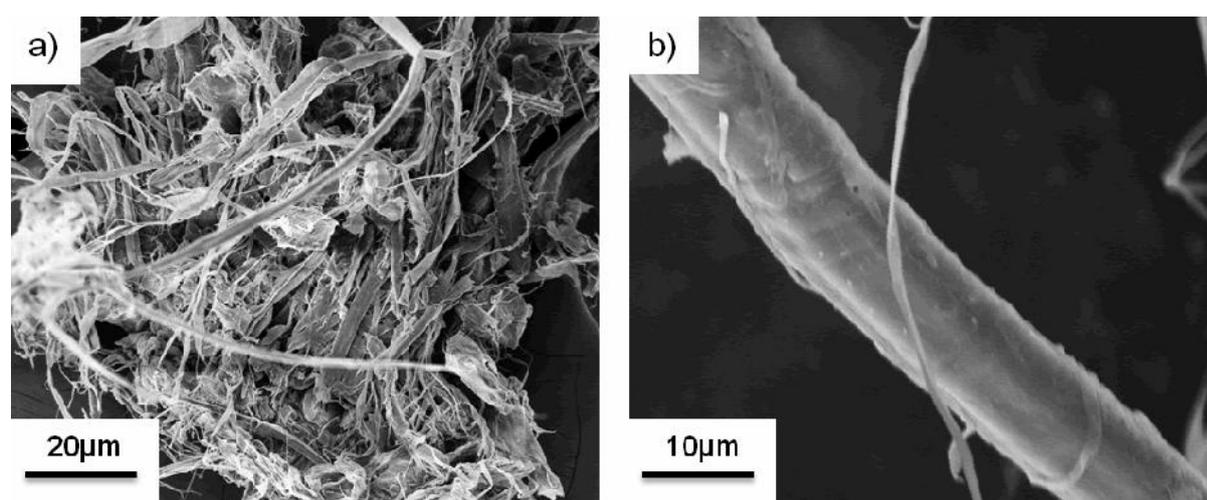


Figure 6: SEM images of a) the processed fibres b) single fibre after combination of acid hydrolysis and ultrasonic treatment.

3.1.3 Combined Processing of Acid Hydrolysis and Ultrasonic Treatment

Figure 6 shows the processed bamboo fibres after combined treatments of acid hydrolysis and ultrasonication. Many separated fibres were obtained using this processing technique (Figure 6a). Figure 6(b) show a SEM image of a typical single fibre with a diameter of $\sim 12 \mu\text{m}$ and some irregular small pores on the surface. This method gave whiter fibres than the other two methods. The yield of fibres was 63~ 65%.

3.2 UV-blocking Ability after Processing

Figure 7 compares the UV absorbance of ultrasonic treated bamboo in NaOH, enzymes and H_2O_2 solutions with untreated bamboo and cotton. It is evident that the UV absorbance of treated bamboo was similar to or slightly higher than the untreated bamboo but significantly higher than cotton. After shaker milling, the NaOH, enzyme, H_2O_2 and water treated samples showed slightly lower UV absorbance than the untreated bamboo but had similar values to each other (Figure 8). These bamboo samples had remarkably higher UV absorbance than cotton.

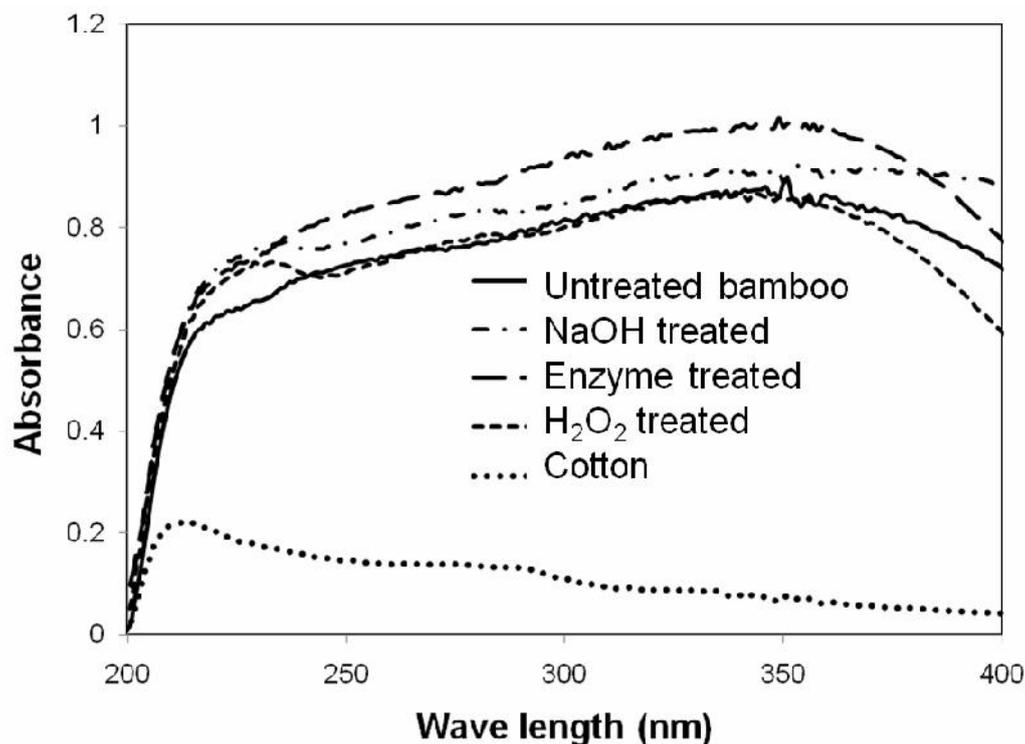


Figure 7: Comparison of UV absorbance among bamboo samples before and after ultrasonic treatment with cotton.

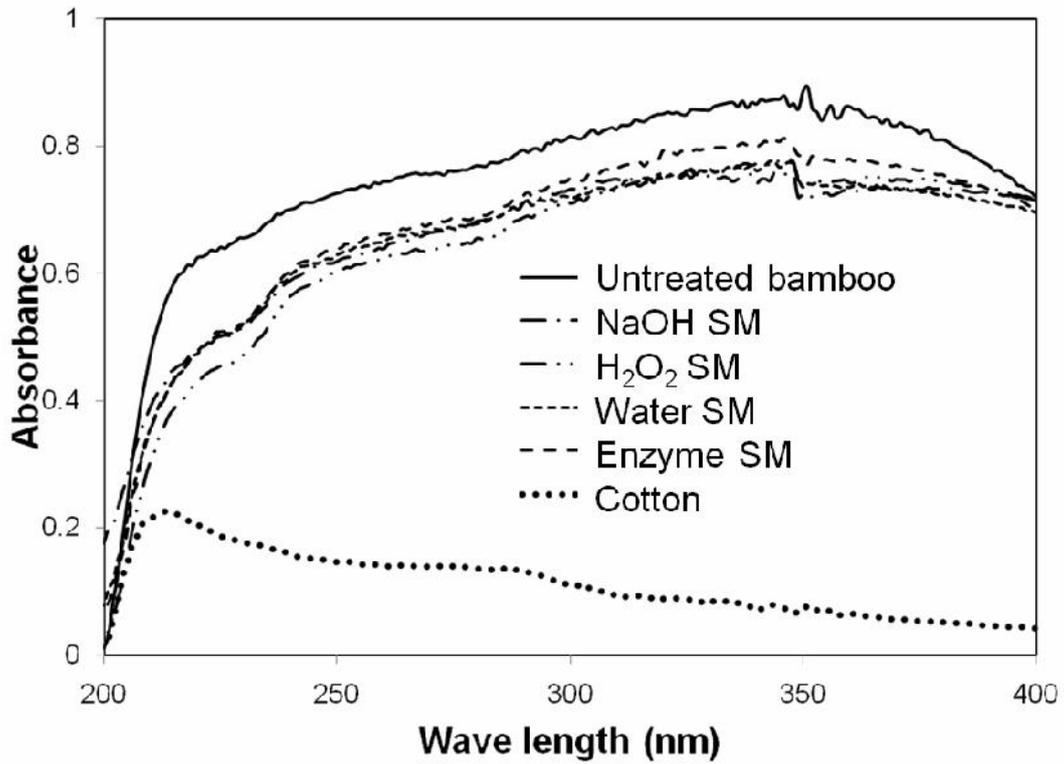


Figure 8: Comparison of UV absorbance among bamboo samples before and after shaker milling (SM) with cotton.

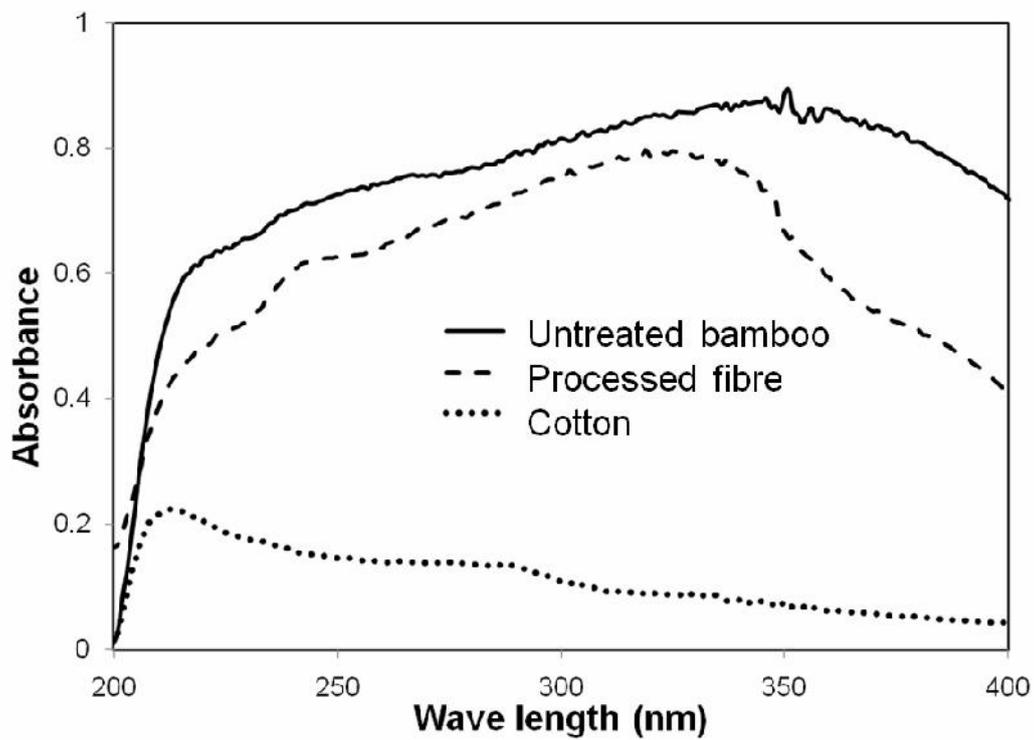


Figure 9: Comparison of UV absorbance among untreated bamboo, processed bamboo fibres and cotton.

Combination of acid hydrolysis and H₂O₂ ultrasonic treatment gave better fibre separation and appearance. It is assumed that the acid weakened the bonds between lignin and cellulose. In fact, inorganic acids are used in many test procedures (Cohen & Dadswell 1931; Tappi method T 222 om-06 2006) to quantify the lignin content in plants. It is thought that H₂O₂ decomposed lignin through oxidation reactions (i.e. bleaching) from the outer surface of the separated fibre cells. After processing, the fibres became very white and had a colour similar to the bleached cotton samples used in this study. The UV absorbance of those processed fibres showed higher values than cotton and slightly lower than the untreated bamboo (Figure 9). The difference in UV absorbance between the treated and untreated bamboos was largest in the spectral range between 350 and 400 nm.

4. Conclusions

This article describes several approaches to produce natural bamboo fibres from raw bamboo plants by means of ultra-sonication, shaker milling and combined acid hydrolysis and ultra-sonication. It was found that ultra-sonication was not sufficient to process bamboo into fibres even with the aid of chemicals such as NaOH. The effect of shaker milling (high energy ball milling) was studied for the first time to process a ligno-cellulosic material into fibres and it was found that raw bamboo plants could be processed into fibres only with the aid of water and mechanical forces. The combination of mild acid hydrolysis and H₂O₂ treatment gave more separated fibres with a better appearance (whiter in colour) than the other two methods. All of the processed bamboo showed significantly higher UV blocking ability than cotton. The process optimisation and spinnability study of the processed fibres are currently on-going. The effect of these processes on parenchyma cells and vessel elements is a subject of further study.

The bamboo fibres produced using the newly developed methods would be useful to produce natural textiles that have high UV screening abilities.

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References

- Afrin, T, Tsuzuki, T & Wang, X 2009, 'Bamboo fibres and their unique properties', in CM Wilson & RM Laing (eds), *Combined (NZ and Aus) Conference of The Textile Institute*, Dunedin, NZ, pp. 77-82.
- Afrin, T, Tsuzuki, T & Wang, X 2010, 'Bamboo : a distinctive green fibre', in ASMT Amin (ed.), *ICTA 2010 : Recent Developments and Challenges of Textile and Apparel Industry : Proceedings of the 1st International Conference on Textile and Apparel*, Dhaka, Bangladesh pp. 14-19.
- Afrin, T, Tsuzuki, T & Wang, X 2011, 'UV absorption property of bamboo', *Journal of the Textile Institute*, DOI:10.1080/00405000.2011.580543
- Afrin, T, Tsuzuki, T, Kanwar, RK & Wang, X 2011, 'The origin of the antibacterial property of bamboo', *Journal of the Textile Institute*, DOI:10.1080/00405000.2011.614742
- ARPANSA 2003, *Resource Guide for UV Protective Products*, Australian Radiation Protection and Nuclear Safety Agency, Canberra

- Cohen, WE & Dadswell, HE 1931, *A Study of the Lignin Determination*, The Chemistry of Australian Timbers, Division of Forest products.-Technical Paper No. 3, Council for Scientific and Industrial Research, Melbourne.
- Gritsch, CS & Murphy, RJ 2005, 'Ultrastructure of Fibre and Parenchyma Cell Walls During Early Stages of Culm Development in *Dendrocalamus asper*', *Annals of Botany*, vol. 95, pp. 619-29.
- Hidalgo-Lopez, O 2003, *Bamboo - The Gift of the Gods*, O. Hidalgo-Lopez, Bogota, Colombia.
- Jain, S, Kumar, R & Jindal, UC 1992, 'Mechanical behavior of bamboo and bamboo composites', *Journal of Material Science*, vol. 27, no. 17, pp. 4598-604.
- Li, Q, Hurren, CJ, Ding, C, Wang, L, Lin, T & Wang, X 2011, 'Ultrasonic scouring of wool and its effects on fibre breakage during carding', *Journal of the Textile Institute*, vol. 102, no. 12, pp. 1059-64.
- Liese, W 1998, *The anatomy of bamboo culms*, International Network for Bamboo and Rattan (INBAR), Beijing, China.
- Liese, W 2003, 'Structures of a Bamboo Culm Affecting its Utilization', in *International Workshop on Bamboo Industrial Utilization*, China, pp. 6-8.
- Okumura, RS, Queiroz, RAD, Takahashi, LSA, Santos, DGCD, Lobato, AKdS, Mariano, DdC, Aves, GA & Filho, BGdS 2011, 'Bamboo: Plant morphology, agronomic aspects, human utilization and perspective', *Journal of Food, Agriculture & Environment*, vol. 9, no. 2, pp. 778-82.
- Parameswaran, N & Liese, W 1976, 'On the Fine Structure of Bamboo Fibres', *Wood Science and Technology*, vol. 10, pp. 231-46.
- Rao, KMM & Rao, KM 2005, 'Extraction and tensile properties of natural fibers: Vakka, date and bamboo', *Composite Structures*, vol. 77, pp. 288-95.
- Tappi Method T 222 om-06 2006, *Acid-insoluble lignin in wood and pulp*, TAPPI Press, Atlanta, GA.
- Ueda, K 1960, *Studies on the Physiology of Bamboo with Reference to Practical Application*, Resources Bureau, Science and Technics Agency, Prime Minister's Office, Tokyo.

Bamboo (*Guadua angustifolia*) fibres for strong light composite materials

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Abstract

The overall advantages and the good mechanical properties of natural fibres such as bamboo fibres are not exploited yet for high performance materials. For practical applications of bamboo fibre composites (BFC), still some obstacles need to be characterized and overcome such as thermal degradation during composite production, where the matrix is still limited to fairly low melting temperature thermoplastics (e.g. polypropylene) and moisture sensitivity; especially during composite service life. In this study, and to have a better understanding of the final composite behaviour, single bamboo fibres were characterized and exposed to different temperature-time ranges. For unidirectional (UD) bamboo fibre thermoplastic composites (BTC) (with Polypropylene and Maleic Anhydride grafted Polypropylene matrices), flexural tests with two fibre orientations were performed. Also, unidirectional bamboo/epoxy composites (BEC) were characterized after exposure to different humidity levels. The results show that the strength value of the fibre is around 800 MPa and a Young's modulus of 43 GPa is obtained by using a novel green mechanical extraction process, although these properties start to degrade at temperatures above 170°C. Mechanical properties evaluated by 3-point bending tests on BTC show that the material performance was reasonable, but further work is necessary to improve fibre-matrix interfacial strength. In BEC, very good properties were obtained; a statistically relevant increase is observed in both failure strain and strength properties when the relative humidity is raised.

Keywords

Bamboo technical fibres, bamboo fibre composites, mechanical properties.

Introduction

Natural fibres are environmentally friendly, not only during their growth stage but also after their lifetime because they are biodegradable. A comparative study including life cycle assessment (LCA) studies of natural fibres and glass composites carried out by Joshi et al (2004), reveals that natural fibre composites are likely to be environmentally superior to glass fibre in most cases because: 1) their production has a low environmental impact, 2) the light-weight natural fibre composites improve fuel efficiency and reduce emissions in the use phase of the component, especially in automotive applications; and 3) end of life incineration of natural fibres results in recovered energy and carbon credits; these materials are basically carbon neutral. Carbon dioxide fixation of natural fibres is an important issue in the reduction of the greenhouse effect.

Studies setting out the utilization of bamboo *G. angustifolia* fibers as the reinforcement of polymeric matrices are practically non-existent, even though it is the most important bamboo species of America and one of the three largest bamboos in the world. This prominent role is due to its size, high performance, and impact for the local economy where it grows (Londoño 2001, Young and Judd 1992). It also has a high growth rate, between 11 and 21 cm per day, reaching its definitive height 6–7 months after the shoot emerges, coming into maturity when it is 4–6 years old (Londoño 1998). This bamboo is one of the tropical species that has been identified as having a great potential to fix atmospheric carbon dioxide, which makes it an effective plant in terms of global warming prevention and a suitable resource for fiber production (Riaño et al. 2002).

Regarding the production of natural fibres, the energy consumption required to produce a natural fibre mat (9,7 MJ/Kg), including cultivation and harvesting, amounts to just less than one-fifth of the energy required to produce glass fibre mat (54,8 MJ/Kg) (Schlöesser 2004). Additionally, unlike the traditional engineering fibres, e.g. glass and carbon fibres, and mineral fillers, these lignocellulosic fibres cause low machine wear during processing, no health hazards due to fibre dust and the hollow nature of vegetable fibres may impart acoustic insulation or damping properties to certain types of matrices (Herrera and Valadez 2005).

To avoid confusion, the distinction between a technical fibre and an elementary fibre must be made. An elementary fibre is the mesoscale constituent of the fibre that can be extracted when it undergoes a chemical treatment. These are typically about several millimeters long. The technical fibre on the other hand is the collection of elementary fibres, held together by a gluish medium. Technical fibres are commonly used as reinforcement elements in a polymer matrix. In this paper, the term ‘fibre’ implies ‘technical fibre’.

Bamboo fibres are often called “natural glass fibres” because of their properties (Dieu et al. 2004; Okubo and Fujii 2002). Among the well-known natural fibres, bamboo has one of the most favourable combinations of low density and high mechanical strength. The specific tensile strength of bamboo, however, is generally reported to be less than for glass fibres (Jindal 1986). The fibres are the most important structural part of the bamboo culm and are responsible for this outstanding performance. They are distributed through the culm, being more numerous in the outer part and becoming scarcer towards the inner part of the wall to withstand extreme environmental (bending) conditions.

Several studies have been published of other bamboo fiber reinforced composites using thermoset (Osorio et al. 2011; Shin 1989), thermoplastic (Trujillo et al. 2010; Katayama et al. 2002) and biodegradable matrices (Kumar et al. 2005; Shibata et al. 2004). The results of these researches

suggest that there is a good potential for this reinforcing material to be used in light, high strength polymeric composites.

It is technically difficult and expensive to extract fine, long and straight technical bamboo fibres. Only few efforts have been carried out worldwide to extract long bamboo fibres from the culm. Actual processes are currently applied at laboratory scale and use either chemicals or high pressure, negatively affecting the quality of the fibres (Ray et al. 2004; Deshpande et al. 2000). A novel green mechanical extraction process has been developed in the Composite Materials Group at KU Leuven (Osorio et al. 2011), see Figure 1. The final goal is to provide an alternative for glass fibres, whose annual global production reaches about 5 million tons. For that, among other barriers (e.g. variability in properties (Gassan and Bledzki 1999)), two important aspects, particularly thermal degradation and moisture absorption should be studied.

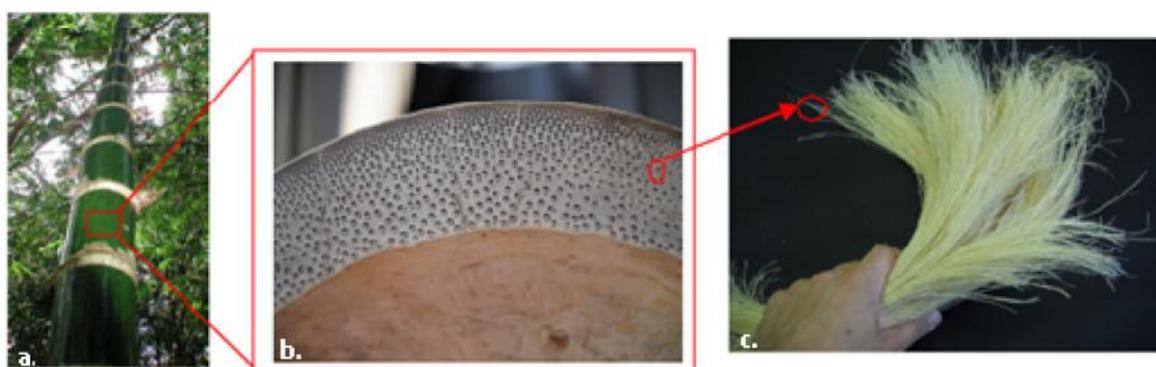


Figure 1. Bamboo (*Guadua angustifolia*) fibres extracted from bamboo culm. From left to right: bamboo culm – cross section of the culm – bamboo technical fibres after mechanical extraction

As an organic material, natural fibres have a hydrophilic nature, which decreases the compatibility with hydrophobic polymeric matrices (particularly some thermoplastics). Moisture sensitivity causes fibre swelling, which may be reduced if better adhesion with the matrix is reached. Consequently, in this field most of the research has been focused on improving interfacial properties between the polymer matrices and natural fibres, in order to enhance the physical and mechanical properties of the end products (Lee et al. 2006). According to Jindal et al (1986), moisture absorption is a major obstacle which prevents extensive applications of natural fibre composites. Reducing the amount of hemicellulose (the substance which holds the primary fibres together) reduces the swelling capacity of lignocellulosic fibres (Pott 2004). This aspect is also related to the quality of the fibre extraction process, because the process determines the absence of defects along the fibre length as well as the non-presence of lignin, hemicellulose and impurities on the fibre surface.

As is well known, thermal stability is one of the major drawbacks of natural fibres where the first degradation occurs typically at temperatures above 180°C. It has been established that there is no degradation taking place until 160°C. Above this temperature thermal stability is gradually decreasing and decomposition of the fibres occurs. Hemicelluloses are generally thought to decompose first, followed by cellulose and lignin. In addition, the amount of impurities may accelerate the beginning of thermal degradation (Wielage et al. 1999).

Materials and methods

Bamboo fibres of the species *Guadua angustifolia* (Figures 1a-b) were obtained from well defined locations in Colombia. Technical fibres (which will be referred to as fibres in this paper, as mentioned before), were extracted from the bamboo culms using a newly developed and proprietary extraction process, giving a maximum fibre length between 20 and 35 cm. Figure 1c shows a group of mechanically extracted fibres whose diameter has a range between 90 and 250 μm ; the main diameter concentration is around 150 μm . Before tensile testing, the fibres were visually selected in order to verify the absence of defects along the length of the fibres.

After this, the cross sectional area of each individual fibre was determined using both the apparent density (1,4 gr/cm^3) and the weight as well as the length of the fiber. After this they were glued in between two paper frames to assure a good gripping and straight position in the test clamps. The opening of the paper frame determines the gauge length; for this experiment it was set at 5, 10, 25 and 40 mm. Single fibre tensile tests were performed on a mini tensile testing machine with a loadcell of 200N where the crosshead speed was set at 1 mm/min; the load and the displacement are registered during the complete test. Because an extensometer cannot be used during the test, a theoretical correction for the machine compliance developed by Defoirdt et al (2010) was applied in order to determine the real elongation of the specimens. By plotting modulus versus $1/\text{span}$, extrapolating to $1/\text{span} = 0$ (infinite fibre length) provides the material modulus for which slip and machine compliance may be ignored. When thus the real material modulus is known, an estimation can be made for the machine compliance. Knowing this compliance, strain values can be corrected. The correction was done for every single experiment.

Thermal degradation of single bamboo fibres was studied by observing their tensile behaviour after exposure at two different temperatures. The fibres were put into a vacuum oven and heated at two temperatures (150 $^{\circ}\text{C}$ and 170 $^{\circ}\text{C}$) and left in the oven for 5, 30 and 60 minutes. After this, the treated fibres were conditioned at room temperature and 50% relative humidity, weighed and measured individually to determine their cross sectional area before being placed into a paper frame. The average diameter was $134 \pm 18 \mu\text{m}$. Approximately 30 successful tests were carried out for each thermal treatment.



Figure 2. Unidirectional bamboo fibre - PP prepreg

Bamboo fibre/polypropylene composites were prepared by compression moulding of stacks (7 layers) of bamboo prepreps (see Figure 2), each consisting of untreated fibres and PP film (Polypropylene and Maleic Anhydride grafted Polypropylene (MAPP)). To obtain good impregnation, the temperature used was about 25 $^{\circ}\text{C}$ higher than the melting temperature of the polymer and a pressure of 15 bars was used. In all cases, the natural fibres and prepreps were dried overnight at 65 $^{\circ}\text{C}$ to prevent problems due to moisture. Fibre volume fraction was set at 45% by weight measurements. Flexural strength and

Young's modulus for UD composites with longitudinal disposition of the fibres were evaluated by 3 point bending tests on a universal testing machine (Instron 4426) based on ASTM D790M.

The Light RTM technique was used to prepare BEC samples with good surface quality, dimensional accuracy and good degree of alignment. Fibre volume fraction of approximately 40% was reached by weight measurement. The multicavity mould had the final sample dimensions according to the standard ASTM 3039, in order to avoid flaws (cracks) that can act as stress concentrations along the edges after cutting. In order to study the influence of moisture on the BEC samples it was opted to test samples at very low moisture level ($\sim 10\%$ rh), at standard moisture level ($\sim 50\%$ rh) and at very high moisture level ($\sim 100\%$ rh). For every condition 6 samples were tested. To achieve full acclimatization, samples were conditioned for 4-5 weeks. A wide range in humidity conditions was created using saturated salt solutions in closed containers. Tensile tests were performed on an Instron 4467 and 5567 with a 30kN loadcell. The gage length was set by the extensometer at 2,5cm. Crosshead speed was 2mm/min.

Results and discussion

Single Bamboo Fibre

Single fibre tensile tests for untreated bamboo fibres were performed at different spans lengths. The values for tensile strength and Young's modulus, after the machine compliance correction was applied (see methodology), are shown in Figure 3. Strain to failure remains around 1.9% on average. The modulus showed an extrapolated value of 43 GPa. Under tensile load bamboo fibres show a clean fracture instead of defibrillation or splitting. The clean fracture shows that there is a good bond between the elementary fibres and the technical fibre matrix (typically consisting of lignin; as is generally known, a vegetable fibre is in itself a composite). The small variation in tensile strength at different span lengths (Figure 3) could be an indication of the low presence of defects along the length. This means that the mechanical extraction process that was applied in this case did not significantly affect the fibre quality. With a bamboo fibre density of 1.4 gr/cm^3 , specific mechanical properties are very close to these of glass fibre.

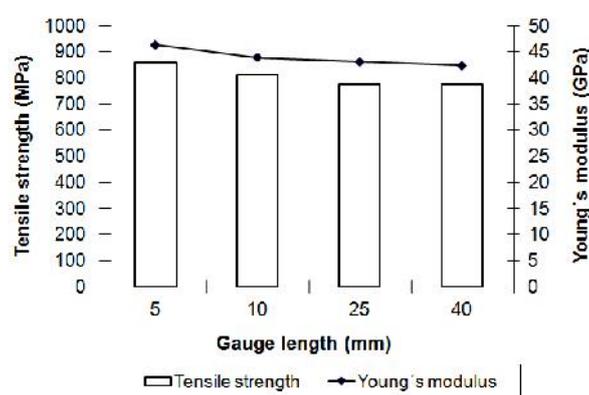


Figure 3. Tensile single fibre properties of bamboo fibres (*Guadua angustifolia*), after machine compliance correction

Figure 4 shows a general decrease in fibre tensile strength after thermal treatment while the tensile modulus increases initially (Figure 5). When bamboo fibres are exposed at 150°C for maximum 30 min, the strength remains almost constant; then a significant decrease is presented. At 170°C the decrease is more dramatic even at 5 min exposure with a reduction of $\sim 18\%$ of the fibre strength. The stiffness of the fibre at 150°C increases 19% after 30 min, then, when the time is doubled, it reduces.

Nevertheless, it is still 9% greater than for the non-treated fibre. At 170°C during 5 min exposure an increase of 7.7% is observed. The thermal treatment of the fibres produces stiff and evidently more brittle fibres. These results are in agreement with Rong et al (2001), where after thermal treatment the higher fibre stiffness is explained by the increase in crystallinity of the cellulose.

The obtained values of the effect of temperature on bamboo fibres are helpful to establish process parameters for the bamboo fibre composites fabrication, avoiding damage to the fibres, especially when thermoplastic polymers are used.

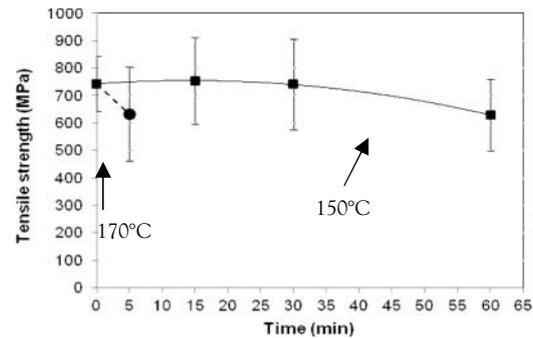


Figure 4. Bamboo fibres tensile strength after thermal treatment

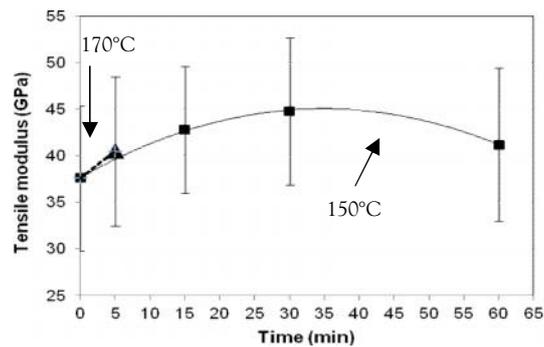


Figure 5. Bamboo fibre Young's modulus after thermal treatment

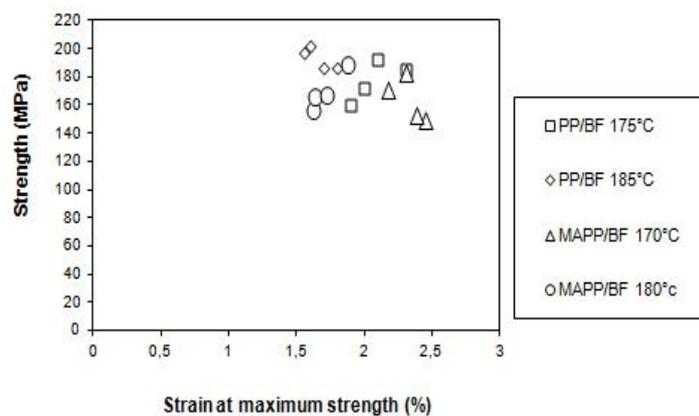


Figure 6. Flexural strength and failure strength for BTC at different processing temperatures

Unidirectional thermoplastic bamboo fibre composites (BTC)

For unidirectional bamboo fibre thermoplastic composites loaded in the longitudinal direction of the fibres, it was found that for both Polypropylene and MAPP the consolidation temperature has a clear effect on the final behaviour of the composite.

There is no clear difference in maximum strength between PP and MAPP, as all samples are roughly situated on one line as a function of processing temperature. In both cases, not only a shift to the upper part of the spectrum when the temperature is raised by 10 degrees is observed, but also there is a reduction in strain at maximum stress. Both combined result in a modulus increase (see Figure 6).

Young's modulus for PP composites goes from 15.6 to 19.3 GPa and for MAPP composites from 11.8 to 16.4 GPa, improving 24 and 39% respectively, again for the same increase in temperature.

This increase may be explained because the higher the temperature, the lower the viscosity of the polymer, so it will flow around the fibres more easily. Young's modulus is particularly indicative of the quality of impregnation. Theoretically, at 45% fibre volume fraction, one would expect a longitudinal modulus of about 20 GPa. Thus, it can be hypothesized that for PP at 185°C (20° above the melting temperature), the wetting is probably quite good. The theoretical longitudinal strength is about 360 MPa. Whereas, for epoxy resins these strength values have been approached, for PP and MAPP the strength remains relatively low, indicating that probably significant improvements in interfacial bond strength are still possible and desirable. From transverse properties (Table 1), it is clear that even with an improvement in wetting; the interface remains relatively weak, since the transverse strength values are lower than the strength values of the pure polymers.

Table 1. Results for transverse flexural strength of bamboo fibre/thermoplastic composites

Transverse flexural strength BFC (MPa)	Consolidation temperature			
	165 °C	170°C	175°C	180°C
Polypropylene matrix	17 ± 1.8	20 ± 0.5	18 ± 2.2	17 ± 1
MAPP matrix	16 ± 1.4	19 ± 0.8	18 ± 0.8	20 ± 0.3

There is also very little difference between the results with PP and MAPP. We have indications that the surface of our bamboo fibres is covered with lignin (Fuentes et al. 2011), which is apparently not highly compatible with either PP or MAPP.

Unidirectional bamboo fibre/epoxy composites (BEC) for moisture sensitivity

The mechanical properties of the bamboo/epoxy composite, when conditioned to different humidity levels, is given in Table 2. The composite strength and stiffness values have are normalized to a fibre volume fraction of 40%.

Table 2: Mechanical properties of bamboo/epoxy composite at different humidity levels

Humidity condition (%rh)	Strength (MPa)	Stiffness (GPa)	Strain to failure (%)	N° of samples
20	210±13	19.9±4	1.02±0.16	6
50	206±11	17.5±3	1.41±0.17	6
100	255±9	18.3±1	1.88±0.04	6

To study the statistical significance of the results, a hypothesis test with a 0,05 significance level is used to compare the mean mechanical properties of the composite climatized at 20%rh and at 100%rh. From the test it can be concluded that there is a significant difference between the mean values for stress and strain. However, it cannot be assured that the difference in stiffness is significant. Depending on the humidity condition that are applied to the composite samples, the total acclimatization duration varies. Samples kept at 20%rh were climatized for 35 days. Samples at 100%rh were climatized for 25 days.

BEC samples were tested in tension showing linear stress-strain behavior until fracture. The humidity level of the environment has a significant influence on the mechanical properties of the composite. Increase in humidity level up to 100%rh causes an increase in failure strain and strength compared to samples at 20%rh (see Table 2). These tendencies can be explained looking at the influence of humidity on fibre morphology; when absorbing moisture, fibres swell slightly. This closes existing cracks, lowering the probability of a critical flaw to cause failure at low strength levels. Conversely, at low humidity levels the fibres lose moisture causing shrinkage. This introduces micro-cracks in the material making the bamboo/epoxy composite more vulnerable for a critical crack. An increase in strain can be explained due to plasticization of the fibres when absorbing moisture, which is in agreement with Joffe et al (2003).

However, the change in mechanical behavior with humidity is not purely bamboo related. Testing of neat epoxy resin shows that the epoxy is also influenced by the conditioning. Epoxy climatized at 20%rh shows a brittle behavior, failing at failure strains lower than is the case for epoxy treated at 100%rh. At 100%rh there is sign of plasticizing of the epoxy.

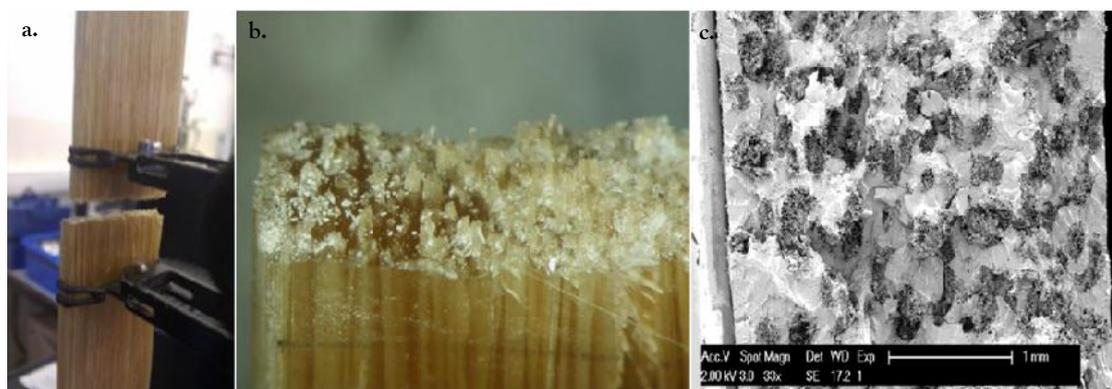


Figure 7. (a) Failure mode of bamboo/epoxy samples, b) stereoscope image: surface of the edge of a broken bamboo/epoxy sample and c) SEM image: surface of the central area of a broken sample (equal fibre spread) (50%rh)

Conclusions

Technical bamboo fibre is difficult to extract undamaged from the culm, but once a good extraction process is used, as developed at KU Leuven, fibres are obtained with specific mechanical properties comparable to glass fibre.

The significant increase in modulus for BTC at higher temperature indicates better wetting and increased effectiveness of the fibers, but it does not necessarily mean an improvement in interfacial bond strength. Further work to improve the compatibility is needed in order to reach the potential of bamboo fibre thermoplastic composites.

The mechanical properties of BEC show an influence by the humidity. High humidity levels have a plasticizing effect on the composite. Also there is a significant increase in strength due to the conditioning which is in agreement with other studies.

Acknowledgements

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References

- Defoirdt, N; Biswas, S; De Vrieze, L, Tran, N; Ahsan, Q and Van Vuure, A. 2010. Assessment of the tensile properties of coir, bamboo and jute fibre. *Composites Part A*, 41: 588–595.
- Deshpande, A; Rao, C and Rao, L. 2000. Extraction of bamboo fibres and their use as reinforcement in polymeric composites. *J Appl Polym Sci*, 76: 83–92.
- Dieu, T; Phai, L; Ngoc, P; Tung, N; Thao, L and Quang, L. 2004. Study on preparation of polymer composites based on polypropylene reinforced by jute fibres. *JSME International Journal, Series A*, Vol. 47, No. 4, pp. 547 – 550.
- Fuentes, C; Tran, L; Dupont-Gillain, C; Vanderlinden, W; De Feyter, S; Van Vuure, AW and Verpoest, I. 2011. Wetting behaviour and surface properties of technical bamboo fibres. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 380 (1-3), 89-99.
- Gassan, J and Bledzki, A. 1999. Possibilities for improving the mechanical properties of jute/epoxy composites by alkali treatment of fibres. *Composites Science and Technology*, Vol. 59, pp. 1303-1309.
- Herrera, P and Valadez, A. 2005. A study of the mechanical properties of short natural-fiber reinforced composites. *Composites Part B*, Vol. 36, 597 - 608.
- Jindal, U.C. 1986. Development and Testing of Bamboo Fibers Reinforced Plastic Composites, 20, 19–29.
- Joffe, R; Andersons, J and Wallström, L. 2003. Strength and adhesion characteristics of elementary flax fibres with different surface treatments. *Composites Part A, Applied Science and Manufacturing Volume 34, Issue 7*, 603-612.
- Joshi, S.V; Drzal, L.T; Mohanty, A.K and Arora, S. 2004. Are natural fiber composites environmentally superior to glass fiber reinforced composites?. *Composites Part A*, Vol. 35, 371-376.
- Katayama, T; Tanaka, T; Suzuki, Y; Nagai, S and Aoyama, E. 2002. Study on injection molding of bamboo fiber reinforced thermo-plastics. In: Brebbia C and de Wilde W(eds), *High performance structures and composites*. Southampton: WIT Press, 87–96.
- Kumar, H; Siddaramaiah and Roopa, S. 2005. Study of chemical and tensile properties of polyurethane and polyurethane/polyacrylonitrile coated bamboo. *J Reinf Plast Compos*, 24(2), 209–213.
- Lee, S and Wandg, S. 2006. Biodegradable polymer/bamboo fiber biocomposite with bio-based coupling agent. *Composites Part A*, Vol. 37, pp. 80–91.
- Londoño, X. 1998. A decade of observations of a *Guadua angustifolia* plantation in Colombia. *Journal American Bamboo Society*, 12, 37–42.
- Londoño, X. 2001. La Guadua un bambú importante de América. In: *Proceedings of First Bamboo Seminar*, Ecuador, 12–18.
- Okubo, K and Fujii, T. 2002. Eco-composites using natural fibers and their mechanical properties. Paper from: *High performance Structures and Composites*, edited by: C. Brebbia and W. de Wilde, 77- 85.
- Osorio, L; Trujillo, E; Van Vuure, AW and Verpoest, I. 2011. Morphological aspects and mechanical properties of single bamboo fibres and flexural characterization of bamboo/epoxy composites. *J Reinf Plast Compos*, 30 (5), 396-408.

- Pott, G. 2004. Natural fibers with low moisture sensitivity. In: Natural fibers, plastics and composites, edited by: F. Wallenberger and N. Weston, 2004, 104-122.
- Ray, A.K, Das, S.K, Mondal, S and Ramachandrarao, P. 2004. Microstructural characterization of bamboo. *J Mater Sci*, 39, 1055–1060.
- Riaño, N; Londoño, X; López, Y and Gómez, J. 2002. Plant growth and biomass distribution on *Guadua angustifolia* Kunth in relation to ageing in the Valle del Cauca – Colombia. *American Bamboo Society*, 16(1), 43–51.
- Rong, M; Zhang, M; Liu, Y; Yang, G and Zeng, M. 2001. The effect of fibre treatment on the mechanical properties of unidirectional sisal – reinforced epoxy composites. *Composites Science and Technology*, 61:1437-1447.
- Schloesser, T. 2004. Natural fiber reinforced automotive parts. In: Natural fibers, plastics and composites, edited by: F. Wallenberger and N. Weston, Chap. 15, 275 –285.
- Shibata, S and Fukumoto, I. 2004. Effects of bamboo and kenaf fibres on the flexural modulus of bio-composites. Department of Mechanical Systems Engineering, University of the Ryukyus, Okinawa, Japan,
- Shin, F; Xian, J; Zheng, W and Yipp, M. 1989. Analyses of the mechanical properties and microstructure of bamboo-epoxy composites. *J Mater Sci*, 24: 3483–3490.
- Trujillo, E; Osorio, L; Fuentes, C; Van Vuure, A and Verpoest, I. 2010. Bamboo fibre thermoplastic for transport applications. In: Proceedings of SAMPE Europe 31st International Technical Conference and Forum, Paris.
- Wielage, B; Lampke, T; Marx, G; Nestler, K and Starke, D. 1999. Thermogravimetric and differential scanning calorimetric analysis of natural fibres and polypropylene. *Thermochimica Acta*, Volume 337, Issues 1-2, 169-177.
- Young, S and Judd, W. 1992. Systematics of the *Guadua angustifolia* complex (Poaceae: Bambusoideae). *Ann Mo Bot Gard*, 79(4), 737–769.

The relation between bamboo fibre structure and mechanical properties

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Abstract

Bamboo technical fibres are an attractive alternative to reinforce polymers in the new era of green materials. Furthermore, bamboo plants have been present for many years in many cultures, where they have played an important role for local economies.

In spite of the well-known usefulness of this plant, its fibres which are the main structural component have been scarcely used due to the difficulty in extracting high quality technical fibres. In the current research project a mechanical process has been developed to extract long bamboo technical fibres from the Colombian species *Guadua angustifolia* with the aim of using them as reinforcement in composite materials as an alternative to replace glass fibres.

Bamboo technical fibre mechanical properties have been studied showing values of strength up to 800 MPa and E-Modulus up to 43 GPa, proving their excellent tensile properties. Therefore, we focus on making this technical fibre suitable as reinforcement in composite materials.

To fully explore the good mechanical properties and to make an adequate use of this new reinforcement, it is indispensable to have a complete understanding of the fibre behaviour as a function of microstructure. Microscopic observations have provided us with a vast knowledge of the complex microstructure of this natural fibre from the macro down to the micro scale level. This information is necessary to find the relationship between the technical fibre microstructure and its mechanical properties.

These analyses are carried out in normal environmental conditions. It is also important to study the effect of more severe conditions, like the presence of moisture, on the mechanical behaviour of bamboo technical fibres. This is related to the morphology and chemical composition and has a direct effect on the performance of the technical fibre as reinforcement in polymeric matrices. First results from moisture sensitivity studies are presented.

Introduction

Composites allow light-weight design and are as such environmentally friendly materials. However, there is an increasing interest in so-called renewable materials, to which category natural fibres clearly belong. Natural fibres can, if mechanical recycling into secondary applications is not feasible, be thermally recycled or if a biodegradable matrix is used, be landfilled for biodegradation. Both last options are largely CO₂ neutral. Natural fibres like bamboo also require much less energy for cultivation and extraction than is used for the synthesis of glass and particularly carbon fibre, which gives them a very favourable carbon footprint. Added to that, their suitable mechanical properties, large availability and low cost make them a real alternative in the market of composite materials. Table 1 presents some literature values for mechanical properties of natural fibres compared to glass and carbon fibres.

Table 1. Mechanical properties of some plant fibres compared to glass and carbon fibres (Bledzki and Gassan 1999; Gomes et al. 2004; Brouwer 2004; Murali and Mohana 2007; Cao et al. 2006; Okubo and Fujii 2002)

Fibre	Density (gr/cm ³)	Diameter (µm)	Elongation at failure (%)	Tensile strength (MPa)	E-Modulus (GPa)
Bagasse	-	490	-	70	-
Coir	1.2	-	30	175	4.0 - 6.0
Cotton	1.5 - 1.6	20	7.0 - 8.0	287 - 597	5.5 - 12.6
Curaua	1.38	66	3.9	913	30.3
Flax	1.5	50-100	2.7 - 3.2	345 - 1035	50 - 70
Hemp	1.10	120	1.6	389 - 900	35
Henequen	-	180	3.7 - 5.9	430 - 570	10.1 - 16.3
Jute	1.3	260	1.5 - 1.8	393 - 773	26.5
Kenaf	1.31	106	1.8	427 - 519	23.1 - 27.1
Pineapple	1.32	-	2.4	608 - 700	24.7 - 29.3
Ramie	1.50	34	3.6 - 3.8	400 - 938	24.4 - 32
Sisal	1.5	50-80	2.0 - 2.5	337 - 413	8.3 - 9.9
Bamboo	0.88 - 1.1	100-200	-	391-713	18-55
E-Glass	2.5	9-15	2.5	1200 - 1500	70
Carbon (PAN)	1.4	5-9	1.4 - 1.8	4000	230 - 240

When discussing natural fibres, different hierarchical levels should be considered. On the micro level, the elementary fibres are composed of cellulose microfibrils embedded in a hemicelluloses/lignin matrix. In the meso level, a technical fibre or fibre bundle is a group of elementary fibres that are glued together by an amorphous material called middle lamellae. The technical fibres are generally obtained after an extraction process and can be used as such to reinforce polymers. Their composition and structure is completely adapted to their function in the plant (Baillie and Nishinko 2004; Mohanty and Misra 2005).

In natural fibres, the cell wall structure of the elementary fibre is the most important feature since its constitution determines all mechanical properties. In other words, the structure of the fibre bundle and the layering structure and thickness of the cell wall are of special interest. Some plant fibres like bamboo, flax, kenaf and oil palm among others have been studied and characterized by using special

microscopic techniques (Charlet 2008; Béakou et al. 2008; Khalil et al. 2009). The knowledge of the technical fibre structure gives us the understanding of the mechanical behavior i.e. the technical fibre modulus is strongly affected by the distribution of the microfibrils in the cell wall structure. This information is important to establish a correct use of this technical fibre as reinforcement in composite materials.

Moreover, natural fibres are very susceptible to their environment. The mechanical properties are not only greatly influenced by the growing cycle and moment of cultivation, but also by the conditions they are used in. Because of this, it is also necessary to study the influence of these factors on the mechanical behaviour. Depending on the type of natural fibre a decreasing (flax) or stable tensile strength (kenaf, sisal, jute, flax, coir, abaca) is measured (Symington et al. 2009) with increasing humidity level. In the work of Nilsson (2006) an increase in strength is measured for flax technical fibres with the presence of moisture. No conclusive results are found for the stiffness and ultimate strain of natural fibres. When considering low moisture contents or drying of the technical fibres a more distinctive conclusion can be made. The effect was studied for flax technical fibres (Efremov 2002). Drying of natural technical fibres introduces micro-cracks, leading to a decrease in strength.

Among the well-known natural fibres, bamboo has one of the most favourable combinations of low density and high mechanical properties, that is, it has high specific stiffness and strength. In terms of specific properties, it is claimed they can be compared with glass fibres (Trujillo et al. 2010).

Bamboo fibre bundles are distributed densely in the outer region of the culm wall and sparsely in the inner region, and also concentrated in the upper part of the culm compared with the base. Elementary fibres in such a bundle consist of thick and thin layers with different cellulose microfibrillar orientation. In the thick layers, the microfibrils are oriented at a small angle to the fibre axis, whereas the thin ones show mostly a more transverse orientation. This structure does not exist in the cell walls of fibres of normal wood and leads to an extremely high tensile strength of the culm. (Liese 1998, Murphy; Alvin 1992). In the case of Bamboo *Guadua angustifolia*, the average value of the tensile strength of the culm is 190 MPa (Ciro 2005). This is the reason why the fibres, the structural part of the culm, are often called 'natural glass fibres' (Okubo and Fujii 2002; Dieu T et al. 2004). The elementary fibre length and fibre diameter for this species are on average 1.6mm and 11 μm , respectively, and they constitute about 40–50wt% of the total bamboo plant tissue and between 60 and 70wt% of the total culm tissue (Londoño et al. 2002).

In this paper, characteristics of bamboo technical fibres from the species *Guadua angustifolia* at the meso and micro level, are studied with special emphasis on the technical fibre, together with the characterization of mechanical properties from single technical fibre tensile tests and the subsequent study of the effect of moisture on the mechanical properties.

Materials and methods

The bamboo culms (species *Guadua angustifolia* Kunth) were extracted from a typical bamboo plantation in Colombia, specifically from the Coffee Region at the National Research Center for Guadua where the environmental conditions are: 1.240 meters above the sea level, annual average temperature of 25°C and relative humidity of 80%. Technical fibres were mechanically extracted, from the bamboo culms.

Microscopic observations

For light microscopy (LM), thin sections (20 µm) of bamboo tissue were prepared using a sliding microtome (Reichert, Vienna, Austria). A mixture of safranin and alcian blue (35 : 65, v/v) was used as staining solution.

After staining, sections were washed with distilled water, dehydrated with ethanol and treated with clearing agent Parasolve (Prosan, Merelbeke, Belgium). The sections were embedded in Euparal (Agar Scientific Ltd, Essex, UK). LM-observations were carried out with a Dialux 20 (Leitz, Wetzlar, Germany).

For the scanning electron microscope, small blocks (\pm 5 mm thick) were cut and attached to stubs using electron-conductive carbon paste. The samples were sputter coated with gold. Observations were carried out at the Laboratory of Plant Systematics (K.U. Leuven) with a Jeol JSM 6360 SEM (Jeol Ltd., Tokyo, Japan).

Bamboo technical fibres

Bamboo technical fibres were extracted from the bamboo culms giving a maximum technical fibre length between 20 and 35 cm. Figure 1 shows a group of mechanically extracted technical fibres whose diameter has a range between 90 and 250 µm; the main diameter concentration is around 150 µm.



Figure 1. Bamboo (*Guadua angustifolia*) technical fibres ; each fibre bundle in the culm wall, splits into a few technical fibres

Determination of the transverse area of bamboo technical fibres

Before tensile testing, every technical fibre was weighed in order to determine the transverse area. The length of the technical fibre was kept constant. The area of each technical fibre was determined using

both the density and the weight as well as the length of each technical fibre using the following equations:

$$\rho = \frac{m}{V} \quad (\text{Eq. 1})$$

which is equivalent to:

$$\rho = \frac{m}{A \cdot L} \quad (\text{Eq. 2})$$

where: m= mass, V= volume, A= area, L= length and ρ = density.

Thus the effective cross-sectional area of the technical fibres is calculated. The apparent density of the *Guadua angustifolia* technical fibre was determined by the method of the displacement of volume (pycnometer) and then confirmed by using a Sartorius balance, where the sample is weighed in a liquid and in air; both techniques gave the same value for the density and it was determined as 1,44 g/cm³.

Sample preparation and tensile test for single technical fibres

The technical fibres are selected before the tensile test; the selected technical fibres were glued in between two paper frames to assure a good gripping and straight direction in the test clamps (Fig. 2). The paper frame is clamped in the machine at top and bottom and is then cut carefully. The opening of the paper frame determines the gauge length; for this experiment it was set at 5, 10, 25 and 40 mm: at least 30 successful tests were performed for each span length. Varying the span length allows for correcting modulus values for machine compliance and slippage and for evaluating the effect of span length on strength.

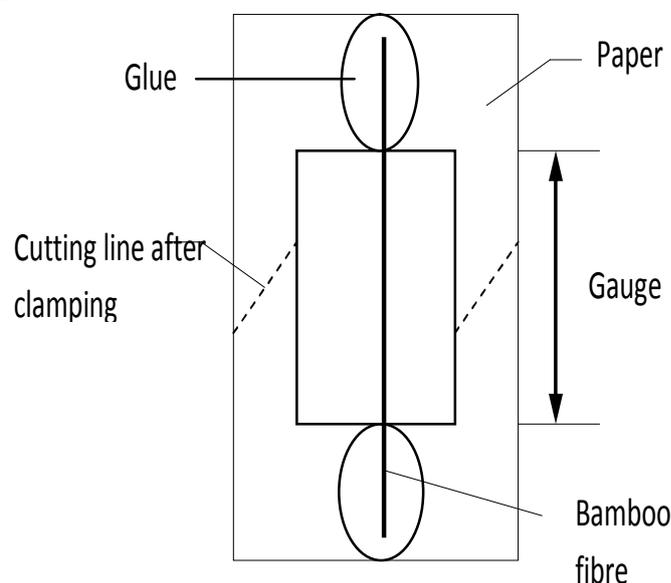


Figure 2. Schematic representation of the paper frame for the single technical fibre tensile test

The tensile test was performed on a mini tensile testing machine developed in the Department of Metallurgy and Materials Engineering at K.U. Leuven. For the conditioning of the samples, all treated and untreated technical fibres remained at least 40 hours in the tensile room before the test.

Water uptake

Humidity tests demand stable humidity conditions at a wide range of humidity levels. Different techniques are available that meet these requirements: desiccators, environmental chambers, saturated salt solutions, dry salts, among others. Due to its versatility but especially portability it was opted to use a combination of saturated and dry salts.

The water uptake test gives information about the maximum amount of water that technical fibres can take up. The amount of moisture the technical fibre has absorbed is defined as:

$$M = \frac{M_{eq} - M_{dry}}{M_{dry}} \quad (\text{Eq. 3})$$

with M the equilibrium moisture content or water concentration, M_{dry} the dry material weight [g] and M_{eq} the material weight when in equilibrium with the environment [g]

Results and discussion

Guadua angustifolia technical fibre

As mentioned earlier in this paper, the density of bamboo technical fibres across the culm wall decreases from the outer to the inner side with a change of morphology of the vascular bundles. In Figure 3a, a picture from the light microscope observations shows this characteristic. In Figure 3b, the SEM picture shows the complete vascular bundle (middle zone) and the detail for the specie *Guadua angustifolia*.

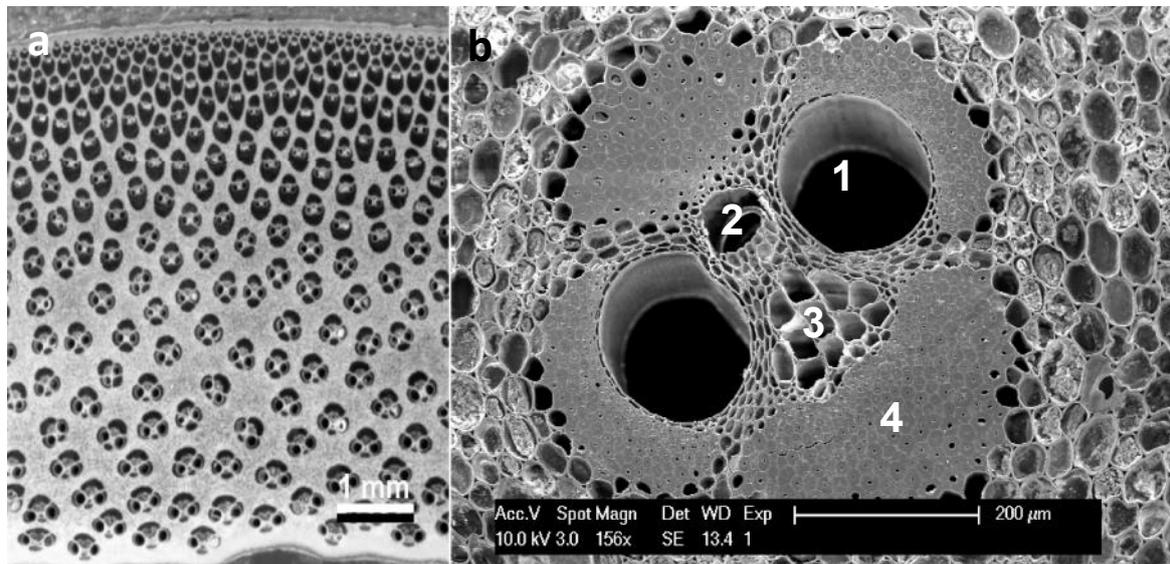


Figure 3. a) Bamboo culm wall b) Vascular bundle and its parts: 1) metaxylem, 2) phloem, 3) protoxylem, 4) fibres.

The fibre bundles (bean-shaped) attached to the conductive tissue are the mechanical support of the bamboo; they consist of many elementary fibres connected by lignin. To characterize the vascular bundle is not only of great importance in taxonomy research, but also to the fibre processing, because the shape and size of the vascular bundles have a strong influence on the difficulty of extraction and the dimensions and quality of the extracted technical fibres.

The elementary fibres represent the main structural component of the bamboo culm, they exhibit a hexagonal or pentagonal shape; the small hole in the centre of each elementary fibre is called lumen

(Fig. 4a). As mentioned before, each elementary bamboo fibre wall possesses a unique multilayer configuration called polylamellate structure which contributes to the strength and modulus of the bamboo culm. This structure determines the mechanical properties of the technical fibres. Figure 4b shows the microfibrils present in *G. angustifolia* elementary fibres, particularly the outer layer with 90° orientation.

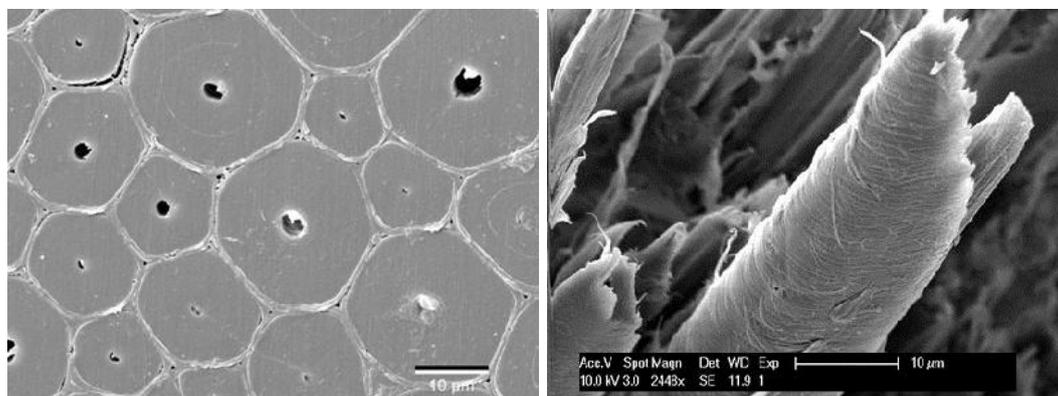


Figure 4. a) *G. angustifolia* elementary fibres b) *G. angustifolia* microfibrils

Bamboo technical fibre mechanical properties

The values for tensile strength and stiffness for untreated bamboo technical fibres at different span lengths are shown in Figure 5. The modulus and strength normalised to density (specific material properties) are similar to the values for glass fibre. Under tensile load bamboo technical fibres present a clean fracture instead of defibrillation or splitting. The clean fracture shows that there is a good bond between elementary fibres and bonding matrix and hence a good stress transfer. Also, the absence of kink bands and defects along the length of the bamboo technical fibres, that can create weak points of stress concentration reducing the strength of the technical fibres, contributes to the high strength of the technical fibres. Because the technical fibres are too thin, an extensometer cannot be used during the test. A theoretical correction for the machine compliance developed by (Defoirdt et al. 2010) was applied in order to determine the true elongation of the specimens.

Furthermore, when the volume (cross-sectional area x tested span length) of the samples is considered, one can observe that there is no effect of this property on the strength (Fig. 6), indicating that the technical fibres are rather homogeneous through their cross section and the presence of defects is not affecting the tensile strength.

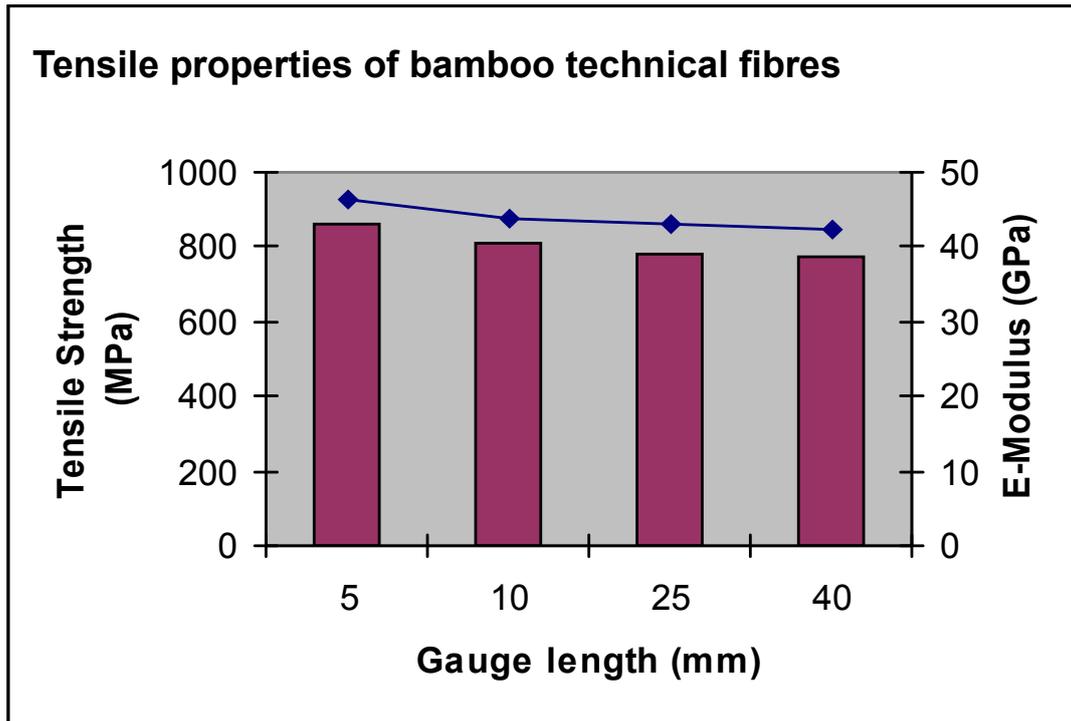


Figure 5. Tensile properties of bamboo technical fibres; bars denote strength values; points denote modulus values

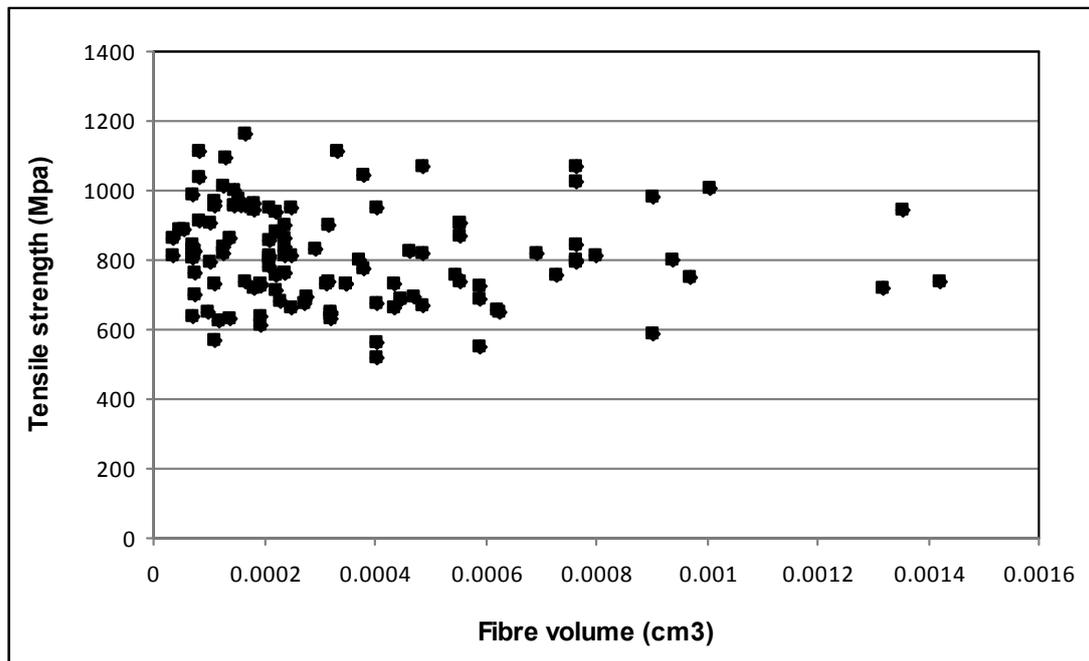


Figure 6. Influence of fibre volume (cross-sectional area x tested span length) on tensile strength of bamboo technical fibres.

Moisture sensitivity of bamboo technical fibres

The moisture uptake rate depends on the relative humidity of the environment. As the concentration gradient is higher with increasing environmental relative humidity level, this was to be expected based on Fick's first law. Figure 7 shows the complete moisture uptake of the technical fibres with time. It is important to note that the rate at which equilibrium is reached decreases with decreasing relative humidity. A consequence of this is that samples at lower relative humidity levels will need longer periods for complete acclimatization. The material reaches its equilibrium moisture content when the water activity in the sample equals the water activity value of the environment.

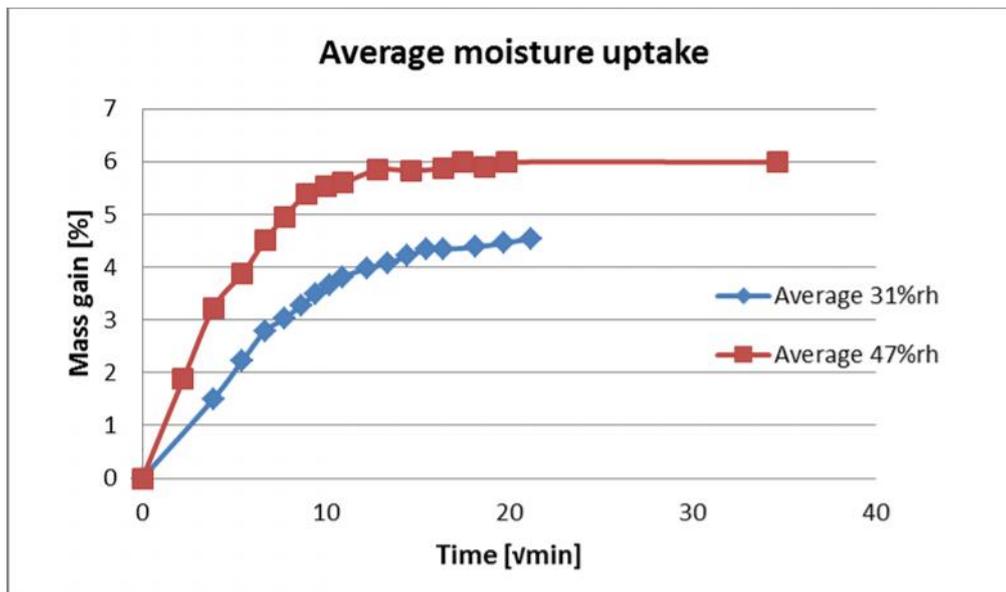


Figure 7. Moisture uptake in function of time of single bamboo technical fibres

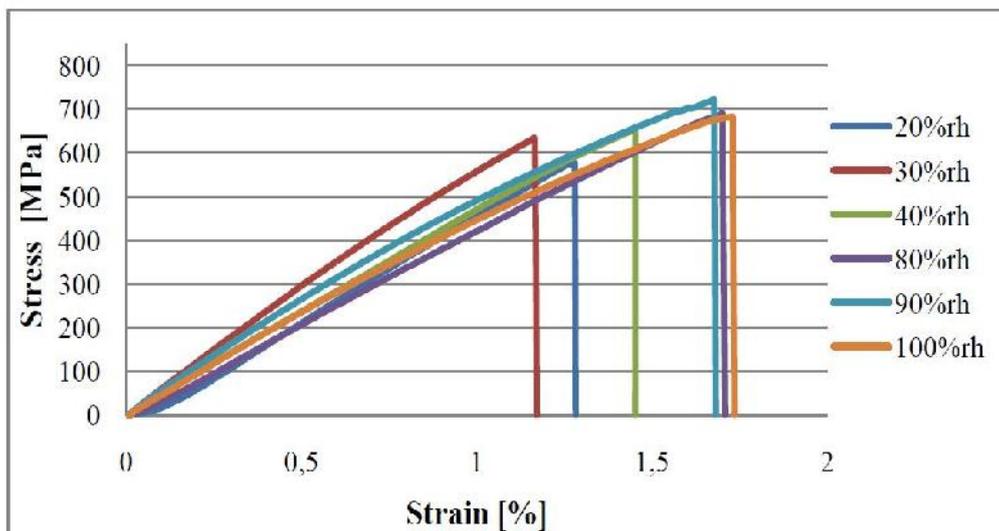


Figure 8. Typical stress-strain curve of climatized bamboo technical fibres

Influence of moisture on the mechanical properties

Figure 8 shows that there is a slight increase in tensile strength with increasing humidity level. This tendency was expected as at low humidity level the technical fibres are prone to micro cracking. On the other hand at higher humidity levels the technical fibres swell slightly closing micro cracks and making the technical fibres less susceptible to a critical defect.

An increase in strain to failure with increasing humidity is caused by plasticization. When the technical fibre absorbs water mostly the interface between elementary fibres is influenced. The elementary fibres become less tightly bonded, resulting in a global plasticizing effect.

Conclusions

A novel mechanical extraction process produces long bamboo technical fibres with excellent mechanical properties with surface characteristics that benefit the performance of the material as reinforcement. The technical fibres are influenced by humidity. It was shown that an increase in moisture level induces an increase in ultimate strain, this due to plasticization of the technical fibres.

In general, the results of this study suggest that there is a good potential for long bamboo technical fibres as reinforcing material for polymeric matrices and that the material could be appropriate to be used for commercial applications, where the fulfilment of environmental regulations and weight reduction are important aspects.

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References

- Baillie, C.; Nishinko, T. 2004. Green composites, Polymer composites and the environment, CRC Press, 49-80
- Béakou, A.; Ntenga, R.; Lepetit, J.; Atéba J.A.; Ayina L.O. 2008. Physico-chemical and microstructural characterization of “*Rhectophyllum camerunense*” plant fibre. Composites: Part A ,39, 67-74.
- Bledzki, A.; Gassan, J. 1999. Composites reinforced with cellulose based technical fibres. Progress in Polymer Science, 24, 221-274 .
- Brouwer, W. 2004. Natural technical fibre composites in structural components: alternative application for sisal? FAO Report Y1873E.
- Cao, Y.; Shibata S.; Fukumoto I. 2006. Mechanical properties of biodegradable composite reinforced with bagasse technical fibre before and after alkali treatments. Composites: Part A ,37, 423-429.
- Charlet, K. 2008. Contribution à l'étude de composites unidirectionnels renforcés par des technical fibres de lin : relation entre la microstructure de la technical fibre et ses propriétés mécaniques. Doctorate thesis of Caen University / Basse – Normandie.
- Ciro, H.; Osorio, J.; Restrepo, J. 2005. Determinación de la resistencia mecánica a tensión y cizalladura de la *Guadua angustifolia kunth*. Revista Facultad Nacional de Agronomía, 58(1), 2709-2715.
- Defoirdt, N.; Biswas, S.; De Vriese, L.; Tran, L.Q.; Van Acker, J.; Ahsan, Q.; Gorbatikh, L.; Van Vuure, A.; Verpoest, I. 2010. Assessment of the tensile properties of coir, bamboo and jute technical fibre. Composites: Part A ,41, 588-595.
- Dieu, T.; Liem, N.; Mai, T.; Tung, N. 2004. Study on fabrication of BMC laminates based on unsaturated polyester resin reinforced by hybrid bamboo/glass fibres. JSME Int J, Ser A, 47(4), 570-573.

- Efremov, G. 2002. Quasistationary method of describing the kinetics of treatment processes for technical fibre materials. *Technical fibre Chemistry*, 34, 372-377.
- Gomes, A.; Goda, K.; Ohgi J. 2004. Effects of alkali treatment to reinforcement on tensile properties of curaua green composites. *JSME International Journal Series A*, 47(4), 541-546.
- Khalil, A. ; Ireana Yusra A.F.; Bhat A.H.; Jawaid, M. 2010. Cell wall ultrastructure, anatomy, lignin distribution, and chemical composition of Malaysian cultivated kenaf fibre. *Industrial Crops and Products*, 31(1), 113–121
- Liese, W. 1998. The anatomy of bamboo culms. INBAR Technical Report, International Network for Bamboo and Rattan, 18, 204.
- Londoño, X. 1998. A decade of observations of a *Guadua angustifolia* plantation in Colombia. *Journal of the American Bamboo Society*, 12, 37-42.
- Londoño, X. 2001. La Guadua un bambú importante de América. in: *Proceedings First Bamboo Seminar*, Ecuador, 12-18.
- Londoño, X.; Camayo, G.; Riaño, N.; López, Y. 2002. Characterization of the anatomy of *Guadua angustifolia* (Poaceae: Bambusoideae) culms. *Bamboo Science and Culture: The journal of the American Bamboo society*, 16, 18-31.
- Mohanty, A.K.; Misra, M.; Drzal, L.T. 2005. *Natural fibres, Biopolymers, and Biocomposites*, Taylor&Francis.
- Murali, K.; Mohana, K. 2007. Extraction and tensile properties of natural fibres: Vakka, date and bamboo. *Composites Structures*, 77, 288-295.
- Murphy, R.; Alvin, K. 1992. Variation in fibre wall structure of bamboo. *IAWA Bulletin* 1992; 13, 403-410.
- Nilsson, T. 2006. Micromechanical modeling of natural technical fibres for composite materials, Licentiate Dissertation, Department of Construction Sciences: Structural Mechanics LTH Sweden.
- Okubo, K.; Fujii, T. 2002. Eco-composites using natural fibres and their mechanical properties. In 'High performance Structures and Composites', C. Brebbia and W. de Wilde, 77- 85.
- Symington, M.; Banks, W.; Opukuro, D. 2009. Tensile testing of cellulose based natural technical fibres for structural composite applications. *Journal of composite materials*, 43, 1083-1108.
- Trujillo, E.; Osorio, L.; Fuentes, C.; Van Vuure, A.; Verpoest, I. 2010. Bamboo fibre thermoplastic for transport applications. In: *Proceedings of SAMPE Europe 31 International Technical Conference and Forum*, Paris, France.
- Young, S.; Judd, W. 1992. Systematics of the *Guadua angustifolia* Complex (Poaceae: Bambusoideae). *Annals of the Missouri Botanical Garden*, 79 (4) 737-769.

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Liquid Hot Water (LHW) pretreatment of bamboo for second-generation (2G) biofuel production

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Abstract

Liquid biofuels produced from plant biomass have the potential to enhance energy security and reduce fossil fuel burning, thereby helping mitigate climate change. Bamboos are a potentially very interesting as a feedstock for second-generation (2G) bioethanol production because of their rapid growth, perennial nature, low management requirements and high sugar content, giving a theoretical maximum ethanol yield of about 450 l/dry tonne of biomass. In our experimental work, low enzymatic saccharification yields from the *Phyllostachys* species indicate the recalcitrance of their cell wall and illustrate the need for an effective pretreatment to improve sugar release. Liquid Hot Water (LHW) pretreatment was explored as a promising process for improving saccharification yields. LHW pretreatments were performed at temperatures between 170 and 190°C for 10 to 30 minutes. The 190°C for 30 minutes condition produced a sugar yield of 66% of initial carbohydrate content, equivalent to a predicted ethanol yield of 246 l/dry tonne of biomass. Further work is focused on optimising saccharification yields along with detailed techno-economic and life cycle assessments of costs and energy inputs in order to develop a comprehensive model for sustainable bamboo-to-bioethanol processing systems.

Keywords: Bamboos, bioethanol, pretreatment, sugar yields, ethanol yields

Introduction

The use of liquid biofuels in the transportation sector is becoming increasingly important due to environmental concerns of climate change, increasing price of oil and energy security (IEA 2006). In the EU, road transport accounts for over one-fifth of the total carbon dioxide emissions, suggesting its significant contribution to global warming (European Commission 2011). Biofuels produced from plant biomass is believed to be the leading renewable primary energy resource that can provide a short-term alternative to current transportation fuels (Alvira et al. 2010). Due to debate regarding the sustainability of biofuels produced from food crop starch and sugars, current research is focusing on the production of lignocellulosic biofuels produced from cell wall sugars (Sims et al. 2010). Cellulose, the main component of biomass, is the most abundant polymer on the planet, and with an estimated annual worldwide production of 10-50 billion dry tonnes of biomass, it represents a significant resource for fuel production (Naik et al. 2010; Galbe 2007).

Due to the recalcitrant structure of lignocellulose, the biochemical conversion processes into ethanol typically involve three main stages: (1) Pretreatment of biomass to convert material into a cellulase-digestible substrate, (2) enzymatic saccharification to produce monomeric sugars from biomass, and (3) fermentation to convert monomeric sugars into fuel such as ethanol. Although the pretreatment step is regarded as one of the technical bottlenecks within the process due to high costs and energy inputs, it is essential to ensure the production of fermentable sugars from saccharification within an industrially acceptable set of conditions (Jorgensen et al. 2007). Using a combination of high temperature, pH and pressure, pretreatments aim either solely or in combination to:

- 1) Increase accessible biomass surface area,
- 2) partially/fully remove lignin and/or hemicellulose,
- 3) reduce cellulose crystallinity
- 4) interrupt cell wall component interactions (Kumar et al. 2009; Pederson and Meyer 2010).

Liquid Hot Water (LHW) pretreatment was selected for investigation with bamboo due to its potentially low energy and chemical requirements, which reduce overall costs, environmental impacts and chance of equipment corrosion (Cybulska et al. 2009).

The chemical composition of bamboos has been reported to range from 40-48% cellulose, 24-28% hemicellulose and 20-26% lignin by oven-dry mass, suggesting that with the appropriate technology there is an abundant pool of sugars available for ethanol production (Yamashita et al. 2010). Bamboo stands can be highly productive which allows them to attain 7-30% greater biomass production than other fast-growing plants (INBAR 2009). Their extensive rhizome system allows for: 1) Efficient storage of nutrients year-round, limiting the amount of nutrient inputs required and thereby reducing overall agricultural costs and environmental impacts; 2) re-growth of new shoots, leading to rapid regeneration in short time periods; 3) reduction in soil erosion, which poses one of the largest environmental issues in countries such as China (Kobayashi et al. 2004; Potters et al. 2010). Their ability to be grown on marginal/low nutrient land can bring degraded land back into production and help minimise potential indirect land-use change. The optimal harvesting season can occur over a 6-month period and the biomass stored for about 3-months, providing a nearly year-round supply of biomass for ethanol production (Potters et al. 2010).

The aims of the present study were to investigate the polymeric composition of the bamboos *Phyllostachys spp* and to determine how varying severities of LHW pretreatment influenced the accessibility of the cell wall material to enzymatic saccharification. Particular attention was given to

providing definitive data for calibrating changes in the biomass composition during pretreatment and to considering the results in the context of potential biorefining and biofuels production from bamboo.

Materials and Methods

Plant material

Five culms of *Phyllostachys dulcis* and three culms of *Phyllostachys viridi-glaucescens* were randomly selected from Kew Gardens during October 2010. All culms were 5 years of age and were harvested at ground level. Branches and leaves were removed and each culm was separated into nodes and internodes and left to air-dry in the laboratory for 2 weeks.

Compositional Analysis

A two-step extraction step using deionised water followed by 95% ethanol was performed according to the National Renewable Energy Laboratory (NREL) LAP protocol “Determination of extractives in biomass (Sluiter et al. 2005) using an Dionex Accelerated Solvent Extractor (ASE) 200. Samples were air-dried, re-weighed and moisture contents calculated to determine the percentage extractives in the biomass. Compositional analysis procedures were based on the NREL LAP protocol “Determination of structural carbohydrates and lignin in biomass” (Sluiter et al. 2008).

Pretreatment

LHW pretreatment was also carried out using the ASE 200. Replicate bamboo samples (2.0 g oven-dry weight) were pretreated in triplicate under the conditions shown in Table 1:

Table 1: Liquid Hot Water pretreatment conditions

Temperature (°C)	170, 180, 190
Time (minutes)	10, 20, 30
Pressure (psi)	500
Heat-up time (minutes)	7, 8, 9
Flush volume (%)	100
Purge time (seconds)	120

Enzymatic Saccharification

Enzymatic saccharification followed the NREL LAP protocol “Enzymatic saccharification of lignocellulosic biomass” (Selig et al. 2008). All saccharification samples were treated with an enzyme loading of 60 FPU/g glucan of a cellulase enzyme mixture containing cellulase (Celluclast 1.5L) and β -glucosidase (Novozym 188) in a volume ratio of 1:1. The pH was adjusted using sodium citrate buffer to 4.8 after 400 μ g of tetracycline and 300 μ g of cycloheximide were added. An Agilent 1200 series HPLC was used to analyse sugar concentrations of each sample by comparing with prepared glucose and xylose standards (0.1, 1, 2 and 4 mg/mL). Total glucose release was measured in samples containing enzyme, and soluble (non-enzyme-derived) glucose was measured in samples lacking enzyme; by subtracting soluble glucose from total glucose the amount of enzyme-derived glucose was calculated.

Results and Discussion

Analysis of raw *Phyllostachys spp.* bamboo material

The average chemical composition and saccharification yield of raw *P. viridiglaucescens* and *P. dulcis* was obtained to establish a baseline value for comparison with pretreated material. These values listed (Table 2) are at the low end of the range stated in Scurlock (2000) for other *Phyllostachys* species, and are comparable to ranges reported for softwoods and hardwoods.

Table 2: Composition of raw bamboo material

Composition	% Dry matter
Glucan	38.4 ± 0.47
Xylan	20.5 ± 0.47
Galactan	3.6 ± 0.26
Arabinan	1.8 ± 0.15
Lignin	20.8 ± 0.52
Ash	0.9 ± 0.22
Extractives	13.5 ± 1.77
Mass closure	99.4 ± 0.19

The cellulose-to -glucose conversion efficiency by enzymatic saccharification of raw bamboo was 5.7% of the total ODW of the raw biomass. This indicates that about 15% of the cellulose was enzymatically converted into glucose without pretreatment, representing about 9% of the initial total carbohydrate content (~65% of the ODW) of raw bamboo. This means that over 90% of the total carbohydrate in the raw biomass remained inaccessible to enzymatic conversion into monomeric sugars when no pretreatment was used. These results demonstrate a clear need for a pretreatment step in order to have an effective yield of sugars from bamboo biomass using enzymatic conversion.

Effect of LHW pretreatment on biomass composition

Compositional analysis of the pretreated biomass shows the effect of each LHW pretreatment condition on the bamboo biomass. At increasing pretreatment severities (higher temperatures and residence times) the amount of biomass solubilised into the liquid hydrolysate (shown as ‘mass loss’ in Figure 1) increases linearly up to 190°C where it reaches a maximum level of 47% of the initial biomass. Under the most severe pretreatment condition of 190°C for 30 minutes, the initial glucan (~cellulose) content is reduced from 38.4% in the raw biomass to 31.1% with reference to its amount in the original biomass (an 18.9% reduction in glucan). The glucan content in the residual biomass under this condition is in fact effectively ‘enriched’ (largely through the solubilisation and removal of the hemicellulose fractions from the biomass) and now represents about 60% of the solids in the pretreated biomass. The maximum lignin removal is observed under the 190°C for 10 minutes condition from 20.8% to 16.0% of the initial biomass (a 23.3% reduction in lignin).

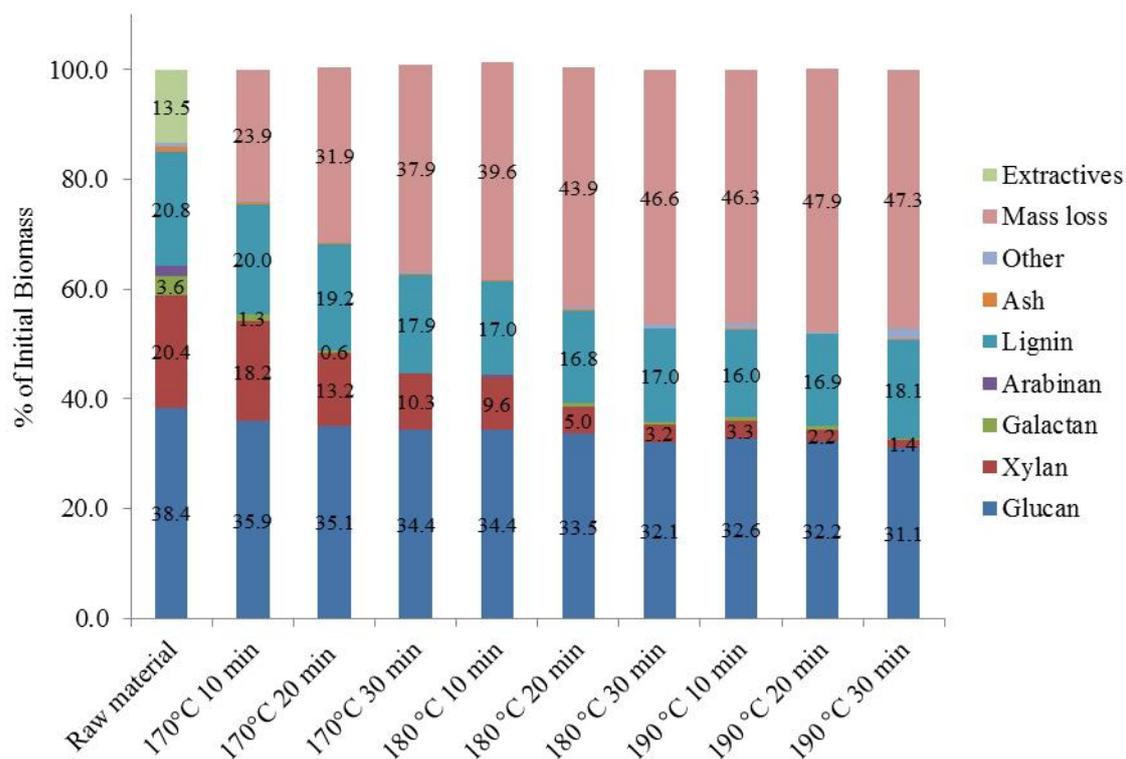


Figure 1: Mass closures for various LHW pretreatment conditions (% of the initial biomass input).

The hemicellulose component is the one most significantly affected by LHW pretreatment, as shown by the complete removal of galactan and arabinan at most pretreatment conditions, as well as xylan which is reduced from 20.4% to 1.4% of the initial biomass under the 190°C for 30 minute condition (a 93.3% removal of xylan). At each temperature, the increase in time from 10 to 30 minutes led to a corresponding rise in solubilised xylan. The mechanism of hemicellulose solubilisation is related to the autohydrolysis of water when it is maintained at liquid state by high pressures. Autohydrolysis creates an acidic environment through the formation of hydronium ions which hydrolyse the glycosidic linkages and acetyl groups of hemicellulose, leading to depolymerisation and further catalytic cleavage of the links between hemicellulose and lignin (Carvalho et al. 2008; Cybulska et al. 2010). The result is a hemicellulose-rich liquid fraction that can be directly fermented into fuel, as well as a cellulose-rich solid that can be enzymatically digested to produce monomeric sugars for fermentation.

Effect of pretreatment on predicted ethanol yields

Enzymatic saccharification of the pretreated solid residue was performed and the predicted ethanol yield per tonne ODW equivalent of original bamboo biomass was generated from the combined sugar release from both the pretreatment liquid hydrolysate and the enzymatic saccharification of the pretreated biomass solids. The predicted ethanol yield is based on the conversion of one glucose molecule into two molecules of ethanol and assumes a 95% conversion efficiency of glucose to ethanol by *Z. mobilis* (Aden et al. 2002). The xylose conversion into ethanol is based on the 85% conversion efficiency of 5 moles of xylose into 3 moles of ethanol by *Z. mobilis*. The total yields (Figure 2) show the maximum predicted ethanol yield of 246 litres from the 190°C for 30 minute pretreatment condition, which is equivalent to 66% of the initial carbohydrate content converted into monomeric sugars. This value falls within the upper range of typical ethanol bioconversions reported

from agricultural feedstocks of between 110 and 270 l/tonne of dry matter or for forestry residues of between 125 to 300 l/tonne of dry matter (Sims et al. 2010).

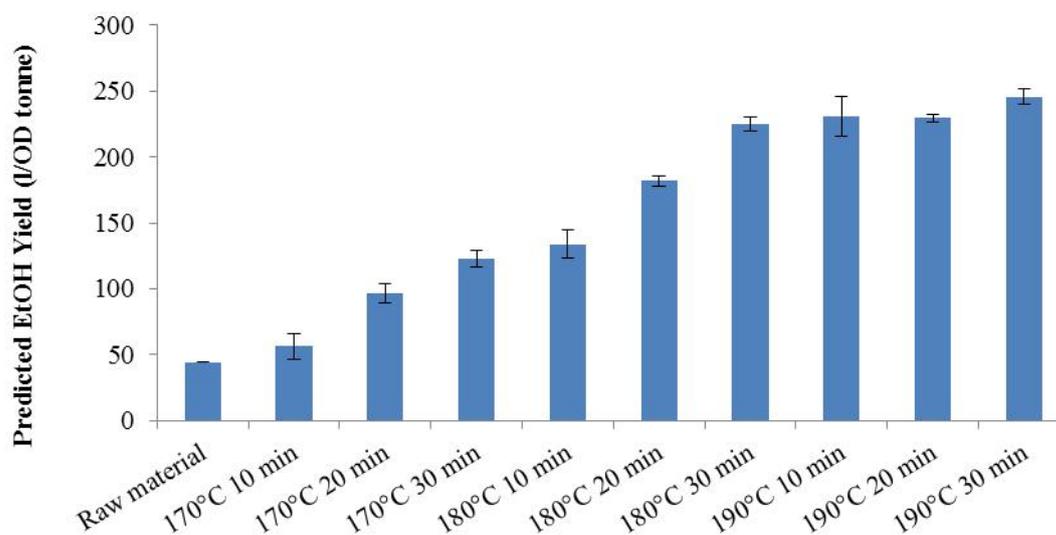


Figure 2: Predicted ethanol yields (litres per OD tonne biomass) from glucose and xylose release from pretreatment and enzymatic saccharification.

The highest predicted ethanol yield of 246 litres is a significant improvement from the 44 litres estimated from raw bamboo, which clearly supports the results reported by Pérez et al. (2008) stating that removal of hemicellulose as well as cleavage of bonds linking hemicellulose to lignin and cellulose, is a key factor to improving cellulose digestibility.

It is uncertain whether a 66% release of the carbohydrate content is the maximum yield that can be achieved in bamboo with LHW pretreatment. However future research will focus on optimising downstream process conditions to evaluate whether these can also contribute to higher yields. Aside from successfully improving yields in bamboo, the avoidance of chemical inputs and the minimal sugar degradation of LHW pretreatment (data not shown), position it as one of the leading processes in the bioconversion of lignocellulose to ethanol. With these improvements, the economic and environmental merits of LHW pretreatment suggest that it has strong potential to be a component of successful biorefining of bamboo and development of sustainable bamboo-to-biofuels processes.

Conclusions

Our results demonstrate the prospects that Liquid Hot Water pretreatment has as an aid to the release of sugars from bamboo as part of a biorefining and/or bamboo-to-biofuel strategy. Pretreatment of bamboo at 190°C for 30 minutes reduced the xylan content of the biomass by 93%, which led to a significant improvement in the total sugar release from 9% to 66% of the initial carbohydrate content, resulting in a predicted ethanol yield of 246 l/tonne of dry matter. Current efforts aim to focus on the downstream enzymatic saccharification process through optimising enzyme loadings which may enhance total sugar yields for future commercialisation. These and other data (e.g. estimations of biomass yield, transportation, co-product quantities and values) are being incorporated into a techno-economic model to provide an overall assessment of cost and energy inputs, as well as generating estimated bioethanol costs for bamboo-to-bioethanol processing systems to assess the potential for bamboo biorefining and biofuels competitiveness with other supply chains.

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References

- Aden, A., Ruth, M. Ibsen, K., Jechura, J., Neeves, K., Sheehan, J. & Wallace, B. 2002. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. NREL. Available: <http://www1.eere.energy.gov/biomass/pdfs/32438.pdf>
- Alvira, P., Pejo-Tomas, E., Ballesteros, M. & Negro, M.J. (2009) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*. 101, 4851-4861.
- Carvalho, F., Duarte, L.C. & Girio, F.M. (2008) Hemicellulose biorefineries: a review on biomass pretreatments. *Journal of Scientific & Industrial Research*. 67, 849-864.
- Cybulska, I., Lei, H. & Julson, J. 2009. Hydrothermal Pretreatment and Enzymatic Hydrolysis of Prairie Cord Grass. *Energy & Fuels*, 24, 718-727.
- Galbe, M.Z. 2007. Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production. *Adv. Biochem. Engin./Biotechnol*, 108, 41-65.
- European Commission. 2011. Reducing emissions from transport. Available: http://ec.europa.eu/clima/policies/transport/index_en.htm
- IEA. 2006. Key world energy statistics. Available: <http://www.iea.org/textbase/nppdf/free/2006/key2006.pdf>
- INBAR. 2009. Bamboo and rattan trade database [Online]. Beijing: INBAR. Available: www.inbar.int/trade/main.asp.
- Jorgensen, H., Kristensen, J.B. & Felby, C. 2007. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels, Bioproducts & Biorefining*, 1, 119-134.
- Kobayashi, F., Take, H., Asada, C. & Nakamura, Y. 2004. Methane production from steam-exploded bamboo. *Journal of Bioscience and Bioengineering*, 97, 426-428.
- Kumar, P., Barrett, D.M., Delwiche, M.J. & Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research*, 48, 3713-3729.
- Naik, S.N., Goud, V.V., Rout, P.K. & Dalai, A. K. Production of first and second generation biofuels: A comprehensive review. *Renewable & Sustainable Energy Reviews*, 14, 578-597.
- Pederson, M. & Meyer, A.S. 2010. Lignocellulose pretreatment severity - relating pH to biomatrix opening. *New Biotechnology*, 27, 739-750.
- Perez, J.A., Ballesteros, I., Ballesteros, M., Saez, F., Negro, M.J. Manzanares, P. 2008. Optimizing Liquid Hot Water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production. *Fuel*, 87, 3640-3647.
- Potters, G., Brems, A., Valacke, R., Dewil, R., D'Haese, L., Samson, R. & Gielis, J. Energy crops in Western Europe: is bamboo an acceptable alternative? VIII World Bamboo Congress 2010. 22-30.
- Scurlock, J. 2000. Bamboo: an overlooked biomass resource? *Biomass & Bioenergy*, 19, 229-244.
- Selig, M., Weiss, N. & Ji, Y. 2008. Enzymatic saccharification of lignocellulosic biomass. NREL. Available: <http://www.nrel.gov/biomass/pdfs/42629.pdf>
- Sims, R., Mabee, W., Saddler, J. & Taylor, M. 2010. An overview of second generation biofuel technologies. *Bioresource Technology*, 101, 1570-1580.
- Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J. & Templeton, D. 2005. Determination of extractives in biomass. NREL. Available: <http://www.nrel.gov/biomass/pdfs/42619.pdf>

- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. & Crocker, D. 2008. Determination of structural carbohydrates and lignin in biomass. NREL. Available: <http://www.nrel.gov/biomass/pdfs/42618.pdf>
- Yamashita, Y., Shono, M., Sasaki, C. & Nakamura, Y. 2010. Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo. *Carbohydrate Polymers*, 79, 914-920.

Applications for biomass out of bamboo: Pyrolysis oil, activated carbon, pretreatments for digestion.

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Abstract

The use of bamboo as an energy crop is of high interest because of various beneficial properties, including its fast growth, capability to grow on marginal soils (hence not competing with food crops for land usage), low maintenance requirements and ease of storage after harvest. The conversion of bamboo to liquid or gaseous biofuels is specifically attracting much attention. This can be achieved by a thermochemical (pyrolysis or gasification) or biochemical (anaerobic digestion) conversion technique. Some aspects of both techniques are investigated in this paper. While bio-oil is in most cases the primary product from pyrolysis (to be used as a fuel or, after separation, as a source for renewable chemicals - the latter being still in the research phase), also a solid char fraction is generated as a by-product. This paper assesses its use as a low cost adsorbent for wastewater treatment or air purification, after steam activation. The adsorption capacity of the obtained activated carbon was rather low, compared to commercially available products, but nevertheless it can be used for applications with lower purification demands. As for anaerobic digestion, this study mainly focuses on pre-treatment methods to improve the biodegradability of the bamboo, and hence increase the biogas production. A screening of different techniques was carried out, including oxidative, acid and thermal hydrolysis. The effects on the bamboo were characterized by Van Soest fractionation and the solubilisation of organics (as measured by the COD of the liquid phase).

Keywords

Biomass; Bamboo; Pyrolysis; Activated carbon; Digestion pretreatments; Biogas production.

Abbreviations

HMF	Hydroxymethylfurfural
PAA	Peracetic acid
TOC	Total Organic Carbon (mg/L)
COD	Chemical Oxygen Demand (mg O ₂ /L)
NDF	Neutral Detergent Fiber fraction (%)
SDS	Sodiumdodecylhydrogensulphate
DTA	Ethylenediaminetetraacetic acid
ADF	Acid Detergent Fiber fraction
a20	Acid treatment at 20°C
a80	Acid treatment at 80°C

St	Steam treatment
oxLow	Oxidative treatment low concentration
oxHigh	Oxidative treatment high concentration
BD	Biodegradability
ND	Not defined

Introduction

The large dependency on depleting fossil fuels to satisfy the global energy demand (also leading to global warming), necessitates the further development of renewable energy sources. It is generally accepted that a full transition to renewables, will be only feasible if a broad mixture of available technologies is applied including solar energy, wind energy and energy from biomass. The latter is of specific importance because a continuous power generation is possible (in contrast to other technologies). Bamboo has several features, making it very attracting as an energy crop. First of all it is barely used as a food crop, so there is minimal risk of unhealthy competition between its use for industrial purposes and for nutrition. It is fast growing (leading to a high biomass yield per hectare) and its ability to grow on marginal soils (Scurlock et al. 2000; Shanmughavel and Francis 2001) omits competition for land use with other food crops. A bamboo plant typically requires 5 years to reach the stage of fully grown plant. During this period little fertilization is needed and the general costs of maintenance are low. Once the plant is fully grown it is self-sustaining and does not need further maintenance. Bamboo can be harvested during a period of about 6 months and can be stored for 3 months after being harvested (Gielis 2000; Temmerman et al. 2005). Therefore it is possible to supply bamboo to the industry for 9 months a year.

The conversion of lignocellulosic material (including bamboo) to liquid or gaseous biofuels is attracting wide attention. In this paper some aspects related to the thermochemical conversion by pyrolysis and the biochemical conversion by anaerobic digestion will be investigated.

Pyrolysis

Pyrolysis is a thermochemical conversion method during which biomass is converted into a liquid (bio-oil), solid (ashes and char) and gaseous (non-condensable gasses) (Van de Velden et al. 2010). The bio-oil is mostly applied as a liquid fuel, but also contains various components that are interesting to be used as renewable feedstock in the chemical industry (the required separation is, however, very difficult and still in the research phase). It can be more readily stored and transported and it has a higher volumetric energy content per unit of volume compared to raw biomass. The pyrolysis process is usually optimized towards the production of bio-oil (Bridgewater 2004; Lievens et al. 2008, 2009). Since process conditions (temperature, residence time, ...) have a large influence on the yield of the various fractions, extensive research has been conducted to determine the conditions which maximizes the yield of the liquid phase, and it was seen that an intermediate temperature (823 K) and a short residence time (less than 2 s) promote a large oil fraction. Because of the short residence time, the technology is referred to as "fast pyrolysis". To avoid unwanted secondary reactions for which the char acts as a catalyst, the bio-oil needs to be separated from the char fraction as soon as possible after reaction (Bridgewater 2003; PyNe 2006; Van de Velden et al. 2010). According to Onay and Koçkar (2004), secondary reactions such as recondensation, repolymerization and cracking reactions can be avoided when a heating rate of 300 K min⁻¹ is used. In order to limit the presence of oxygen, a nitrogen atmosphere is created inside the reactor (Onay and Koçkar 2004). Apart from temperature, residence time and (nitrogen) gas flow, the particle size is also an important parameter. Too large particles result

in the presence of a temperature gradient inside the particle during the heating process and hamper a fast temperature increase within the particle, leading to less favorable process conditions. Van de Velden et al. (2010) have proven theoretically that the size of the particles should be less than 200 μm . Practical tests by Ensöz et al. (2000) and Onay and Koçkar (2004) have shown that 600–1800 μm is the ideal particle size.

Activated Carbon

The char fraction is a by-product of pyrolysis, but not necessarily a waste product. In some types of pyrolysis reactors it is burned to generate the heat for sustaining the (endothermic) process (Bridgewater et al. 1999; BTG). A higher value added approach is to convert this fraction to activated carbon, which is widely used for the removal of unwanted chemicals by adsorption (Abdel-Nasser et al. 2001). The large specific surface of activated carbon, generated by the very open internal structure (containing a large amount of micro- and meso-pores) results in a high adsorption capacity. This structure can be generated in a thermal (using steam) or chemical way (e.g., by KOH application). Abdel-Nasser et al. (2001) have shown that chemically activated carbon has the highest porosity because of the large fraction of mesopores, while activated carbon produced by steam activation contains mostly micropores.

Pretreatment Methods for Biogas Production

A second approach is the anaerobic digestion of bamboo. Because of the hemicellulose and lignin ‘shielding’ the cellulose, the latter is not easily accessible for the enzymes excreted by the micro-organisms during the hydrolysis stage (i.e. the first stage of anaerobic digestion). This renders the digestion process slow, and hence not economically feasible. These shortcomings can, however, be overcome by a pretreatment method, which releases the cellulose from the plant fibers to increase its bio-availability (Kumar et al. 2009). Various pre-treatment methods have been developed, all of which are applied to remove or dissolve the hemicellulose and lignin. In this paper five methods have been tested: two acid, two oxidative and one steam pretreatment.

The *acid pretreatment* method degrades the hemicellulose to smaller monomers through hydrolysis reactions. One disadvantage of this method is the formation of by-products such as furfural, HMF (hydroxymethylfurfural) and other volatile compounds, which are inhibitors for micro-organisms (Fengel and Wegener 1984; Ramos 2003; Hendriks and Zeeman 2009), although anaerobic micro-organisms can sustain relatively high concentrations of furfural after a period of acclimatization (Hendriks and Zeeman 2009). Also, dissolved lignin inhibits the digestion process, and this is why it should be precipitated after its release. A precipitation occurs in acidic conditions, starting from 72 % sulfuric acid (under formation of the so-called Klason-lignin) (Monties 1984). It was previously shown that cellulose accessibility increases for a decreasing pH. In practical applications, however, more dilute solutions are preferred because fewer secondary reactions take place and hence fewer inhibitory products are formed. Phosphoric acid is preferred over sulfuric or nitric acid because this minimizes the formation of H_2S and N_2 (decreasing the energy content of the biogas) during the anaerobic phase, respectively (Hendriks and Zeeman 2009).

The second method investigated is an *oxidative pretreatment*. The most commonly used chemicals here are hydrogen peroxide or a mixture of peracetic acid (PAA) and acetic acid. The specificity of these reactions is also low and various reactions take place, such as displacement of side chains and electrophilic substitutions (Hon and Shiraishi 2001). These give rise to the formation of side product, which are similar to those formed under acidic treatment conditions. PAA is preferred over hydrogen peroxide because it is quite lignin-selective and therefore causes less loss of carbon during the

pretreatment. Also, the molecule eventually breaks down to water and acetic acid which is further biologically degradable and enhances the biogas production (Appels et al. 2010).

The third method applied is a *steam treatment*, during which an aqueous solution is brought to high pressure and temperature, to break the hemicellulose bonds (Laser et al. 2002). As the temperature rises, first hemicellulose is dissolved, then lignin (Bobleter 1994; Garrote et al. 1999). The major drawback of this method is the incomplete cleavage of the bonds in the lignin matrix and the production of inhibitory compounds such as toxic phenols (Gosset et al. 1982).

To achieve maximum availability of cellulose, the following parameters should be optimized: reaction time, temperature, particle size and moisture percentage (Duff and Murray 1996; Wright 1998). These parameters differ from crop to crop and should be determined experimentally. According to Kobayashi et al. (2004) the application of a proper pretreatment method is a prerequisite to obtain methane production from bamboo.

Material and methods

Activated carbon

The bamboo material consisted of three year old culms of field grown *Phyllostachys humilis*, harvested in July, dried for at least three months in a barn, and then stored indoors until use (for at least 9 months). Average moisture content after drying was 15%. The material was cut into small pieces (± 0.5 cm), put into an Erlenmeyer flask (3/4 filled) and closed off with punctured tin foil preventing air pressure to build up. Next, the bamboo was heated at 450 °C for 30 minutes. Afterwards the 'coal' was ground with a mortar and pestle.

To activate the coal according to the first method, a glass tube - melted and closed on one side - was used. An amount of ground coal was put into the open side of the tube and an equal amount of distilled water was added. Then the tube was sealed by melting the tip, in such a way that it contains one part coal, one part water and one part air. The glass tube was then put at 150 °C for 30 minutes; afterwards the tube was broken and the wet coal oven-dried at 100 °C. The coal could be filtered first to accelerate the drying process.

Given that the glass tube tended to break because of the pressure buildup inside, a second method was used to obtain activated carbon. An equal amount of ground coal and distilled water was added to a pressure bottle and put into an autoclave for 30 minutes at 120 °C - 130 °C. Afterwards the coal was yet again dried in an oven at 100 °C.

To determine the activity of the obtained activated carbon, its adsorption capacity for acetone was tested. In parallel test tubes, 0.2 g of activated carbon was added to 14 mL of a 5% acetone in water solution. At various times the activated carbon was removed by filtration and the remaining acetone concentration in the water was measured by a TOC-analyzer (TOC-5000A Total Organic Carbon Analyzer, Shimadzu). This way, the decrease in acetone (expressed in terms of TOC) concentration could be plotted as a function of time. The amount adsorbed on the carbon can further be calculated from the difference between initial and final acetone concentration in the water phase.

Pretreatment for digestion

The same ground bamboo material as described before was used in these experiments. Two samples were subjected to an acid treatment at two different temperatures to determine the influence of temperature on the treatment. First distilled water was added to 2 g of ground bamboo, so that all the bamboo was submerged. Then a 96% sulfuric acid solution was added until a pH=3 was reached. One treatment was placed at room temperature (20 °C) on a stirred plate and one on a hotplate stirrer at 80 °C, both for one hour. A third bamboo sample was subjected to a steam pretreatment, with the use of an autoclave. 2 g of ground bamboo was put into a pressure bottle and submerged in distilled water.

The closed bottle was autoclaved for 30 minutes at 120 °C – 130 °C. Two more samples undergone an oxidative treatment, using 15% peracetic acid (PAA) solution (Brentagg) at two different concentrations. The used PAA solution is a mixture of 15 % peracetic acid, 10 % hydrogen peroxide, 36 % acetic acid and 39 % water. The low concentration treatment was prepared by adding 40 g PAA/kg dry matter to 2 g ground bamboo. The high concentration was made by adding 100 g PAA/kg dry matter to 2 g ground bamboo (Appels et al., 2011). It was diluted with distilled water until all of the bamboo was submerged. Both pretreatments were carried out on a stirred plate at room temperature (20 °C), for one hour. A last sample was left untreated to serve as control.

After completion of the pretreatment reactions, a Büchner funnel was used to filter off the bamboo. Each pretreatment sample was filtered and the filtrate was used to determine the Chemical Oxygen Demand (COD) of the liquid fraction as a measure of the organic matter solubilization, hence representing the effectiveness of the pretreatment. The effects of the pretreatment on the remaining solid fraction were characterized by a Van Soest fractionation, using the Fiber Bag system of Gerhardt. This method enables to measure the lignin, hemicelluloses, cellulose and the water soluble fraction in the material.

The experiments should be considered to be a screening of different pretreatment techniques, and the experiments have not been duplicated. Therefore, the observed trends should be taken into account, rather than the absolute values of the obtained experimental results. Based on the identified trends, further research will be carried out to further elaborate the most promising techniques.

Results

Activated carbon

Possible useful applications for the char, created during the pyrolysis, is as activated carbon. Two different methods were used in an attempt to activate the char: one by heating a mixture of water and char in a glass tube, the other by steam activation in an autoclave. The steam activation method allowed more accurate determination of the duration of contact, because the filtering process was faster in this case and takes a similar amount of time for each test. The results are presented as the amount of TOC removed from the water/acetone mixture, after contact with the activated carbon (see Tables 1 and 2).

Table 1. Rate of carbon elimination caused by activated carbon (heating in a glass tube)

Time (min)	Carbon elimination ± 0,1 (ppm)
5.17	618.6
5.25	628.8
4.67	597.0
6.48	605.2
6.48	601.5
8.50	600.0
7.48	610.6
7.50	624.8
10.42	618.7
13.67	638.4
15.67	649.5
22.32	622.9

Table 2. Rate of carbon elimination caused by activated carbon (steam activation)

Time (min)	Carbon elimination ± 0,1 (ppm)
0.50	494.4
1.00	451.7
1.50	510.0
2.00	496.9
3.00	432.7
4.00	407.5
5.00	412.4
8.00	456.9
11.50	526.0
15.00	440.2

Statistical tests show that the data are normally distributed. Two straight lines were determined for each method of activation. The p-value is higher than 0.05. This means time is not a significant factor, after a contact of at least half a minute. Hence, the average value can be used and calculated for each method.

$$\text{carbon elimination (heating in a glasstube)} = (618 \pm 5)\text{ppm}$$

$$\text{carbon elimination (steam activation)} = (460 \pm 10)\text{ppm}$$

A comparison of the two methods shows a significant difference (see Figure 1). Both methods appear to result in the formation of activated carbon. The carbon elimination is $(86.5 \pm 0.01)\%$ and $(64.4 \pm 0.01)\%$ with respect to the initial concentration for, respectively, heating in a glass tube and steam activation. The results show that the carbon residue that underwent heating in a glass tube is more activated than the carbon activated by steam in an autoclave.

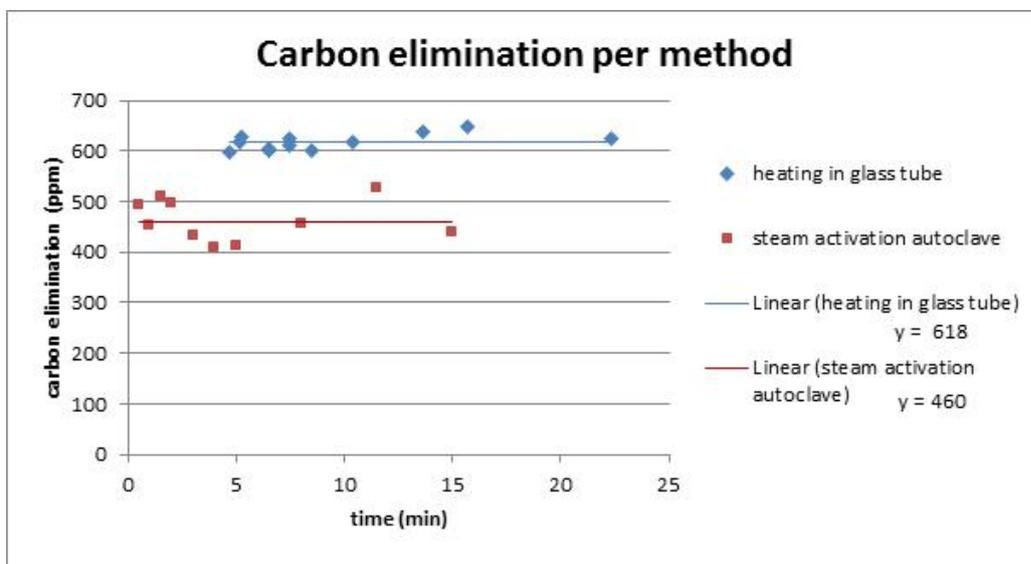


Figure 1.

Carbon elimination as a function of time (activation + filtration) for the two different methods. Error bars are not shown.

Next, results were compared to those gathered from another study conducted by Jiun-Horng et al. (2008). In this study the authors compared the adsorption capacity of 4 different types of activated carbon in relation to the temperature. They tested 4 different concentrations of acetone. These four types were a commercially available activated carbon, two activated carbon fibers and a sludge adsorbent. Considering bamboo has fibers, the present results were compared to those gathered from the two types of activated carbon fibers. The results were first converted into adsorption capacities (Table 3) (Jiun-Horng et al. 2008).

Table 3. Adsorption capacity per method

Method	Adsorption capacity
Heating in a glass tube	$43 \pm 2 \text{ mg/g}$
Steam activation	$32 \pm 2 \text{ mg/g}$

The results were also plotted side to side with those of the study by Jiun-Horng et al. (2008) for different concentrations of acetone (Figure 2). The initial concentration of acetone in our test was 714.3 ppm. The experiments described here were conducted at room temperature, $(22.0 \pm 0.5) \text{ }^\circ\text{C}$, whereas the experiments of Jiun-Horng et al. (2008) were performed at $30 \text{ }^\circ\text{C}$. Their research demonstrated an inverse correlation between adsorption capacity and temperature.

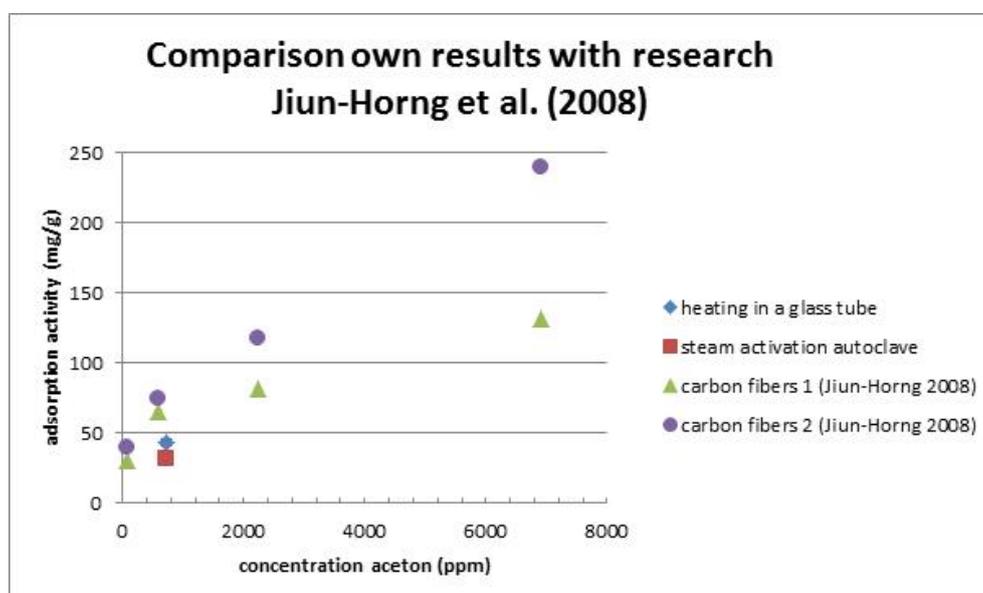


Figure 2. Results from the research of Jiun-Horng et al. (2008) with present study results for comparison. Error bars are not shown.

The carbon was respectively 40% and 55% less activated by the method of heating in a glass tube and by steam activation compared with the results of Jiun-Horng et al. (2008).

Activated carbon originating from bamboo char clearly shows a smaller adsorption activity, but still it achieves a relatively good elimination of carbon. This makes the use of activated carbon from bamboo a viable option if the purification demands are not too high. When a high level of purification is needed, other types of activated carbon are required or a large quantity of bamboo activated carbon needs to be used. Also the char fraction can be used as a fuel in the heating for the pyrolysis process (Bridgewater et al. 1999; BTG). It remains to be determined whether it is more useful to activate the char, or use it in the pyrolysis process as a fuel.

For the activation of char obtained from the pyrolysis of bamboo, the method of heating in a glass tube should be used whenever the goal is to achieve the highest possible adsorption capacity.

Pretreatments for digestion

By applying different methods of pretreatment, the fractions of the different compounds of the cell wall remaining in the solids component can be changed. The following pretreatments were tested:

abbreviation

1. Acid treatment at 20°C a20
2. Acid treatment at 80°C a80
3. Steam treatment St
4. Oxidative treatment low concentration oxLow
5. Oxidative treatment high concentration oxHigh
6. No treatment: control control

By using the Van-Soest-method the following fractions are determined in succession: soluble, hemicellulose, cellulose and lignin.

Table 4. Weighing of each step after using the Van-Soest-method.

Pretreatment	start (g)	step 1 (g)	step 2 (g)	step 3 (g)	step 4 (g)
1	1.72	1.58	1.11	1.17	0
2	1.64	1.52	1.09	0.48	0.01
3	1.79	1.77	1.23	1.58	0.02
4	1.62	1.5	1.05	0.86	0.01
5	1.62	1.46	1.19	0.46	0
6	1.6	1.35	0.95	0.88	0.03

For a few of these results, the masses are larger than in the proceeding step; this is most likely due to an incomplete evaporation of liquid during the drying process. These values are equalized to zero in the following calculations.

$$fraction\ 1 = \frac{m_{begin} - m_{step1}}{m_{start}} = 1 - \frac{m_{step1}}{m_{start}} \quad (Eq. 1)$$

$$fraction\ 2 = \frac{m_{step1} - m_{step2}}{m_{start}} \quad (Eq. 2)$$

The same was applied to fractions 3,4 and 5.

Table 5. Bamboo composition after pre-treatment. Values indicate the percentage of each cell wall component that remains in the solid fraction after treatment, ± technical error.

Pretreatment	water-soluble	Hemi-cellulose	cellulose	lignin	ash
Acid 20 °C	8.1±0.8%	27.3±0.8%	0.0±0.8%	64.5±0.9%	0.0±0.8%
Acid 80 °C	7.3±0.8%	26.2±0.9%	37.2±0.9%	28.7±0.9%	0.6±0.9%
St	1.1±0.8%	30.2±0.9%	0.0±0.9%	67.6±0.9%	1.1±0.9%
Ox. low conc.	7.4±0.8%	27.8±0.9%	11.7±0.9%	52.5±0.9%	0.6±0.9%
Ox. high conc.	9.9±0.8%	16.7±0.9%	45.1±0.9%	28.4±0.9%	0.0±0.9%
Control	15.6±0.8%	25.0±0.9%	4.4±0.9%	53.1±0.9%	1.9±0.9%

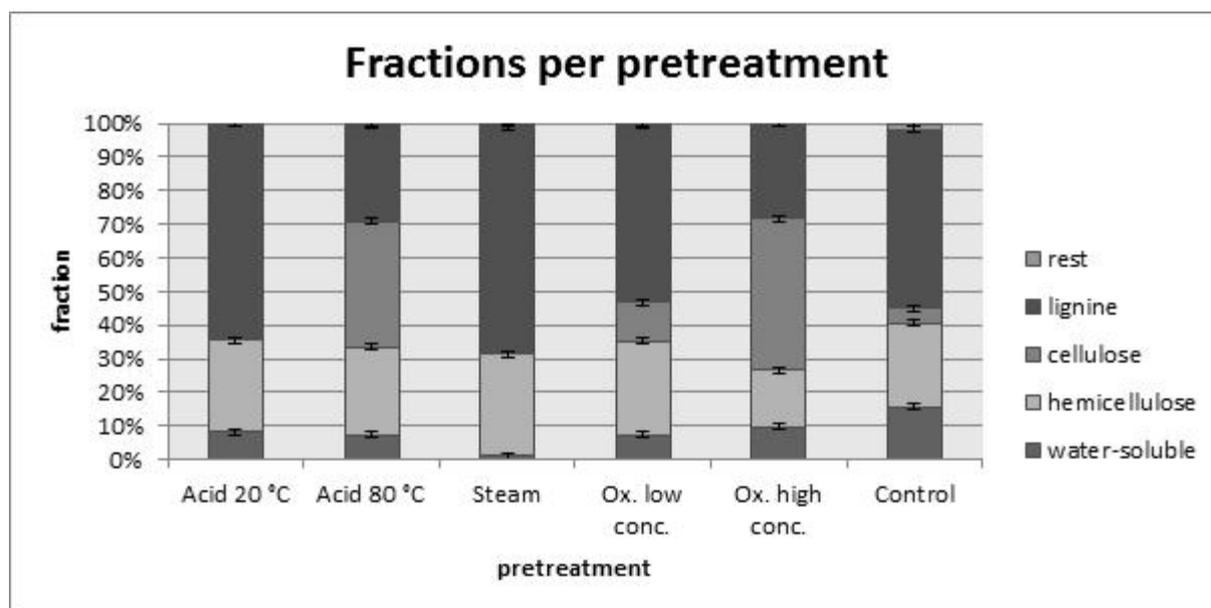


Figure 3. Distribution of the different fractions after applying the Van-Soest-method for each pretreatment.

The first fraction consists of all the water soluble components and the percentages are almost equal for pretreatment a20, a80, St and oxHigh. Steam pretreatment induces a small first fraction while bamboo from the control sample has a larger first fraction. For all treatments, the fractions of hemicellulose are comparable, except for the oxidative pretreatment with a high concentration of PAA. Pretreatments a20 and St have a negative effect on the amount of cellulose in comparison with the control sample. The control sample itself has a small cellulose fraction, which is strange, given the fact that several published amounts for bamboo point at 40% of cellulose in bamboo. We suspect a technical error during the fractionation step, leading to inaccurate estimates of the absolute values for the fraction yield. Also, given the fact that only one measurement was performed, the outcome of this procedure needs to be treated with care.

A large increase of the cellulose fraction is observed when bamboo is treated in acidic conditions at 80 °C or with oxidative agents. The lignin fractions decrease under these conditions. The control sample and the oxidative pretreatment aka low PAA concentration consist of almost twice as much lignin than the samples mentioned above. The last two treatments, a20 and St yield an even bigger lignin fraction. In other words, a smaller cellulose fraction corresponds with a larger lignin fraction. The residual fraction, consisting of inorganic residues, is almost negligible for all pretreatments. Statistical analysis of these results was not undertaken because these are single determinations. As a consequence the value of these results is merely indicative. (Van Soest and Mcqueen 1973; Figure 3; Table5).

The final goal of the digestion of bamboo is to achieve a maximum amount of biogas. This biogas should also be produced as efficiently as possible. There are numerous factors to measure the efficiency: biodegradability (BD), Chemical Oxygen Demand (COD) and available cellulose after the pretreatment are some of the determining factors (Buffiere et al. 2006; Kumar et al. 2009).

The different applied methods of pretreatment have the common aim to release cellulose from the plant fibres so that micro organisms have better access to it (Kumar et al. 2009). As all the other fractions are broken down, the cellulose fraction increases. It is preferable to have a large cellulose fraction in order to conduct a successful pretreatment and later on a successful digestion. The acid pretreatment at 80 °C causes the cellulose fraction to increase 8 times in comparison with the control sample. The strong oxidative pretreatment even causes an increase of 10 times the original fraction of cellulose.

Komilis and Ham (2003) have proven the positive effect of the water soluble fraction and the negative effect of a large lignin fraction on the organic degradability. In our experiment, the acidic pretreatment at 80 °C and the strong oxidative pretreatment give the best results regarding the small lignin fractions. The water soluble compounds fractions are too small in all of the pretreatments to have a positive effect, especially in the steam treatment where it is the lowest (Komilis en Ham 2003; Figure 3; Table 5).

Buffiere et al. (2006) investigated if there is a link between the lignocellulosic fraction and the biodegradability. Biodegradability is defined as the ratio of the achieved methane production and the maximum possible methane production. In this research an indication of a negative link was found between the lignin proportion and the biodegradability. When this theory is reflected on the experiment, the acidic treatment at 80°C and the oxidative treatment are again among the better ones. The significance in the experiments of Buffiere (2006) being low, the results should rather be interpreted as indicative.

Aside from the determination of the different fractions of the solid phase, the COD of the liquid phase was also measured.

Table6. COD values of the liquid fraction for the different pretreatments

Pretreatment	COD (mgO ₂ /L)
1	352
2	614
3	ND
4	230
5	582
6	10

Biodegradability is the amount of methane released during digestion over the maximum possible amount of methane, the last one being the amount released if all of the dissolvable material were converted into methane. The biodegradability always has a value between 0 and 1. In the research of Buffiere (2006) the biodegradability was calculated using the following formula:

$$BD = \frac{\text{biomethane production (CH}_4\text{/gsolidmatter)}}{350 \cdot \text{COD (gCOD/gsolidmatter)}} \quad (\text{Eq. 3})$$

The more biogas produced, the better the digestion. Both a higher biodegradability and a higher COD value result in an increased biogas production.

Each treatment causes an increase of the COD value (Eq. 3). Of the different tested pretreatments, the oxidative treatment at low concentration has the lowest COD, followed by the acidic treatment at 20 °C. The acidic pretreatment at 80 °C and the oxidative pretreatment at high concentration result in the highest COD values. Probably these last two treatments will result in the highest biogas yield.

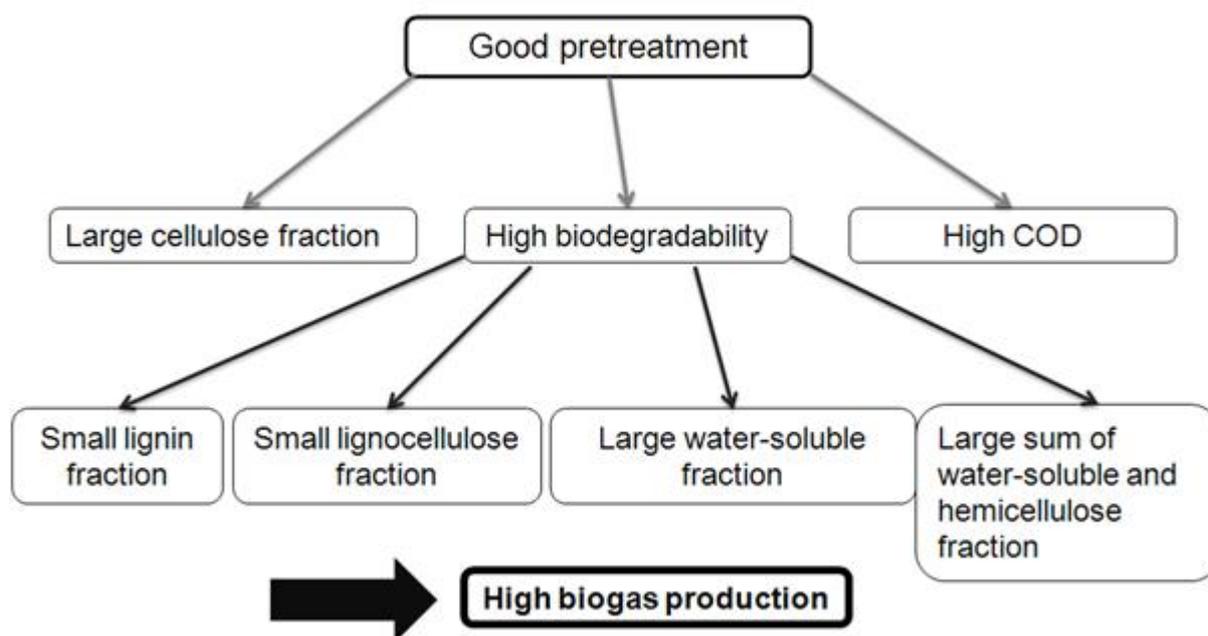


Figure 4. Diagram used for assessing the pretreatment.

Conclusions

There is a significant difference between the two methods used to activate the carbon acquired from the pyrolysis of bamboo. Char, when heated in a glass tube, has an adsorption capacity of 43 milligram carbon per gram activated carbon, while the adsorption capacity of the steam activated char is 32 milligram carbon per gram activated carbon. In comparison to other studies, bamboo char performs less well than some other substrates. The results for bamboo char are 40 % lower in adsorption capacity for heating in a glass tube and a 55 % lower for steam activation in an autoclave compared to activated carbon from carbon fibers as observed by Jiun-Horng et al. (2008). Nevertheless, the activated carbon from bamboo are likely to be useful when the demands for purification are relatively modest.

Five methods of pretreatment prior to anaerobic digestion for biogas production were discussed in this work. Taking the results into account, an indicative order in terms of biodegradability can be established (biodegradability being defined here as the amount of biogas produced over the maximum possible biogas production). The steam treatment does not yield any good results in any of the tests and can therefore be considered as the worst method. The acidic treatment (pH 3) at 20 °C did not yield any better results: both the fractionation and COD value were poor, which means that this is not a recommended method either. The oxidative treatment at a low PAA concentration (40 g PAA/kg DW) yields better results in comparison with the control sample, but the differences in terms of biodegradability are small. In two samples a reasonable increase in biodegradability was observed: the acidic treatment (pH 3) at 80 °C and the treatment at high PAA concentration (100 g PAA/kg DW). These two treatments almost always give the best results on the experimented parameters and they will probably have a positive influence on the biodegradability and the biogas production in comparison with the control sample. After the acidic treatment at 80 °C a cellulose fraction of 37.2 % and a COD value of 614 mg O₂/L were determined. The strong oxidative treatment resulted in a cellulose fraction of 45.1 % and a COD value of 582 mg O₂/L.

A choice between the acidic pretreatment at 80 °C and the oxidative treatment with high PAA concentration, can be based on the reaction conditions and the chemicals and energy needed (to

achieve these conditions). The use of a PAA mix limits the formation of toxic by-products that may have an inhibitory effect on biogas production. Furthermore, the use of PAA as an oxidative medium results in water and acetic acid, the latter having a positive influence on the digestion as a nutrition source for microorganisms. Moreover, the oxidative treatment can be executed at room temperature whereas an extra energy cost is needed for the acidic treatment at 80 °C. For these reasons the choice of the strong oxidative pretreatment for biogas production through digestion seems preferred. This conclusion, though, can only be evidenced after the execution of the actual anaerobic digestion and determination of biogas yields.

References

- Abdel-Nasser, A.; Samra, S.E. , Girgis, B.S. 2001. Adsorption characteristics of activated carbons obtained from corncobs. *Colloids and surfaces A: Physicochemical and Engineering Aspects*, 180 (3), 209-221.
- Appels, L.; Van Assche, A.; Willems, K.; Degève, J.; van Impe, J.; Dewil, R. 2010. Peracetic acid oxidation as an alternative pre-treatment for the anaerobic digestion of waste water activated sludge. *Bioresource technology*, 102, 4124-4130.
- Bobleter, O. 1994. Hydrothermal degradation of polymers derived from plants. *Prog. Polym. Sci.*, 19, 797-841.
- Bridgewater, A.V. 1999, Meier D., Radlein D. An overview of fast pyrolysis of biomass. *Organic Geochemistry* 30, 1479-1493.
- Bridgewater, A.V. 2003. Renewable fuels and chemicals by thermal processing of biomass. *Chem Eng J*, 91, 87-102.
- Bridgewater, A.V. 2004. Biomass Fast Pyrolysis, *Thermal Science*, 8, 21-49.
- BTG – Biomass Technology Group. www.btgworld.com, last consulted on 12 October 2011
- Buffiere, P.; Loisel, D.; Bernet, N.; Delgenes, J.P. 2006. Towards new indicators for the prediction of solid waste anaerobic digestion properties. *Water Science & Technology*, 53 (8), 233-241.
- Duff, S.J.B.; Murray, W.D. 1996. Bioconversion of forest products industry waste cellulose to fuel ethanol: A review. *Bioresource Technology*, 55, 1-33.
- Ensöz, S.; Angın, D.; Yorgun, S. 2000. Influence of particle size on the pyrolysis of rapeseed (*Brassica napus* L.): fuel properties of bio-oil. *Biomass and Bioenergy*, 19, 271-279.
- Fengel, D.; Wegener, G. 1984. *Wood: Chemistry, Ultrastructure, Reactions*. De Gruyter, Berlin.
- Fox, M.H.; Noike, T.; Ohki, T. 2003. Alkaline subcritical-water treatment and alkaline heat treatment for the increase in biodegradability of newsprint waste. *Water Sci. Technol.*, 48 (4), 77-84.
- Garrote, G.; Dominguez, H.; Parajo, J.C. 1999. Hydrothermal processing of lignocellulosic materials. *Holz Roh Werkst.*, 57, 191-202.
- Gielis, J. 2000. Future possibilities for bamboo in European agriculture. Short report “Bamboo for Europe” Project, Oprins Plant, Rijkvorschel, Belgium.
- Gossett, J.M.; Stuckey, D.C.; Owen, W.F.; McCarty, P.L. 1982. Heat treatment and anaerobic digestion of refuse. *J. Environ. Eng. Div.*, 108, 437-454.
- Hendriks, A.T.W.M.; Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 100, 10-18.
- Hon, D.N.S.; Shiraishi, N. 2001 *Wood and Cellulosic Chemistry*. Second ed. Dekker, New York.
- Jiun-Horng, T.; Hsiu-Mei, C.; Guan-Yinag, H.; Hung-Lung, C. 2008. Adsorption characteristics of acetone, chloroform and acetonitrile on sludge-derived adsorbent, commercial granular activated carbon and activated carbon fibers. *Journal of Hazardous Materials*, 154, 1183-1191.
- Kobayashi, F.; Take, H.; Asada, C.; Nakamura, Y. 2004. Methane Production from Steam-Exploded Bamboo. *Journal of Bioscience and Bioengineering.*, 97 (6), 426-428.

- Komilis, D.P.; Ham R.K. 2003. The effect of lignin and sugars to the aerobic decomposition of solid wastes. *Wastes Management*, 23, 419-423.
- Kumar, P.; Barrett, D.M.; Delwiche, M.J.; Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research*, 48 (8), 3713-3729.
- Laser, M.; Schulman, D.; Allen, S.G.; Lichwa, J.; Antal, Jr M.J.; Lynd, L.R. 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. *Bioresource Technology*, 81 (3), 33-44.
- Lievens, C.; Carleer, R.; Cornelissen, T.; Yperman, J. 2009. Fast pyrolysis of heavy metal contaminated willow: Influence of the plant part. *Fuel*, 88, 1417-1425.
- Lievens, C.; Yperman, J.; Vangronsveld, J.; Varleer, R. 2008. Study of the potential valorisation of heavy metal contaminated biomass via hytoremediation by fast pyrolysis: Part I. Influence of temperature, biomass species and solid heat carrier on the behaviour of heavy metals. *Fuel*, 87, 1894-1905.
- Monties, B. 1984. Determination of acid-insoluble lignin – effect of pretreatment by acid hydrolysis on the klason lignin in wood and straw. *Agronomie* 4, 387-392
- Noike, T., Niigata Engineering. 2001. Micromolecularization of undegradable organic substances in methanedigestion. Development of the Waste Treatment System for Recycling Society. Waste Research Foundation, Tokyo.
- Onay, O.; Koçkar, O.M. 2004. Fixed-bed pyrolysis of rapeseed (*Brassica napus* L.). *Biomass and bioenergy*, 26, 289-299.
- PyNe – Pyrolysis Network of IEA Bioenergy. 2006. www.pyne.co.uk, last consulted on 15 may 2011.
- Ramos, L.P. 2003. The chemistry involved in the steam treatment of lignocellulosic materials. *Quim. Nova*, 26 (6), 863–871.
- Scurlock, J.; Dayton, D.; Hames, B. 2000. Bamboo: an overlooked biomass resource? *Biomass and Bioenergy*, 19, 229-244.
- Shanmughavel, P.; Francis, K. 2001. *Physiology of Bamboo*. Scientific Publishers, Jodhpur. 154 pp.
- Temmerman, M.; Van Belle J.; Delcarte J.; Gielis J.; Brias V. 2005. Bamboo thematic network: Bamboo as a source of bioenergy. Technical paper, CRAW Walloon Agricultural Research Center & Oprins Plant NV.
- Toles, C.A.; Marshall, W.E.; Johns, M.M. 1997. Granular activated carbons from nutshells for the uptake of metals and organic compounds. *Carbon*, 35, 1407-1414.
- Van de Velden, M.; Baeyens, J.; Brems, A.; Janssens, B.; Dewil, R. 2010. Fundamentals, kinetics and endothermicity of the biomass pyrolysis reaction. *Renewable Energy*, 35, 232-242.
- Van Soest, P.J.; Mcqueen R.W. 1973. Symposium of 'fibre in human nutrition'. The chemistry and estimation of fibre. *Proceedings of the nutrition society*, 32, 123-130.
- Wright, J.D. 1998. Ethanol from biomass by enzymatic hydrolysis. *Chemical Engineering Progress*. 84, 62-74.

Evaluation of *in vitro* antiproliferative activity of bamboo leaf extracts

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Abstract

Several bamboo species have been used in traditional medicine for the treatment of various diseases, including cancer. The present study evaluates the *in vitro* antiproliferative properties of 12 heterogeneous bamboo species, both traditional used species as species without medicinal uses.

The extracts were evaluated for their growth inhibitory activity on human breast (MCF-7/AZ & MCF-7/6 cells) and human colon (HCT-8E11 & HCT-8E11R1) cancer cells. HPLC-DAD and LC-MS/MS analyses were performed for the phytochemical characterization of the extracts. *Fargesia robusta* 'Pingwu', *Fargesia rufa* 'Green Panda' and *Pseudosasa japonica* caused a growth inhibition of all cell lines. *Bambusa balcooa* showed selectivity towards breast cancer cells.

A combined action of different phytochemicals, including the flavones tricetin and luteolin-6-C- β -boivinopyranoside, seems to be responsible for the observed activity.

Keywords

bioactivity, antiproliferative, HPLC-DAD, LC-MS/MS, flavones

Abbreviations

DAD	diode array detection
DMEM	dulbecco's modified eagles medium
HPLC	high performance liquid chromatography
IC ₅₀	concentration which results in an inhibition percentage of 50%
LC	liquid chromatography
MS	mass spectrometry
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide
NMR	nuclear magnetic resonance
QTOF	quadrupole-time of flight
SPE	solid phase extraction
SRB	sulforhodamine B
UV	ultraviolet

Introduction

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008. Deaths from cancer worldwide are projected to continue to rise to over 11 million in 2030 (WHO 2011).

Interest in chemoprevention as an alternative approach to the control of cancer has gained increased attention due to the fact that deaths from the common epithelial cancers, including pancreas, ovary, colon, lung, breast and prostate, are still rising and the lack of a 'magic' cure (Sporn and Suh 2000; Sporn and Suh 2002). Chemoprevention is defined as a pharmacological approach used to arrest or reverse the process of cancer development before invasion and metastasis occur (Sporn 1991).

A large number of epidemiological studies have shown a protective effect of vegetables and fruits against cancer (AICR 1997). As demonstrated by a series of *in vitro* and *in vivo* studies, naturally occurring plant compounds, particularly those present in dietary and medicinal plants, may inhibit various stages in the cancer process (Wattenberg 1992; Surh 1999; Bode and Dong 2004; Galati and O'Brien 2004; Kang et al. 2011).

Limited information exists on the traditional use of bamboo as anticancer therapeutic. *Sasa spiculosa*, *Bambusa arundinaceae*, *Bambusa spinosa* and *Bambusa vulgaris* are bamboo species with reported traditional use as anticancer agent (Duke 1994). In addition, several studies have been recently published demonstrating anticancer properties of several *Sasa* and *Phyllostachys* species (Ando et al. 2004; Ren et al. 2004; Kim et al. 2007; Lin et al. 2008).

Our study was conducted to obtain a more general insight into the potential antiproliferative activities of bamboo species and to compare traditionally used species with species without medicinal application. Twelve morphologically heterogeneous bamboo species were evaluated for their growth inhibitory potential on human breast and colon cancer cells. In addition, bioactivity guided fractionation was applied for the characterization of bioactive compounds.

Two types of human cancer cell lines were used. The first are human mammary carcinoma cells, namely MCF-7/AZ and MCF-7/6. MCF-7/AZ cells differ from MCF-7/6 cells in that they are not invasive *in vitro* when tested in the precultured chick heart invasion assay (Bracke et al. 1991) or the matrigel chemoinvasion assay (Simon et al. 1992). HCT-8E11 and HCT-8E11R1, the second couple of invasive/non-invasive human cancer cells, are subcloned from the HCT-8 human colon carcinoma cell line. HCT-8/E11R1 cells are α -catenin-deficient and are invasive in precultured chick heart fragments. HCT-8E11 cells have a epitheloid morphology with dome formation, while the invasive HCT-8E11R1 have a round morphology (Vermeulen et al. 1995).

For the evaluation of *in vitro* growth inhibition, two assays were used, namely the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and the sulforhodamine B (SRB) assay. Both assays have been widely used to evaluate anticancer effects of extracts and compounds on different types of cancer cells (Liu et al. 1997; Papazisis et al. 1997; Houghton et al. 2007).

Materials and Methods

Plant Material

A voucher specimen of each species was deposited in the Herbarium of the Ghent University Botanical Garden, Faculty of Sciences, Ghent University. Each species was provided with an accession number: 2009-1565 G (*Fargesia robusta* 'Pingwu'), 2009-1552 G (*Phyllostachys nigra*), 2009-1557 G (*Sasa veitchii*), 2009-1551 G (*Dendrocalamus hamiltonii*), 2009-1547 G (*Dinochloa scandens*), 2009-1549 G (*Guadua amplexifolia*), 2009-1556 G (*Phyllostachys humilis*), 2009-1563 G (*Pleioblastus variegatus*), 2009-1555 G (*Pseudosasa japonica*), 2009-1564 G (*Fargesia rufa* 'Green Panda'), 2010-2642 G (*Arundinaria gigantea*), and 2010-2641 G (*Bambusa balcooa*). The bamboos

were *in vitro* propagated and kindly provided by Oprins Plant NV. In table 1, the twelve species are depicted with the corresponding harvest times of the leaves.

Table 1. Selected bamboo species with their collection times.

Species	Origin	Collection time
<i>Arundinaria gigantea</i>	North-America	May 2010
<i>Bambusa balcooa</i>	India	April 2009
<i>Dendrocalamus hamiltonii</i>	India	November 2009
<i>Dinochloa scandens</i>	Java	May 2010
<i>Fargesia robusta</i> 'Pingwu'	China	October 2009
<i>Fargesia rufa</i> 'Green Panda'	China	September 2009
<i>Guadua amplexifolia</i>	Central-America	April 2010
<i>Phyllostachys humilis</i>	China	September 2009
<i>Phyllostachys nigra</i>	China	November 2008
<i>Pleioblastus variegatus</i>	Japan	September 2009
<i>Pseudosasa japonica</i>	Japan	October 2009
<i>Sasa veitchii</i>	Japan	November 2008

Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sulforhodamine sodium salt (SRB), glacial acetic acid, trichloroacetic acid (TCA) 6.1 N, trizma base, vinblastine sulfate salt, and CD₃OD were purchased from Sigma-Aldrich (Bornem, Belgium). Tricin was previously isolated from bamboo leaves. HPLC solvents were purchased from Biosolve (Valkenswaard, The Netherlands).

Extraction of bamboo leaves

Fresh leaves were harvested and freeze dried. The milled leaves were successively extracted three times with MeOH-H₂O (1/1, v/v) at 40°C in a sonication bath (Bandelin sonorex) for 30 min. After removing the solvent under reduced pressure, the extracts were freeze dried.

HPLC analysis

The chromatographic profiles of the extracts and fractions were determined by HPLC with DAD-detection consisting of a Waters 2695 Alliance separations module and 996 photodiode array detector (Waters, Milford, MA) in combination with a phenomenex Luna 5µ C18 column (i.d.: 4.6 mm, length: 250 mm). The UV detection range was set from 200 to 800 nm. The mobile phase consisted of 0.025% HCOOH in H₂O (solvent A), 0.025% HCOOH in CH₃CN (solvent B) and 0.025% HCOOH in MeOH (solvent D). The elution program was: 0-3 min – isocratic at 85% A (7.5% B and D); 3-8 min. – linear gradient from 85 to 76% A; 8-11 min. – isocratic at 76% A; 11-18 min – linear gradient from 76 to 66% A; 18-28 min. – linear gradient from 66 to 56% A; 28-36 min. - linear gradient from 56 to 19% A; 36-42 min. from 19 to 5% A; 42-50 min. – isocratic at 5% A; 50-57 min. – linear gradient from 5 to 85% A; 57-60 min. – isocratic at 85% A. The flow rate was 0.7 mL/min., injection volume 10 µL and the column oven temperature was set at 35°C.

LC-MS/MS analysis

LC-MS/MS analyses of the fractions were performed using a Waters 2695 Alliance separations module coupled to a Q-TOF micro (Waters, Milford, MA) mass spectrometer. Chromatographic separations were obtained using the same column, same elution program and the same mobile phase as for HPLC-DAD analysis but with 0.1% HCOOH in H₂O (solvent A) and 0.1% HCOOH in MeOH/CH₃CN (1/1) (solvent C) as mobile phases. The flow rate was 0.7 mL/min with a split ratio of ¼ and the injection volume was 10 µL. MS data were acquired in the positive and in the negative

mode. A cone voltage of 25 V and a capillary voltage of 2.5 kV were used in both positive and negative mode. The desolvation temperature was set to 300°C and the source temperature to 120°C. MS and MS/MS spectra were acquired over a m/z range of 50-950. The MCP detector potential was set to 2200 V both for positive and negative mode.

Bioactivity guided fractionation

Dried crude extract was dissolved in H₂O-MeOH (9/1, v/v) and applied to an activated and conditioned SPE column (5000 mg/20 ml, GracePure™, Alltech Associates, Lokeren, Belgium). Four fractions were eluted with 100% H₂O (20 ml), 30% MeOH (30 ml), 60% MeOH in H₂O (30 ml) and 100% MeOH (40 ml). The eluents were acidified with 0.025% HCOOH. Each fraction was dried under nitrogen for further use in the assays.

Isolation and identification of antiproliferative compounds

Tricin (**1**) was previously isolated and identified in our laboratory.

The 100% MeOH SPE fraction (120 mg) of *F. robusta* 'Pingwu' was subjected to two consecutive cycles of semi-preparative HPLC, by subsequent elution with 60% MeOH in H₂O and 30% MeCN in H₂O, respectively. This resulted in the isolation of luteolin-6-*C*- β -boivinopyranoside (**2**) and a third compound with a similar structure (**3**).

Luteolin-6-*C*- β -boivinopyranoside (**2**): t_R : 35.7 min.; UV λ_{max} (MeOH-H₂O): 257 (sh), 271 and 346 nm; ¹H and ¹³C NMR, Table 5; positive ESIMS, m/z (rel int): 417.2296 [M+H]⁺ (20), 381 [M + H-36]⁺ (100).

Compound **3**: t_R : 36.5 min.; UV λ_{max} (MeOH-H₂O): 257 (sh), 271, 343 nm; ¹H NMR, table 5; positive ESIMS, m/z (rel int): 417.1857 [M+H]⁺ (100), 399 [M+H-18] (60).

Cell culture

Human breast adenocarcinoma MCF-7/6 and MCF-7/AZ cell lines were kindly provided by the laboratory of Experimental Cancer Research (Ghent University Hospital, Belgium). MCF-7/6 cells were cultured in DMEM/F12 (ham) (1/1) (Invitrogen, Merelbeke, Belgium) containing L-glutamine and supplemented with 10% FBS (Greiner Bio-One, Wommel, Belgium) and 50 IU/mL penicillin and 50 μ g/mL streptomycin (Invitrogen, Merelbeke, Belgium). MCF-7/AZ cells were cultured in DMEM (Invitrogen, Merelbeke, Belgium) containing L-glutamine and supplemented with 10% FBS (Greiner Bio-One, Wommel, Belgium) and 50 IU/mL penicillin and 50 μ g/mL streptomycin (Invitrogen, Merelbeke, Belgium). Cell cultures were maintained at 37°C in a 5% CO₂ atmosphere with 95% humidity.

Human colon carcinoma HCT-8 E11 and HCT-8 E11R1 cell lines were cultured in RPMI 1640 (Invitrogen, Merelbeke, Belgium) supplemented with 10% FBS (Greiner Bio-One, Wommel, Belgium), 1 mM sodium pyruvate (Invitrogen, Merelbeke, Belgium) and 50 IU/mL penicillin and 50 μ g/mL streptomycin (Invitrogen, Merelbeke, Belgium). Cell cultures were maintained at 37°C in a 5% CO₂ atmosphere with 95% humidity.

Subconfluent cells (80%) were passaged with a solution containing 1% trypsin and 0.02% EDTA.

Antiproliferative assays

MTT-assay

The *in vitro* growth inhibitory activity of bamboo extracts was assessed using the 3-(4,5-dimethyl 1-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye. Cells (5000/well) were seeded into a transparent flat-bottomed 96 well plate (Nunc A/S, Roskilde, Denmark) (100 μ L/well) and allowed to attach. After 48 h, the extracts were added to the wells. Extracts were initially dissolved in DMSO or

H₂O (100 mg/mL). The extracts were further diluted with medium to produce a concentration range of 2.5, 25, 50, 250 and 500 µg/mL. 100 µL/well of each concentration was added to the wells in three replicates. The final dilution used for treating the cells contained no more than 0.5% of the initial solvent, this concentration being used in the solvent control wells. Vinblastine sulfate (20 nM) was used as the positive control. A blank, containing medium without cells was also measured within each assay. After 48 h of incubation with the extracts, medium was replaced with 100 µL of MTT solution (5mg/mL in PBS) and incubated for 2 h at 37°C in a 5% CO₂ atmosphere with 95% humidity. After removing the remaining solution, the formazan crystals were dissolved in 200 µL DMSO and the absorbance of the solution was read at 570 nm and 650 nm (reference wavelength) using an ELISA plate reader (Safire2™, Tecan, Männedorf, Switzerland). The absorbance is directly proportional to the number of metabolic active cells (Mosmann 1983). The results were expressed as a percentage of metabolic activity compared to non-treated cells (negative control).

SRB- assay

The SRB assay was the second test performed to assess growth inhibition. Briefly, cells (5000/well) were seeded into a transparent flat-bottomed 96 well plate (100 µL/well). After 48 h, the extracts were added to the wells. The same concentrations of extract as in the MTT assay were used. Vinblastine sulfate (20 nM) was used as positive control. After 48 h of incubation, medium was removed and the cells were fixed with 50 µL 50% TCA. Cells were incubated at 4°C for 1 h, after which plates were washed five times with distilled water and dried in an oven (37°C). 200 µL of SRB solution (0.4 % in 1% glacial acetic acid) was then added and left in contact with the cells for 30 min.. After staining, the plates were washed 4 times with glacial acetic acid and dried in an oven (37°C). 200 µL Tris base (10mM) was added to the dried plates to solubilize the dye. The absorbance of each well was read on an ELISA plate reader (Safire2™, Tecan, Männedorf, Switzerland) at 570 nm and 650 nm (reference wavelength). A wavelength of 490 nm instead of 570 nm was used at high cell density. Cell protein content was measured as the percentage absorbance compared to the negative control.

Results

The leaf extracts of the 12 selected bamboo species were evaluated for their antiproliferative activity using the MTT and SRB assays. Of these, *F. robusta* 'Pingwu', *F. rufa* 'Green Panda', *B. balcooa* and *P. japonica* showed significant growth inhibitory activity. The other species showed no significant inhibition on cell growth or survival.

F. robusta 'Pingwu'

Crude extract

F. robusta 'Pingwu' leaf extract showed statistically significant growth inhibition starting from a concentration of 250 µg/mL (Figure 1). The IC₅₀ values of *F. robusta* 'Pingwu' extract towards the 4 cell lines are summarized in table 2, with indication of cell number in the negative control wells at the end of the experiment.

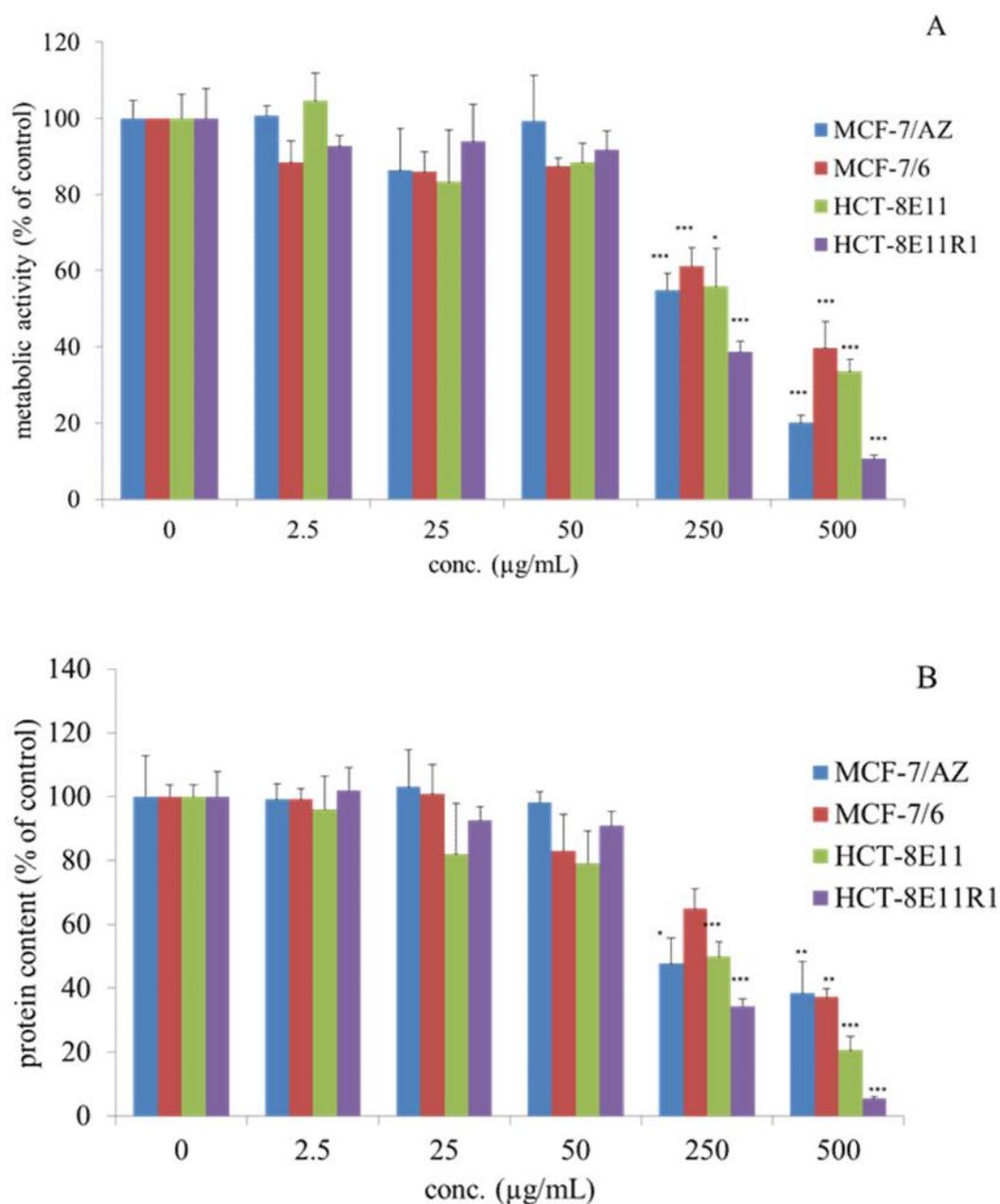


Figure 1. Metabolic activity (A) and protein content (B) of cancer cells after exposure (48 h) to *F. robusta* 'Pingwu' leaf extract. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 2. IC_{50} values (µg/mL) of *F. robusta* 'Pingwu' extract

	MTT	SRB	cell number
MCF-7/AZ	305 ± 11	334 ± 10	14376
MCF-7/6	314 ± 9	318 ± 25	20497
HCT-8E11	348 ± 2	222 ± 14	36124
HCT-8E11R1	169 ± 17	133 ± 4	67774

SPE fractions

The crude extract of *F. robusta* 'Pingwu' (126 mg) was divided into 4 fractions: a 100% H₂O (58%), 30% MeOH (9%), 60% MeOH in H₂O (27%) and a 100% MeOH fraction (6%). Of the 4 fractions, the 30% MeOH and 60% MeOH fractions exerted minor activity, while the 100% MeOH fraction (Figure 2) showed more potent antiproliferative activity. HPLC-DAD and LC-MS/MS analysis of the 100% MeOH fraction revealed the presence of flavones.

Isolated compounds

Tricin (compound **1**) (Figure 3) was identified as one of the constituents of the 100% MeOH fraction. Tricin was evaluated for its antiproliferative activity on the four cell lines. From table 3, it is clear that triclin exert antiproliferative activity towards the 4 cell lines. However, the growth inhibitory effect of *F. robusta* 'Pingwu' could not be explained only by the action of triclin. From its concentration in the crude extract (6.6 μ M in 500 μ g/mL) and its activity profile on the four cell lines, we can calculate that triclin is responsible for 4-24 % of the observed activity of the total extract at 500 μ g/mL.

Table 3. IC₅₀ values (μ M) of triclin.

cell line	MTT	SRB	cell number
MCF-7/AZ	92.5 \pm 8.9	86.8 \pm 5.4	37545
MCF-7/6	117.5 \pm 11.3	90.2 \pm 8.1	56846
HCT-8E11	59.3 \pm 2.0	80.5 \pm 1.4	37631
HCT-8E11R1	49.9 \pm 3.8	57.3 \pm 4.0	90456

Luteolin-6-*C*-boivinopyranoside (compound **2**) (Figure 3) was another compound isolated from the MeOH fraction and identified through comparison of its UV, MS and NMR data (table 5) with literature (Wang et al. 2008; Lee et al. 2010). It also showed antiproliferative activity towards the four cell lines (table 4). From its concentration in the crude extract (4.3 μ M in 500 μ g/mL) and its activity profile on the four cell lines, we could calculate that this compound accounts for 1-16% of the activity of total extract at 500 μ g/mL, except for on HCT-8E11R1 cells, where its concentration is too low for a contribution into the growth inhibition.

Leaving possible antagonistic, synergistic or other effects aside, we can conclude from the above calculations that triclin and luteolin-6-*C*-boivinopyranoside account together for up to 40% of the observed growth inhibitory effect of *F. robusta* 'Pingwu' leaf extract.

Table 4. IC₅₀ values (μ M) of luteolin-6-*C*-boivinopyranoside.

cell line	MTT	SRB	cell number
MCF-7/AZ	76.9 \pm 3.8	61.5 \pm 2.0	45852
MCF-7/6	46.0 \pm 2.6	42.3 \pm 2.8	87063
HCT-8E11	72.4 \pm 5.4	96.9 \pm 2.4	42698
HCT-8E11R1	70.6 \pm 8.2	91.8 \pm 9.9	113700

A third flavone glycoside (compound **3**) with a similar MS and ¹H NMR spectrum as luteolin-6-*C*-boivinopyranoside was also isolated from the 100% MeOH fraction. It has the same pseudomolecular ion [M+H]⁺ = 417, the same UV spectrum and a similar ¹H NMR spectrum (table 5) as luteolin-6-*C*- β -boivinopyranoside.

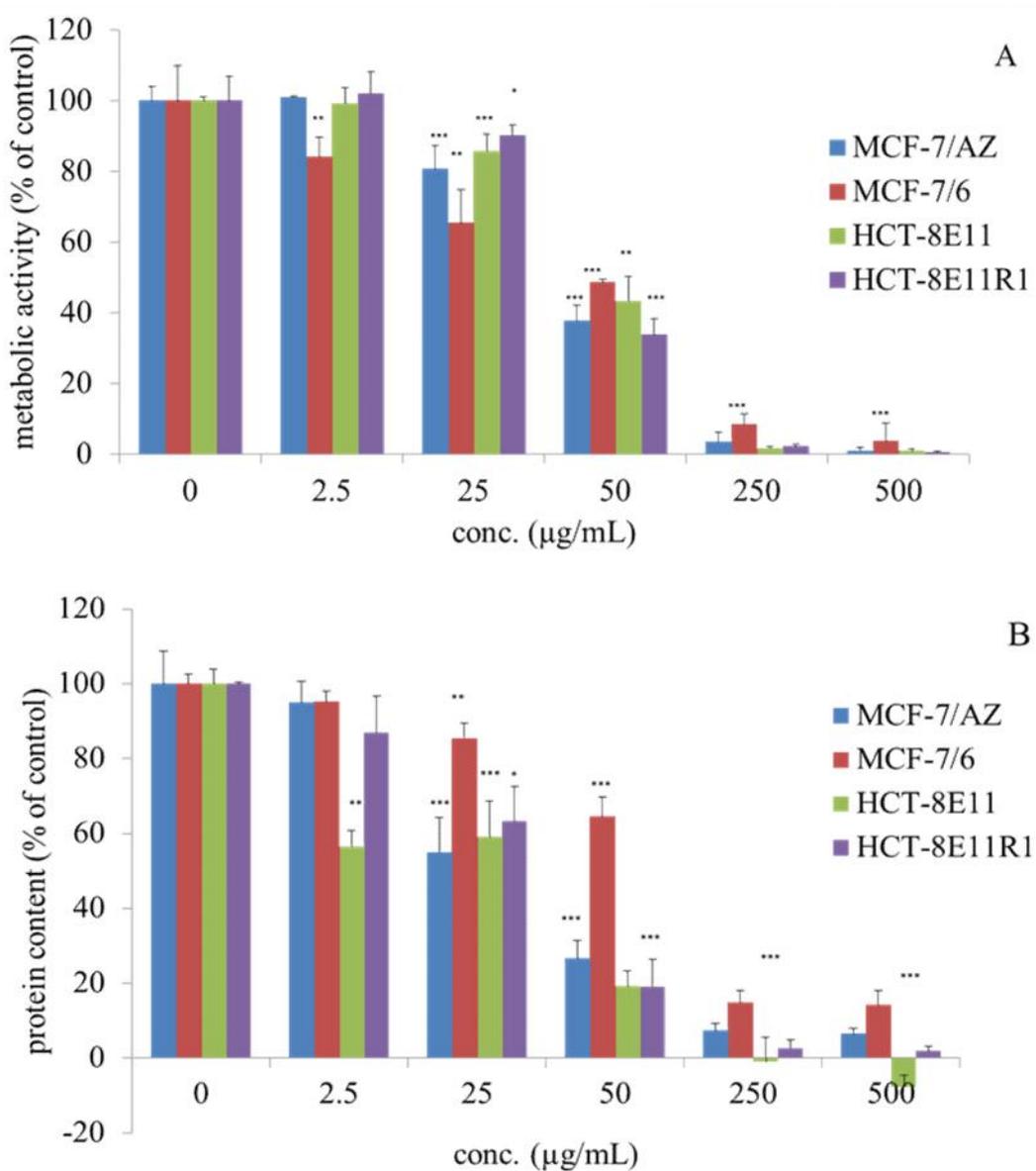


Figure 2. Metabolic activity (A) and protein content (B) of cancer cells after exposure (48 h) to the 100% MeOH fraction of *F. robusta* 'Pingwu' leaf extract. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

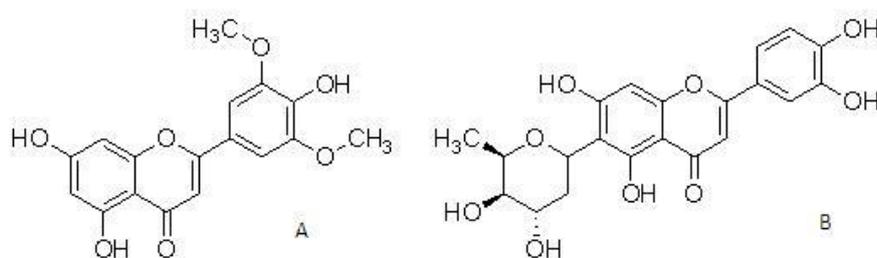


Figure 3. Flavones with antiproliferative activity: A) tricetin; B) luteolin-6-C- β -boivinopyranoside.

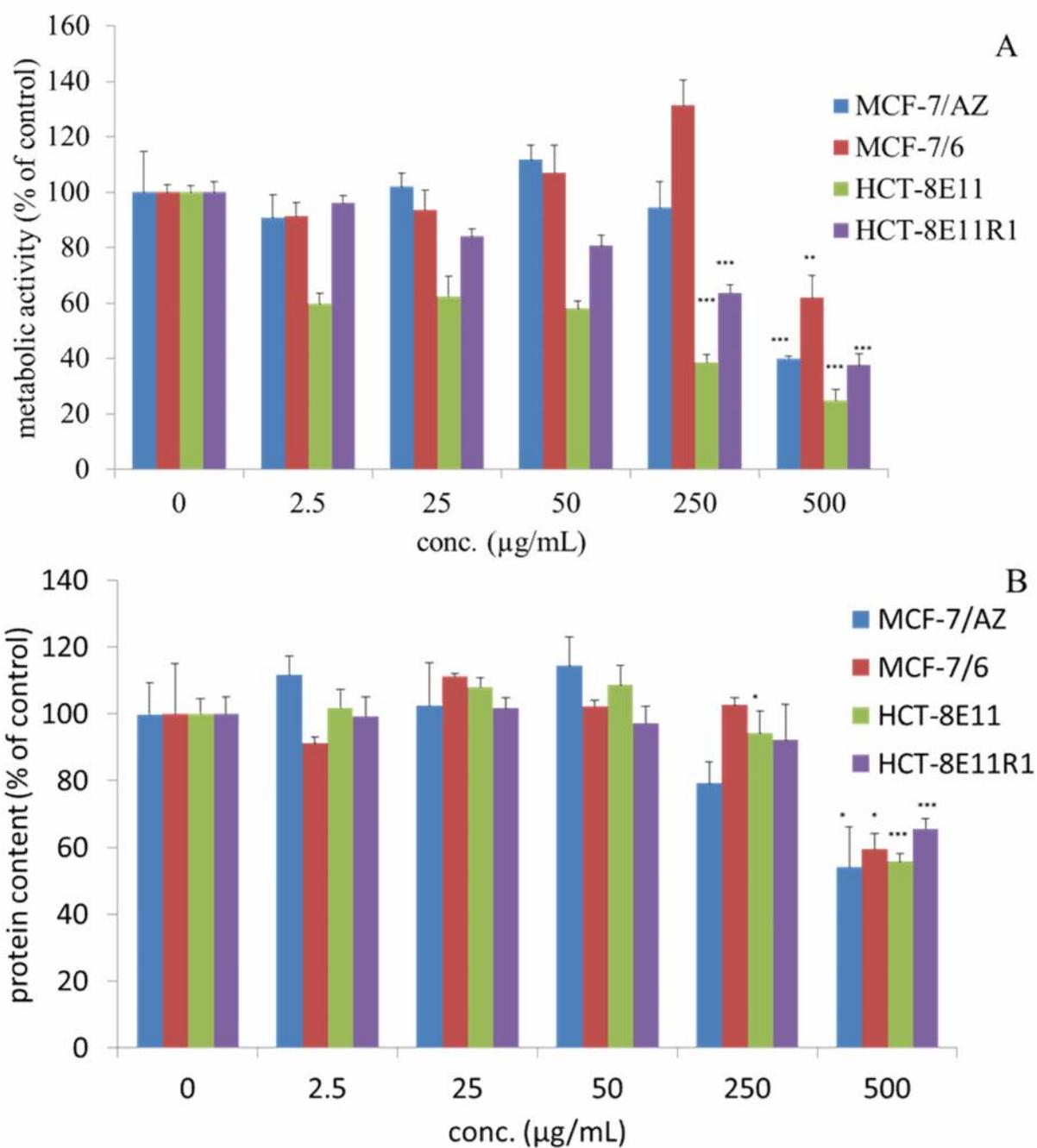


Figure 4. Metabolic activity (A) and protein content (B) of cancer cells after exposure (48 h) to *F. rufa* 'Green Panda' leaf extract. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Its ^1H NMR spectrum showed the presence of a *C*-conjugated luteolin derivative, as indicated by the flavone protons at δ 7.56 (1H, dd, 8.4, 2.1, H-6'), 7.51 (1H, d, 2.1, H-2'), 6.91 (1H, d, 8.4, H-5'), 6.58 (1H, s, H-3), 6.23 (1H, s, H-6/H-8). The ^1H NMR shifts of the sugar unit were similar to those of compound **2** (table 5).

The only difference is that compound **3** has an aromatic singlet at 6.23 ppm compared to 6.49 ppm in compound **2**, which is indicative for a 8-*C*-glycoside (Burns et al. 2007). In the MS spectrum of compound **3**, the protonated molecule was found to be much more stable than was the case for compound **2**. This was also observed for orientin (luteolin-8-*C*-glucoside) compared to homoorientin (luteolin-6-*C*-glucoside) described by Waridel *et al.* (2001). Therefore, this compound is likely to be luteolin-8-*C*- β -boivinopyranoside. When evaluating this compound in the antiproliferative assays, we observed only minor activity ($\text{IC}_{50} > 120 \mu\text{M}$).

Table 5. ^1H and ^{13}C NMR Data (CD_3OD , 300 MHz for ^1H and 75 MHz for ^{13}C) for compounds **2** and **3**^a.

position	2		3
	δ_{C}	δ_{H}	δ_{H}
2	165.3		
3	102.6	6.56, 1H, s	6.58, 1H, s
4	182.9		
5	157.5		
6	110.4		6.23, 1H, s
7	163.4		
8	94.8	8.49, 1H, s	
9	157.1		
10	103.7		
1'	122.4		
2'	113.0	7.38, 1H, m	7.51, 1H, d (2.1)
3'	145.9		
4'	149.9		
5'	115.6	.90, 1H, d (8.7)	6.91, 1H, d (8.4)
6'	119.2	7.41, 1H, m	7.56, 1H, dd (8.4, 2.1)
1''	68.8	1H, dd (12.0; 2.7)	5.69, 1H, dd (12.3, 2.4)
2''	31.6	H, dt (2.7, 14.4) (ax) 1.72, 1H, d (14.1) (eq)	2.42, 1H, dt (2.7, 14.4) (ax) 1.70, 1H, d, (14.4) (eq)
3''	67.6	.00, 1H, d (3.0)	4.06, 1H, d (3.3)
4''	69.4	.39, 1H, d (3.3)	3.46, 1H, d (3.6)
5''	71.5	13, 1H, q (6.6)	4.21, 1H, q (6.6)
6''	16.2	.28, 3H, d (6.9)	1.32, 3H, d (6.6)

^a Values in parentheses indicate coupling constants in Hz.

Fargesia rufa ‘Green Panda’

Crude extract

F. rufa ‘Green Panda’ was a second species showing antiproliferative activity towards the 4 cell lines. It showed activity starting from a concentration of 500 µg/mL, except for HCT-8 cells, where a significant reduction in metabolic activity was seen starting from a concentration of 25 µg/mL (Figure 4). The respective IC₅₀ values are presented in table 6.

Table 6. IC₅₀ values (µg/mL) of *F. rufa* ‘Green Panda’ leaf extract

	MTT	SRB	
MCF-7/AZ	-	-	-
MCF-7/6	-	-	-
HCT-8E11	161 ± 34	259 ± 45	37148
HCT-8E11R1	379 ± 16	-	93020

HPLC-DAD and LC-MS/MS analysis showed a similar chromatographic profile as *F. robusta* ‘Pingwu’. *F. rufa* ‘Green Panda’ has a relative high content of tricetin (17.6 µM in 500 µg/mL in the antiproliferative extract). From its concentration and its growth inhibitory effect on the four cell lines, we could calculate that tricetin accounts for 18-35% of the observed growth inhibition by this extract. The concentration of luteolin-6-C-boivinopyranoside in 500 µg/mL total extract is only 0.25 µM. The contribution of this compound to the overall activity on the four cell lines was calculated as 0-12%.

B. balcooa

Crude extract

B. balcooa leaf extract showed growth inhibitory effect on MCF-7/AZ and MCF-7/6 cancer cell lines, but no significant effect was seen on HCT-8E11(R1) cells (Figure 5). The IC₅₀ values of this extract towards MCF-7/AZ and MCF-7/6 are presented in table 7.

Table 7. IC₅₀ values (µg/mL) of *B. balcooa* extract

	MTT	SRB	cell number
MCF-7/AZ	352 ± 3.8	416.5 ± 9.8	14893
MCF-7/6	445 ± 47	439.6 ± 7.1	31017
HCT-8E11	-	-	-
HCT-8E11R1	-	-	-

SPE fractions

Four SPE fractions of different polarity were obtained from the total extract (110.0 mg): 100% H₂O (84%), 30% MeOH (6%), 60% MeOH in H₂O (8) and 100% MeOH (2%). The growth inhibitory activity of these fractions were evaluated on MCF-7/AZ cells using the same protocol as for the crude extract. Of the four fractions, the 100% MeOH fraction exerted significant antiproliferative activity (Figure 6), while only minor activity was observed for the other fractions. At a concentration of 500 µg/mL, the metabolic activity was decreased with 99.7 % compared to control. In comparison, the same fraction caused only 40.5% decrease in metabolic activity when incubated with HCT-8E11R1 cells (data not shown). This confirmed its selectivity towards MCF-7/AZ and MCF-7/6 cells.

LC-MS/MS analysis in positive ion mode revealed the presence of methoxylated flavone derivatives, which were characterized by the presence of the fragment ion m/z 331 (pos. mode) or 229 (neg. mode) (trihydroxy-dimethoxyflavone) (Grayer et al., 2001) and m/z 345 (pos.) or 343 (neg.) (dihydroxy-

trimethoxyflavone) (Grayer et al., 2001; Lin and Harnly, 2010). The typical loss of one ([M-H-15]) or two methyl radicals ([M-H-30]) further confirmed the presence of methoxylated flavones.

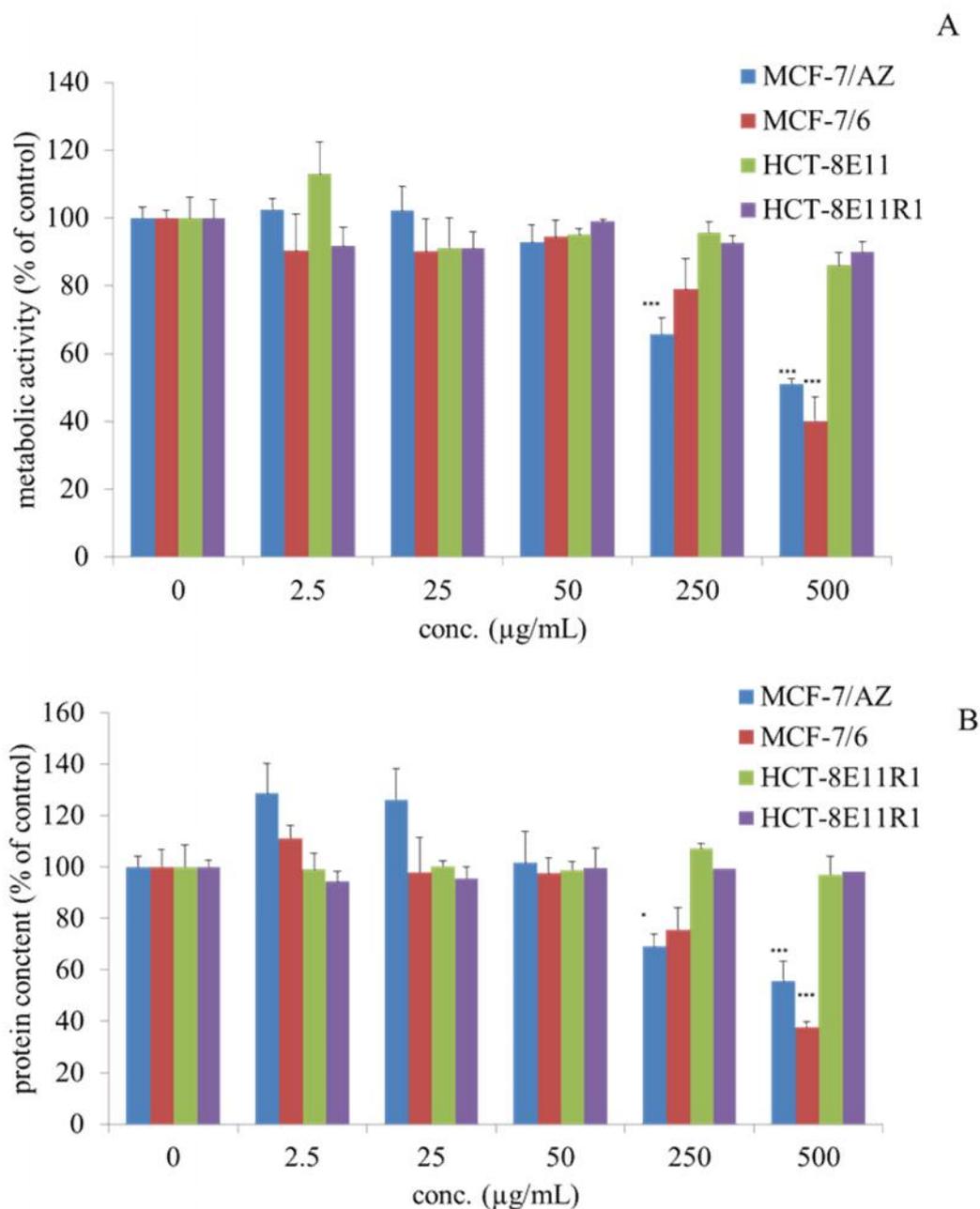


Figure 5. Metabolic activity (A) and protein content (B) of cancer cells after exposure (48 h) to *B. balcooa* leaf extract. * $p \leq 0.05$; ** $p \leq 0.01$; * $p \leq 0.001$.**

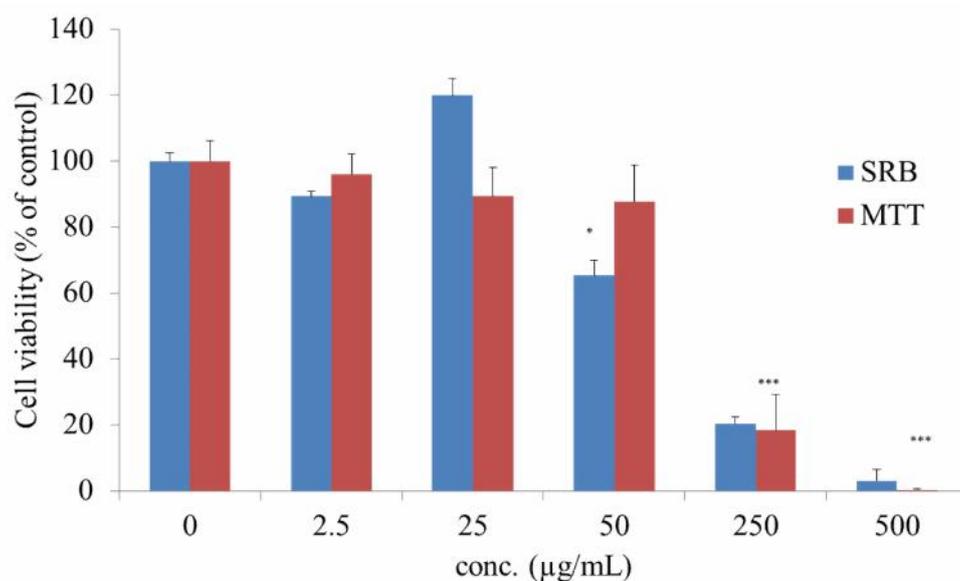


Figure 6. Growth inhibitory effect of the 100% MeOH fraction on MCF-7/AZ cells. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

P. japonica

Crude extract

P. japonica leaf extract caused a significant decrease in metabolic activity and protein content in all 4 cell lines, starting from a concentration of 250 µg/mL (MCF-7/6) or 500 µg/mL (MCF-7/AZ, HCT-8E11(R1)) (Figure 7). IC_{50} values are presented in table 8.

Table 8. IC_{50} values (µg/mL) of *P.japonica* leaf extract

	MTT	SRB	cell number
MCF-7/AZ	355 ± 46	-	12719
MCF-7/6	313 ± 36	298 ± 33	46714
HCT-8E11	-	-	-
HCT-8E11R1	-	-	-

SPE fractions

Analogously as for the above ascribed extracts, 4 SPE fractions were made of the total extract (130.5 mg): a 100% H₂O (39%), 30% MeOH (21%), 60% MeOH in H₂O (30%) and a 100% MeOH fraction (10%). They were evaluated for their growth inhibitory potential on MCF-7/6 cells. The 100% MeOH fraction was the most active one, while the 100% H₂O fraction also showed activity (Figure 8).

HPLC-DAD and LC-MS/MS analysis of the 100% H₂O fraction demonstrated the presence of phenolic acids. HPLC-DAD and LC-MS/MS analysis of the 100% MeOH fraction revealed the presence of triclin as major compound. From its concentration in the crude extract at 500 µg/mL (6.7 µM) and its activity profile on the four cell lines, we could calculate that the contribution of triclin to the observed antiproliferative activity of the total extract is 6-31%.

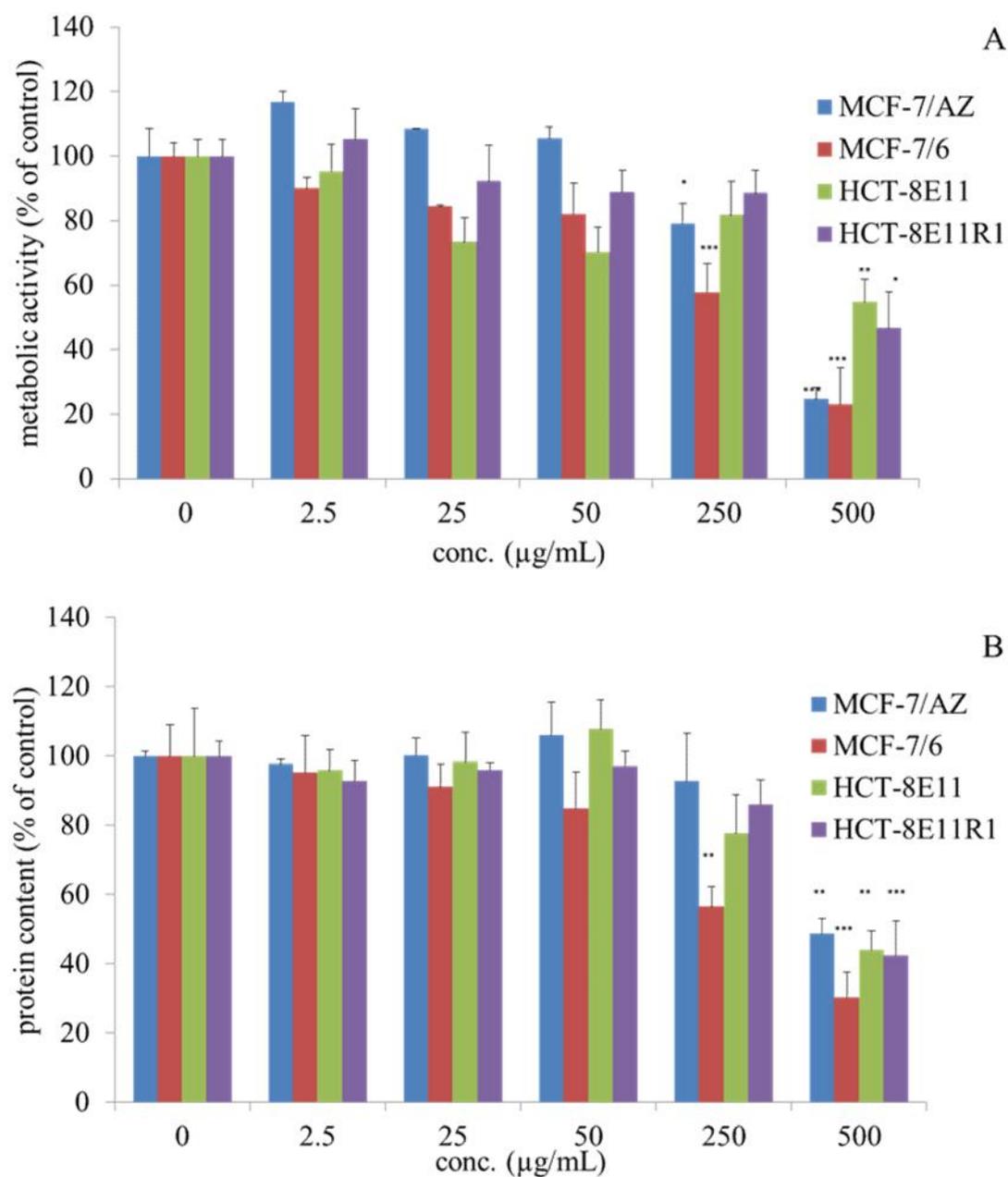


Figure 7. Metabolic activity (A) and protein content (B) of cancer cells after exposure (48 h) to *P. japonica* leaf extract. * $p \leq 0.05$; ** $p \leq 0.01$; * $p \leq 0.001$.**

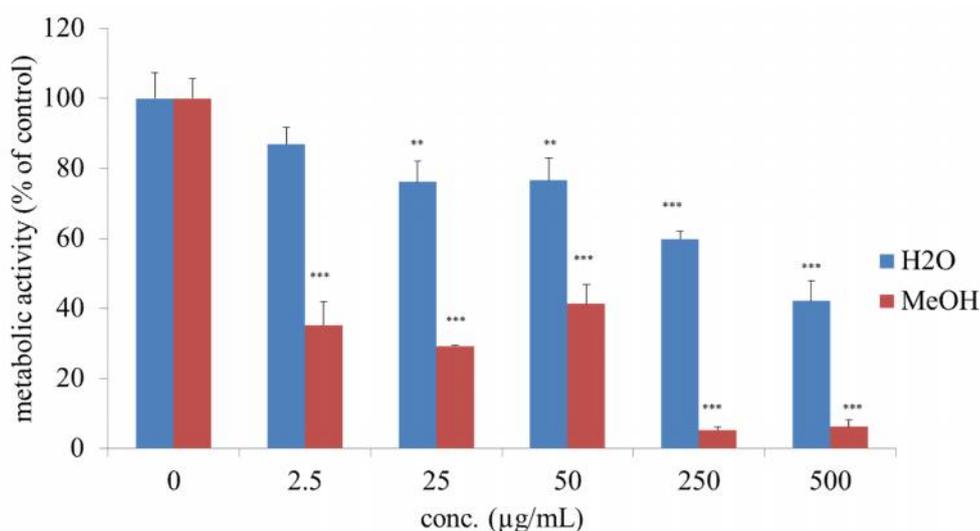


Figure 8. Metabolic activity of MCF-7/6 cells after exposure (48 h) to the 100% H₂O and 100% MeOH fraction of *P. japonica* leaf extract. * $p \leq 0.05$; ** $p \leq 0.01$; * $p \leq 0.001$**

The chromatogram at 450 nm shows the presence of chlorophyll-like (λ_{\max} near 400 and 650 nm) compounds. In several studies chlorophyll and its derivatives have been demonstrated to exert antiproliferative activity (Chiu et al. 2003; Tsai et al. 2010; Chou et al. 2011). It is possible that these compounds contribute to the observed growth inhibitory action of *P. japonica*.

Discussion and Conclusion

The leaf extracts of *F. robusta* ‘Pingwu’, *F. rufa* ‘Green Panda’, *P. japonica* and *B. balcooa* were able to inhibit the proliferation of human breast and/or colon cancer cells.

B. balcooa extract and its 100% MeOH fraction showed selective growth inhibition towards breast cancer cells.

F. robusta ‘Pingwu’ leaf extract demonstrated significant antiproliferative activity towards all cell lines, but the fast growing HCT-8E11R1 cells appeared to be most sensitive.

In the presence of *F. rufa* ‘Green Panda’ leaf extract, the formazan production by HCT-8E11 and HCT-8E11R1 cells was decreased from a concentration of 2.5 and 25 µg/mL onwards, respectively. However, protein content was not affected until a concentration of 500 µg/mL. Apparently, *F. rufa* ‘Green Panda’ affects metabolic activity of these cells, without or before any growth inhibition occurs. In the case of MCF-7/AZ and MCF-7/6 cells, the decrease in metabolic activity and cell number occurred simultaneously at a concentration of 500 µg/mL.

MCF-7/6 cells were more sensitive to growth inhibition by *P. japonica* leaf extract, than the other cells. A significant decrease in metabolic activity and protein content was seen from a concentration of 250 µg/mL onwards, while for the other cell lines significant decreases were measured starting from 500 µg/mL.

Using SPE, it was possible to locate the most active compounds, being almost merely present in the 100% MeOH fraction. The observed activity is apparently due to the combined action of several compounds including, tricetin and luteolin-6-*C*- β -boivinopyranoside, but also further non-identified compounds may contribute to the overall antiproliferative activity.

Tricetin is a flavone, commonly found in species belonging to the Poaceae family (Harborne and Hall 1964). It has been shown to have growth inhibitory effect on human-derived malignant MDA-MB-468

breast cancer cells *in vitro* and *in vivo* (Cai et al. 2004). Additionally, Cai *et al.* (2005) demonstrated the antiproliferative effect of tricetin on intestinal adenomas in Apc(Min) mice.

Luteolin-6-C-boivinopyranoside, has been previously identified in only two other species, namely *Pogonatherum crinitum* (Wang et al. 2008) and *Eremochloa ophiuroides* (Lee et al. 2010). Both species belong the Poaceae family. Flavonoids conjugated with a boivinoside are rare in the plant kingdom, but seem to be common in certain grass species, including several bamboos (Van Hoyweghen et al. in press). Our study is the first report describing the antiproliferative activity of this compound.

In addition to their antiproliferative activity, the two *Fargesia* 's have also good radical scavenging properties (Van Hoyweghen et al. 2010; Van Hoyweghen et al. in press). These data indicate that these species could be interesting as chemopreventive agents.

In conclusion, we demonstrated that some bamboo species, namely *F. robusta* 'Pingwu', *F. rufa* 'Green Panda', *P. japonica* and *B. balcooa*, have antiproliferative activities towards human breast (MCF-7/AZ & MCF-7/6) and/or colon (HCT-8E11 & HCT-8E11R1) cancer cells *in vitro*. A combined action of different phytochemicals, including the flavones tricetin and luteolin-6-C- β -boivinopyranoside, seems to be responsible for the observed activity.

References

- AICR 1997. Food, Nutrition and the Prevention of Cancer: a global perspective. Washington, DC, World Cancer Research Fund/American Institute for Cancer Research.
- Ando, H.; Ohba, H.; Sakaki, T.; Takamine, K.; Kamino, Y.; Moriwaki, S.; Bakalova, R.; Uemura, Y.; Hatate, Y. 2004. Hot-compressed-water decomposed products from bamboo manifest a selective cytotoxicity against acute lymphoblastic leukemia cells. *Toxicology in Vitro* 18(6): 765-771.
- Bode, A. M.; Dong, Z. 2004. Targeting signal transduction pathways by chemopreventive agents. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis* 555(1-2): 33-51.
- Bracke, M. E.; Vanlarebeke, N. A.; Vyncke, B. M.; Mareel, M. M. 1991. Retinoic acid modulates both invasion and plasma-membrane ruffling of MCF-7 human mammary-carcinoma cells in vitro. *British Journal of Cancer* 63(6): 867-872.
- Burns, D. C.; Ellis, D. A.; March, R. E. 2007. A predictive tool for assessing C-13 NMR chemical shifts of flavonoids. *Magnetic Resonance in Chemistry* 45(10): 835-845.
- Cai, H.; Al-Fayez, M.; Tunstall, R. G.; Platton, S.; Greaves, P.; Steward, W. P.; Gescher, A. J. 2005. The rice bran constituent tricetin potently inhibits cyclooxygenase enzymes and interferes with intestinal carcinogenesis in Apc(Min) mice. *Molecular Cancer Therapeutics* 4(9): 1287-1292.
- Cai, H.; Hudson, E. A.; Mann, P.; Verschoyle, R. D.; Greaves, P.; Manson, M. M.; Steward, W. P.; Gescher, A. J. 2004. Growth-inhibitory and cell cycle-arresting properties of the rice bran constituent tricetin in human-derived breast cancer cells in vitro and in nude mice in vivo. *British Journal of Cancer* 91(7): 1364-1371.
- Chiu, L. C. M.; Kong, C. K. L.; Ooi, V. E. C. 2003. Antiproliferative effect of chlorophyllin derived from a traditional Chinese medicine *Bombyx mori* excreta on human breast cancer MCF-7 cells. *International Journal of Oncology* 23(3): 729-735.
- Chou, S. T.; Chan, H. H.; Peng, H. Y.; Liou, M. J.; Wu, T. S. 2011. Isolation of substances with antiproliferative and apoptosis-inducing activities against leukemia cells from the leaves of *Zanthoxylum ailanthoides* Sieb. & Zucc. *Phytomedicine* 18(5): 344-348.
- Duke, J. A. (1994). "Duke's Phytochemical and Ethnobotanical databases."
- Galati, G.; O'Brien, P. J. 2004. Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemopreventive and anticancer properties. *Free Radical Biology and Medicine* 37(3): 287-303.
- Grayer, R. J.; Veitch, N. C.; Kite, G. C.; Price, A. M.; Kokubun, T. 2001. Distribution of 8-oxygenated leaf-surface flavones in the genus *Ocimum*. *Phytochemistry* 56(6): 559-567.
- Harborne, J. B.; Hall, E. 1964. Plant polyphenols.12. The occurrence of tricetin and of glycoflavones in grasses. *Phytochemistry* 3(3): 421-428.

- Houghton, P.; Fang, R.; Techatanawat, I.; Steventon, G.; Hylands, P. J.; Lee, C. C. 2007. The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods* 42(4): 377-387.
- Kang, N. J.; Shin, S. H.; Lee, H. J.; Lee, K. W. 2011. Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis. *Pharmacology & Therapeutics* 130(3): 310-324.
- Kim, S. H.; Kim, T. S.; Lee, H. J.; Yoo, J. C. 2007. Enhancement of 1,25-dihydroxyvitamin D-3- and all-trans retinoic acid-induced differentiation of human leukemia HL-60 cells by *Phyllostachys nigra* var. *henonis*. *Immunopharmacology and Immunotoxicology* 29(1): 119-129.
- Lee, E. M.; Lee, S. S.; Chung, B. Y.; Cho, J. Y.; Lee, I. C.; Ahn, S. R.; Jang, S. J.; Kim, T. H. 2010. Pancreatic Lipase Inhibition by C-Glycosidic Flavones Isolated from *Eremochloa ophiuroides*. *Molecules* 15(11): 8251-8259.
- Lin, L. Z.; Harnly, J. M. 2010. Identification of the phenolic components of chrysanthemum flower (*Chrysanthemum morifolium* Ramat). *Food Chemistry* 120(1): 319-326.
- Lin, Y. L.; Collier, A. C.; Liu, W. Y.; Berry, M. J.; Panee, J. 2008. The Inhibitory Effect of Bamboo Extract on the Development of 7,12-Dimethylbenz[a]anthracene (DMBA)-induced Breast Cancer. *Phytotherapy Research* 22(11): 1440-1445.
- Liu, Y. B.; Peterson, D. A.; Kimura, H.; Schubert, D. 1997. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. *Journal of Neurochemistry* 69(2): 581-593.
- Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival - Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65(1-2): 55-63.
- Papazisis, K. T.; Geromichalos, G. D.; Dimitriadis, K. A.; Kortsaris, A. H. 1997. Optimization of the sulforhodamine B colorimetric assay. *Journal of Immunological Methods* 208(2): 151-158.
- Ren, M. Q.; Reilly, R. T.; Sacchi, N. 2004. Sasa health exerts a protective effect on Her2/NeuN mammary tumorigenesis. *Anticancer Research* 24(5A): 2879-2884.
- Simon, N.; Noel, A.; Foidart, J. M. 1992. Evaluation of in vitro reconstituted basement-membrane assay to assess the invasiveness of tumor cells. *Invasion & Metastasis* 12(3-4): 156-167.
- Sporn, M. B. 1991. Carcinogenesis and cancer - Different perspectives on the same disease. *Cancer Research* 51(23): 6215-6218.
- Sporn, M. B.; Suh, N. 2000. Chemoprevention of cancer. *Carcinogenesis* 21(3): 525-530.
- Sporn, M. B.; Suh, N. 2002. Chemoprevention: an essential approach to controlling cancer. *Nature Reviews Cancer* 2(7): 537-543.
- Surh, Y. J. 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis* 428(1-2): 305-327.
- Tsai, Y.-C.; Wu, W.-B.; Chen, B.-H. 2010. Preparation of Carotenoids and Chlorophylls from *Gynostemma pentaphyllum* (Thunb.) Makino and Their Antiproliferation Effect on Hepatoma Cell. *Journal of Medicinal Food* 13(6): 1431-1442.
- Van Hoyweghen, L.; De Beer, T.; Deforce, D.; Heyerick, A. in press. Phenolic compounds and antioxidant capacity of twelve morphologically heterogeneous bamboo species. *Phytochemical Analysis*.
- Van Hoyweghen, L.; Karalic, I.; Van Calenbergh, S.; Deforce, D.; Heyerick, A. 2010. Antioxidant Flavone Glycosides from the Leaves of *Fargesia robusta*. *Journal of Natural Products* 73(9): 1573-1577.
- Vermeulen, S. J.; Bruyneel, E. A.; Bracke, M. E.; Debruyne, G. K.; Vennekens, K. M.; Vleminckx, K. L.; Berx, G. J.; Vanroy, F. M.; Mareel, M. M. 1995. Transition from the noninvasive to the invasive phenotype and loss of alpha-catenin in human colon cancer cells. *Cancer Research* 55(20): 4722-4728.
- Wang, D. D.; Wang, J.; Huang, X. H.; Tu, Y.; Ni, K. Y. 2007. Identification of polymethoxylated flavones from green tangerine peel (*Pericarpium Citri Reticulatae Viride*) by chromatographic and spectroscopic techniques. *Journal of Pharmaceutical and Biomedical Analysis* 44(1): 63-69.
- Wang, G. J.; Chen, Y. M.; Wang, T. M.; Lee, C. K.; Chen, K. J.; Lee, T. H. 2008. Flavonoids with iNOS inhibitory activity from *Pogonatherum crinitum*. *Journal of Ethnopharmacology* 118(1): 71-78.

- Waridel, P.; Wolfender, J. L.; Ndjoko, K.; Hobby, K. R.; Major, H. J.; Hostettmann, K. 2001. Evaluation of quadrupole time-of-flight tandem mass spectrometry and ion-trap multiple-stage mass spectrometry for the differentiation of C-glycosidic flavonoid isomers. *Journal of Chromatography A* 926(1): 29-41.
- Wattenberg, L. W. 1992. Inhibition of carcinogenesis by minor dietary constituents. *Cancer Research* 52: 2085-2091.
- WHO 2011. Cancer Fact sheet N° 297, World Health Organization: <http://www.who.int/mediacentre/factsheets/fs297/en/>.

Bamboo Shoot as a Resource for Health Food and Socio-Economic development in North-East India

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Abstract

The North-East region of India is a rich resource of biodiversity in general and bamboos in particular. This region has about 43% of the total bamboo wealth of India and plays an important role in the life of the people especially in the rural areas. Though bamboo shoot is not a popular food commodity in other parts of the country, it is a delicacy in the North-Eastern states being used fresh as well as in fermented forms. Bamboo shoots have high content of proteins, carbohydrates, minerals, fiber and vitamins and are even richer in many nutrient components than some of the commonly used vegetables. They are endowed with health enhancing properties due to the presence of phenols, phytosterols and fiber. However, this highly nutritious vegetable, like many others locally cultivated crops (finger millet, buckwheat, taro, amaranth etc) is being neglected and replaced by other food items and its usage in local households is gradually diminishing. Very few people in the region are aware of the nutritive value of the shoots. Concerted efforts are being done to utilize this natural resource not only to meet the increasing demand of food and food insecurity in the region but also to encounter malnutrition widely prevalent in the country and provide income generating avenues to the local people. Despite the enormous production of bamboo shoots in the region, processing and packaging of the shoots is in its infancy with only a few units present in the region. Taking into account the increasing demand of bamboo shoots worldwide, and the enormous economic potential, various developmental programs are being framed/researched for utilizing the vast resource to generate employment opportunities for the weaker sections of the society and help in their social and economic upliftment.

Keywords

Bamboo shoot, North-East India, health food.

Introduction

The North-Eastern region of India comprising of eight states, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim, is geographically nestled in one of the most biodiversity-rich regions of the world. Located at the confluence of the Indo-Malayan, Indo-Chinese and Indian biogeographical realms, the North-East region is unique in providing a profusion of habitats, which features diverse biota with a high level of endemism and is the 6th among the 25 mega diversity hotspots of the world (Myers *et al.* 2000, Chatterjee *et al.* 2006). Now, this region is in the main focus of the leading conservation agencies due to its bio-diversity and endemism. North-East region of India, with 8 per cent (2,62,179 sq km) of the total geographic area of the country is inhabited by 4,55,87,982 (4 per cent of the total population of India, 2011 Census; Table 1) people comprising of diverse ethnic groups or tribes. The region is known for its ancient traditions and culture, dense forest cover, rich biodiversity and unique life style of the people. Still, people mainly depend on forests and natural resources for subsistence. Economy of the region is totally agriculture and natural resource based.

This region is also considered as the “Bamboo Paradise of India” and is a treasure house of bamboo diversity harboring 43% share of the total bamboo wealth of India. Bamboos are intricately associated with the life of the people, especially in the rural areas, because of its multipurpose economic uses which extends from childbirth to death. From time immemorial, people in the region have been using bamboo for various purposes right from building bridges over mighty rivers like Siang to sitting mats, rain coats, carry bags, writing pens, house utensils, cradle, walking stick for old man and finally bier to carry the dead body.

Another important use of bamboo for people in this region is as food, as fresh, fermented or dried. Young juvenile shoots, the new culms just emerged from the ground, constitute a range of traditional delicacies which each tribe or ethnic group have developed. However, there is a lack of integration of this traditional knowledge of using bamboo shoots as food with modern nutrition and food technology and thus people of the region are neglecting this nutritive food item like many other crops. The present paper highlights the nutritional value of bamboo shoots, one of the neglected food item of the region in particular and India in general, and presents various ways of its utilization as a health food as well as an income building resource for people of this region.

Bamboos of the North-East Region of India

The distribution of bamboos in India is fairly wide except in the temperate and alpine zones and the extreme arid regions. Forest area occupied by bamboos comprises 8.96 million hectare constituting 11.71% of the 74.96 million hectare of forest area of the country (Naithani *et al.* 2010). The total growing stock of bamboo in the country is 80.428 million tons out of which approximately 42.27 million tons is in the North-Eastern states only. Globally, India has the second highest number of species (128 species) after China, and within the country, it is North-East states with more than fifty % (54 in number) bamboo species (Nathani 2008; Nimachow *et al.* 2010). The status of bamboos of North-East India has been worked out from time to time by various workers like Biswas (1988); Shukla (1996); Sharma *et al.* (1992); Naithani (2008). States like Manipur and Arunachal Pradesh are the richest states with bamboo diversity with 53 and 42 species, followed by Meghalaya and Assam with 35 and 29 species, respectively (Haridasan *et al.* 1987; Rai and Chauhan 1998; Barooah and Borthakur 2003; Naithani 2007; Bhaumik and Hynniewta 2007; Devi and Devi 1995; Singh *et al.* 2003; Singh *et al.* 2006; Sharma and Borthakur 2007).

Table 1. North-East States of India: population, geographical area, forest area and bamboo stock in the region.

North-East Indian States	Geographic Area (sq km)	Population (2011 census)	Population Density	Forest Area(sq km)	Per cent Forest Area of Total Geographic Area	Bamboo Area (sq km)	Per cent Bamboo area of Total Forest Area	Per cent Bamboo Area of Total Geographic Area	Total Bamboo Growing Stock (million tons)
Arunachal Pradesh	83,743	13,82,611	16	51,540	61.5	4,596	8.52	5.5	1,616
Assam	78,438	3,11,69,272	397	26,832	34.21	1,813	6.56	2.3	9,844
Manipur	22,327	27,21,756	103	17,418	75.01	3,692	21.81	16.5	11,470
Meghalaya	22,429	29,64,007	132	9,496	42.3	3,102	32.67	13.8	4,407
Mizoram	21,081	10,91,014	51	16,717	75.6	9,210	57.80	43.7	10,890
Nagaland	16,579	19,80,602	119	9,222	55.6	758	8.8	4.6	3,657
Sikkim	7,096	6,07,688	85	5,841	82.3	-	-	-	-
Tripura	10,486	36,71,032	350	6,294	60.0	939	14.92	9.0	860
TOTAL	2,62,179	4,55,87,982	1253	1,43,360	486.52	24,110.0	151.08	95.4	4,27,44

With respect to area, bamboo is spread over 9.2 % (24,110 sq km) of the total geographic area of the region, maximum, (9,210 sq km) being in Mizoram, which constitutes around 40 % of the total geographic area of the state. Manipur and Meghalaya have around 21.0 and 32 per cent of their total geographic area covered with bamboo. Clump forming bamboos constitute a large portion of the growing stock with percentage in different states of the regions as follows, Assam (16%), Manipur (14%), Mizoram (14%), Arunachal Pradesh (12%), Meghalaya (6%) and Nagaland (5%). *Bambusa bambos*, *B. pallida*, *B. tulda*, *Dendrocalamus hamiltonii*, *Melocanna baccifera* and *D. strictus* are major species of bamboo growing in the region (Naithani *et al.* 2010). Some species have gained prominence in landscaping and few like yellow bamboo, black bamboo, pitcher bamboo and tiger bamboo are used as ornamentals.

Bamboo shoot as a Health Food in the region

In North-East India bamboo shoots are consumed in fresh, fermented, roasted and pickled form. A considerable quantity of shoots is consumed in the form of fermented products after processing through conventional methods. Soft juvenile bamboo shoots of almost all species are used as food by the tribes and ethnic groups of the region. However, shoots of *Bambusa balcooa*, *B. bambos*, *B. tulda*, *Dendrocalamus giganteus*, *D. hamiltonii*, *Melocanna baccifera*, *Chimonobambusa callosa*, *D. hookerii* and *D. giganteus* are the species mainly used for food (Bhatt *et al.* 2004). *Dendrocalamus* species (*D. hamiltonii*, *D. sikkimensis*, and *D. giganteus*) are the most preferred one and harvested maximum for shoots.

Every tribe or ethnic groups have their own methods of fermentation and cooking. The Khasi people in Meghalaya ferment shoots in plastic or glass bottles filled with water, whereas Meiteis of Manipur ferment them in black clay pots or in bamboo baskets. Generally bamboo shoots, fresh or fermented are used for making pickles, curry like Soibum (by Meiteis) or prepared with pork (by Khasis). However, these cuisines are traditional, being followed for generations and may not be palatable to all. Hence, new cuisines are being developed taking into account the changing food habits around the world as well as in the region. New food items like cookies, curries, bread/chapattis, etc are being prepared and popularized in the region.

Presently, based on nutritional analysis, there are several reports which indicate that bamboo shoots are rich in various nutrients like protein, carbohydrates, minerals and dietary fibers (Shi and Yang 1992; Chen *et al.* 1999; Xu *et al.* 2005; Nirmala *et al.* 2007, 2008, 2011). Species like *Bambusa bambos*, *B. polymorpha*, *B. tulda*, *Dendrocalamus brandisii* and *D. hamiltonii* have more than 3 g/100g fresh weight of dietary fiber which is more than the quantity present in commonly consumed vegetables like spinach, cucumber, pumpkin, beans etc. Shoots have also high content of minerals like potassium, magnesium, sodium and iron (Table 2). The potassium content ranges from 232 to 576 mg/100 g fresh weight which is good for the heart as it helps to maintain normal blood pressure. The shoots are a source of physiologically active compounds referred to as phytochemicals, such as phytosterols and phenols, that have significant health potentials (Nirmala *et al.* 2011). Phytosterols lower cholesterol levels, resulting in significantly reduced risk to heart diseases. Phenols have strong antioxidant properties. These bioactive compounds are potential materials for the development of functional foods. Bamboo shoots are known to protect neurons from oxidative stress and have anti-fatigue activity (Akao *et al.* 2004; Zhang *et al.* 2006). Bamboo shoots help in preventing cardiovascular diseases, cancer and weight loss and improving digestion (Lachanche and He 1998; Hiromichi 2007; Fujimura *et al.* 2005). According Prof. Ana Aslan from the Institute of Geriatric in Bucharest, Romania, bamboo shoot is listed third among six (broccoli, berries, bamboo shoots, garlic and onions, parsley and kiwifruit) most healthy foods in the world (www.squidoo.com/gerovitalromania accessed on 20.9.2011).

Table 2. Comparison of macro-nutrients and mineral elements in bamboo species and some other common vegetables.

Bamboo species and common vegetables		Macronutrients (mg/100g)								Mineral elements (mg/100g)						
Common names	Scientific names	Proteins	Amino Acids	Carbo hydrates	Starch	Fat	Fiber	Vit C	Vit E	Ca	P	K	Fe	Mn	Mg	Na
Lam Saneibi	<i>Bambusa bambos</i>	3.57	3.98	5.42	0.25	0.50	1.90	1.90	0.61	0.36	30.12	576	2.99	0.47	5.38	10.06
Saneibi	<i>Bambusa tulda</i>	3.69	3.65	6.92	0.59	0.48	3.97	1.92	0.61	4.06	19.31	408	3.19	0.7	8.68	12.96
Wanap	<i>Dendrocalamus hamiltonii</i>	3.72	3.18	5.50	0.47	0.41	3.90	2.45	0.71	3.0	28.12	416	2.69	0.16	6.09	9.32
Amaranth	<i>Amaranth gangeticus</i>	4.0	1.3	6.1	--	0.5	1.0	1.0	43.3	397	247	31	1.8	0.36	55	20
Cauliflower	<i>Brassica oleracea var. botrytis</i>	5.9	0.4	7.6	--	0.4	2.0	2.5	46.4	33	57	303	1.23	0.2	15	30
Cabbage	<i>Brassica oleracea var. capitata</i>	1.8	0.3	5.6	--	0.1	1.0	2.6	32.2	47	23	246	0.6	0.18	18	18
Carrot	<i>Daucus carota</i>	0.9	0.2	10.6	--	0.2	1.2	1.2	3.0	80	530	108	1.03	0.16	18	35.6
Radish	<i>Raphnus sativus</i>	0.7	0.1	3.4	--	0.1	0.6	1.6	15.0	35	20	393	1	0.22	10	39

Spinach	<i>Spinacea oleracea</i>	2.0	0.3	2.9	--	0.7	0.2	0.6	28.1	99	49	558	2.7	0.9	79	79
Potato	<i>Solanum tuberosum</i>	1.6	0.2	22.6	15.4	0.1	0.4	0.4	19.7	12	58	421	0.8	0.2	23	11
Ladies finger	<i>Abelomoschus esculantus</i>	1.9	0.3	6.4	--	0.2	1.2	1.2	13.0	66	56	103	0.35	0.19	11	6.9
Tibda	<i>Citrullus vulgaris</i>	1.4	0.2	3.4	--	0.2	1.2	1.0	--	--	--	24	0.9	--	--	35
Cucumber	<i>Cucumis sativus</i>	0.6	0.1	2.5	0.1	0.1	0.4	0.7	3.2	14	25	136	0.9	0.14	12	2
Pumpkin	<i>Cucurbita maxima</i>	1.4	0.2	6.5	--	0.4	1.1	0.7	9.0	21	44	340	0.8	0.1	12	5.6
Bottle gourd	<i>Lagenaria siceraria</i>	0.6	0.4	3.4	--	0.1	0.6	0.6	12.0	26	13	150	0.7	0.1	11	2
Tori	<i>Luffa acutangula</i>	1.2	0.1	3.4	--	0.1	3.3	0.5	5.5	18	26	160	0.46	0.07	14	2.9
French beans	<i>Phaseolus vulgaris</i>	18.8	0.3	20.1	--	2.0	1.8	4.6	--	186	304	1316	3.4	1.2	188	18
Brinjal	<i>Solanum melongena</i>	1.4	0.2	4.0	--	0.3	1.3	1.3	12.0	18	47	200	0.9	0.13	10	3

Bamboo shoots and food security in the region

It is estimated that 1.2 billion people in the world do not have enough food to meet their daily requirements, and a further 2 billion people are deficient in one or more micronutrients, especially in the developing countries (Kotecha 2008). In India, the situation is worst; as according to World Bank Report of 2005, the prevalence of underweight children in India is amongst the highest in the world, and is double that of sub Saharan Africa. Energy, food security and health care are the major challenges faced by people in India. Neglect of the local crops like finger millet, pearl millet, sorghum, taro, buckwheat, amaranth, etc. is one of the main reason for food insecurity in India in general, and North-East region in particular. A shift in paradigm of food production and management has been recommended that includes Non-timber Forest Products (NTFP) to handle the socioeconomic dynamics (Warner 2008). In India, bamboo is one such crop which is recently recognized as NTFP, and plays an important role in the livelihoods of the rural poor, as a source of food, medicine, construction materials, and income.

North-Eastern region of India is abundant in nutritious crops like buckwheat, taro, local rice, bamboo shoots and many wild pulses which are being neglected or are being displaced due to pressure from imported food items. Food basket in the region is becoming narrow day by day due to loss of agrobiodiversity and farmers dependence on a few highly select crop species. Bamboo shoot, like many other local crops, is a neglected crop in the region due to lack of a coherent strategy for evaluation and development. This commodity, if properly utilized, can help meet the increasing demand for food and nutrition, energy, medicine and industrial needs. This food commodity is available at least for five to six months (June to October) in the region and can be preserved by simple fermentation and proper storage for more than one year. Presently, bamboo shoots grow naturally without any ecological disturbance on all types of soils and climatic conditions. Consumption of bamboo shoots as food is now mainly restricted to rural areas particularly among the poor people. Urban people do not prefer it due to reasons like lack of easy availability in the vegetable market, difficult to clean and get the soft edible part from the harvested shoots, short shelf-life, lack of knowledge of utilization of shoots as food, unpleasant smell due to presence of cyanogenic glucoside and homogentisic acid. Moreover, people in cities prefer ready to cook packs which is at present totally lacking for bamboo shoots of the region.

Dependence of people on few select crops has also caused food and nutrition insecurity and poverty to rural and urban communities. Prevailing scarcity of pulses and exorbitant prices in many developing countries including India, have deprived a large population of their protein requirements. Bamboo shoots being rich in various nutrient components and bioactive compounds, and also cheaper than commonly available vegetables (Nirmala *et al.* 2011) can be a very good source of food supplement especially for growing children and elderly people. Thus, bamboo shoot can become a solution for food insecurity as well as for nutritive food for the region (Satya *et al.* 2011).

Bamboo shoot for income generation and community development in the region

Presently, over two million tons of bamboo shoots are consumed in the world annually (Yang *et al.* 2008) and India, even though it is the second largest resource of bamboo in the world, stands nowhere in the bamboo shoot export market in the international scenario. Rather, for its domestic consumption India, imports shoots from countries like Bhutan and Thailand. In the international market, China earns 6500 million Indian rupees annually from export of edible shoots, with USA importing around

44,000 tones accounting for 14.5% of the total world import (Lobovikov 2003). USA imports 30,000 tones of canned bamboo shoots from Taiwan, Thailand and China (Daphne 1996).

Bamboo shoot in the North-East region is mainly a vegetable of rural people and marketed at local level only. Statistics show that about 26.2, 435 and 426.8 tonnes of bamboo shoots are harvested annually in the Northeastern states of India like Sikkim, Meghalaya and Mizoram, respectively, for local consumption (Bhatt *et al.* 2003, 2005). *D. hamiltonii*, *D. giganteus*, *D. sikkimensis*, *M. baccifera*, *D. hookerii*, *B. balcooa*, *Schyzostachyum dulloa* and *Teinostachyum wightii* are the main edible species in the region, and collectively, only 5685 tons are harvested annually as fresh shoots. In the name of canning and processing of shoots, there are just three to four small units located at Dimapur, Nagaland (900 tonnes/year) and Jorhat, (200 tonnes/year) and Bongaigown, Assam (300 tonnes/year) and one in Aizwal, Mizoram (Nimachow *et al.* 2010). Whereas, in China there are around 700 factories involved in canning and export of bamboo shoots to the tune of 2,50,000 tones annually. Comparing this to the fact that China has only 4.2 million hectare bamboo forest area whereas, India has nearly 9 million hectare of bamboo growing forest area. But domestic income of India from bamboo shoots is of only Rs 37 million (Singha *et al.* 2008). Even small region, Prachinburi Province of Thailand has more than 25 bamboo shoot canning industries and Thailand earns around US \$ 31 million from bamboo shoot harvest (Thamincha 1988). The market potential of bamboo in India is estimated to increase to \$66 million by 2015, thus there is a lot of opportunity to tap this unexploited crop to augment the family income without expending a penny on cultivation, as still bamboo grows wild in the region (Farooquee *et al.* 2007)

Conclusion

Bamboo shoots are becoming a popular food item globally mainly due to its nutritive value and health enhancing properties (Chongtham *et al.* 2011; Choudhury *et al.* 2011; Satya *et al.* 2011). There is a growing demand for processed and packaged bamboo shoots in the national and international market as the shelf life of freshly harvested shoots is only 2-3 days. North-East region of India with a total bamboo cover of 24,110 sq km and standing stock of 42.74 million tones has great opportunity to develop bamboo shoot canning and processing industry in the region to meet the growing demands both at home and outside the country. Presently, the world market for bamboo shoots is of around US \$ 10 billion which will be doubled in 2015. India in general, and North-East region in particular, has the opportunity to develop bamboo shoot canning and processing industry in a big way to exploit the untapped and neglected natural resource for good health, food security and income generation for the people of North-East India.

References

- Akao, Y.; Seki, N.; Nakagawa, Y.; Yi, H.; Matusumoto, K.; Ito, Y.; Ito, K.; Funaoka, M.; Maruyama, W.; Naoi, M.; Nozawa, Y. 2004. A highly bioactive lignophenol derivative from bamboo lignin exhibit a potent activity to suppress apoptosis induced by oxidative stress in human neuroblastoma SH-SY5Y cells. *Bio and Med Chem.*, 12, 4791-4801.
- Barooah, C.; Borthakur S. K. 2003. Diversity of Bamboos in Assam. Bishen Singh Mahendra Pal Singh, Dehra Dun.
- Bhatt, B.P.; Singha, L.B.; Sachan, M.S.; Singh, K. 2004. Commercial edible bamboo species of the north-eastern Himalayan region, India. Part I: Young shoot sales, *J Bamboo Rattan*, 3(4), 337–64.
- Bhatt, B.P.; Singha, L.B.; Sachan, M.S.; Singh, K. 2005. Commercial edible bamboo species of the North-Eastern Himalayan region, India. Part II: fermented, roasted, and boiled bamboo shoots sales. *J. of Bamboo and Rattan*, 4(1), 13-31.

- Bhatt, B.P.; Singha, L.B.; Singh, K.; Sachan, M.S. 2003. Some commercial edible bamboo species of North-East India: Production, Indigenous uses, Cost- benefit and Management Strategies, J. Am. Bamboo Soc., 17, 4-20.
- Bhaumik, M.; Hynniewta, T.M. 2007. Status of Bamboo identification-cum distribution as Basis for promotion of Bamboo sector in Meghalaya. Paper presented in workshop “Development of Suitable Strategy for Promotion of Bamboo sector in Meghalaya 21st-22nd August, 2007, Shillong.
- Biswas, S. 1988. Studies on bamboo distribution in north-eastern region of India, Indian For., 144, 514-531.
- Chatterjee, S; Saikia, A; Dutta, P; Ghosh,D; Pangging, G; Goswami, A.K. 2006. Biodiversity significance of Northeast India. Working paper prepared for the World Bank and Ministry of Development of the Northeastern Region (MODONER), Government of India.
- Chen, C.J.; Qiu, E.F.; Huang, R.Z.; Fan, H.H.; Jiang, J.X. 1999. Study on the spring shoot nutrient content of *Phyllostachys pubescens* of different provenances. J Bamboo Res 18:6–11.
- Choudhury, D.; Sahu, J.K.; Sharma, G.D. 2011. Value addition of bamboo shoots: a review. J. Food Sci. Tech. Doi 10.1007/s13197-011-0379-z.
- Daphne, L. 1996. Bamboo shoots: delicious to eat, easy to sell. Washington Tilth, Autumn. pp 7-9.
- Devi, D.L.; Devi, S. 1995. Ethnobotanical study of Bamboos of Manipur. Industrial Printing Works, Keisamthong, Imphal.
- Farooque, N.A.; Dollo, M.; Kala, C.P. 2007. Traditional wisdom of Apatani Community in the management and sharing of natural resources in North Eastern India. In: Mishra KK (ed) Traditional knowledge in contemporary societies: challenges and opportunities. Pratibha Prakashan, Delhi. pp 110-126.
- Fujimura, M.; Ideguchi, M.; Minami, Y.; Watanabi, K.; Tadera, K. 2005. Amino acid sequence and antimicrobial activity of chitin binding peptides, Pp-AMP 1 and Pp-AMP2, from Japanese bamboo shoots (*Phyllostachys pubescens*). Biosci Biotech Biochem, 69, 642-645.
- Haridasan, K.; Beniwal, B.S.; Deori, M.L. 1987. Bamboos of Arunachal Pradesh: Distribution and utilization. A preliminary appraisal. Arunachal Forester News, 5 (1), 23-27.
- Hiromichi, H. 2007. Use of an antitumor agent. European Patent EP1413208.
- Kotecha, P.V. 2008. Micronutrient malnutrition in India. Let us say “no” to it now. Journal Community Medicine, 33, 9-10.
- Lachance, P.A.; He, Y.H. 1998. Hypocholesterolemic compositions from bamboo shoots. PCT Intl. Patent, PCT/US98/12556.
- Lobovikov, M. 2003. Bamboo and rattan products and trade. J Bamboo Rattan, 2(4), 397-406.
- Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; da Fonseca, G.A.B.; Kent, J. 2000. Biodiversity hotspots for conservation priorities. Nature, 403, 853–858.
- Naithani, H.B. 2007. Bamboos of North East India with special reference to Meghalaya. Paper presented in workshop “Development of suitable Strategy for Promotion of bamboo sector in Meghalaya 21-22nd August, Shillong.
- Naithani, H.B. 2008. Diversity of Indian bamboos with special reference to North-East India. The Indian Forester, 134(6), 765-788.
- Naithani, H.B.V.; Bisht, N.S.; Singsit, S. 2010. Distribution of Bamboo species of Manipur. Forest Department of Manipur, Govt. of Manipur.
- Nimachow, G.; Rawat, J. S.; Dai, Y. 2010. Prospects of bamboo shoot processing in North-East India. Curr. Sci., 98, 288.
- Nirmala C.; Bisht, M.S.; Sheena, H. 2011. Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. Comprehensive Reviews Food Science Food Safety, 10, 153-165.
- Nirmala, C.; David, E.; Sharma, M.L. 2007. Changes in nutrient components during ageing of emerging juvenile bamboo shoots. Int J Food Sci Nut 58:345–52.
- Nirmala, C.; Sharma, M.L.; David, E. 2008. A comparative study of nutrient components of freshly emerged, fermented and canned bamboo shoots of *Dendrocalamus giganteus* Munro. J Am Bamboo Soc 2:33–9.
- Rai, S.N.; Chauhan, K.V.S. 1998. Distribution and growing stock of bamboo in India. Ind For., 124 (2), 89-98.

- Satya, S.; Singhal, P.; Bal, L.M.; Sudhakar, P. 2011. Bamboo shoot: a potential source of food security, *Mediterr J Nutr Metab*, DOI 10.1007/s12349-011-0086-3.
- Sharma, B. D.; Hore, D. K.; Pandey, G.; Wadhwa, B. M. 1992. Genetic resources of bamboos in the NE region of India. *Ind J. For.*, 15(1), 44-51.
- Sharma, T.K.; Borthakur, S.K. 2008. Ethnobotanical observations on bamboo among Adi tribes in Arunachal Pradesh, *Ind J Trad Knowl.*, 7(4), 594-597.
- Shi, Q.T.; Yang, K.S. 1992. Study on relationship between nutrients in bamboo shoots and human health. In: *Bamboo and its Use. Proceedings of the International Symposium on Industrial Use of Bamboo*. International Tropical Timber Organization and Chinese Academy, Beijing, China, pp. 338-346.
- Shukla, U. 1996. *Grasses of North-Eastern India*. Scientific Publishers, Jodhpur, India.
- Singh, H.B.; Kumar, B.; Singh, R.S. 2003. Bamboo resources of Manipur: an overview of Management and Conservation. *J. Bamboo and Rattan*, 2 (1): 43-55.
- Singh, K.T.; Singh, K.J.; Singh, S.S. 2006. Bamboo resources of Manipur. Umang: A souvenir. 75 years of Forestry in Manipur. Forest Department, Govt. Of Manipur, Imphal, pp 21-26.
- Singha, L.B.; Khan, M.L.; Devi, R. 2008. Understanding bamboo sector for income generation, employment opportunity and sustainable development of the North-East India. *Indian Forester* 1147-1155.
- Singha, L.B.; Khan, M.L.; Devi, R. 2008. Understanding bamboo sector for income generation, employment opportunity and sustainable development of the North-East India. *Indian Forester*, 134 (5): 1147-1155.
- Thammincha, S. 1988. Some aspects of bamboo production and marketing. In Rao, I.V.R.; Gnanaharan, R.; and Sastry, C.B. ed., *Bamboo: Current Research*. Proceedings of the International Bamboo Workshop, Cochin, 14-18, November, 1988, KFRI and IDRI, Canada. pp. 320-326
- Warner K. 2008. Gaining much from little: How Minor forest products can have a major impact on Poverty alleviation and promote biodiversity conservation. Proceedings of International workshop on the "Role of NTFPs in poverty alleviation and Biodiversity Conservation". Hanoi, February, 2007.
- Xu, S.; Cao, W.; Song, Y.; Fang, L. 2005. Analysis and evaluation of protein and amino acid nutritional components of different species of bamboo shoots. *Food Sci.*, 26, 222-227.
- Yang, Q.; Duan Z.; Wang, Z.; He, K.; Sun, Q.; Peng, Z. 2008. Bamboo resources, utilization and ex-situ conservation in Xishuangbanna, South-eastern China. *J Forest Resource*, 19, 79-83.
- Yang, Q.; Duan Z.; Wang, Z.; He, K.; Sun, Q.; Peng, Z. 2008. Bamboo resources, utilization and ex-situ conservation in Xishuangbanna, South-eastern China. *J Forest Resource*, 19, 79-83.
- Zhang, Y.; Yao, X.B.; Bao, B.L.; Zhang, Y. 2006. Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (*Caulis bambusae in taeniam*). *Phytother Res.*, 20(10), 872-876.

Study on traditional fermentation process of bamboo shoot and nutraceutical characteristics (A field investigation)

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Abstract

Bamboo shoots form an integral part of the local diet of the North-Eastern states of India. Since ancient times, tribal communities in North-Eastern region have been using bamboo shoot in various forms including fermented products. In order to understand traditional (indigenous) fermentation process and products, a field visit was made in Tinsukia District and Jorhat region of Assam. Samples of fermented products from local market were procured for analytical work. These samples were analyzed for pH, titratable acidity, vitamin C, total phenol content and antioxidant activity by ferric reducing power. It was found that with increased fermentation period pH decreases. The fermented samples also exhibited nutraceutical property in terms of total phenolics, vitamin C content and ferric reducing power. The toxic content in fermented product as determined by picrate method was observed within safe limits for human consumption. Upon fermentation the antioxidant activity present in bamboo shoot coupled with reduced toxic content would be popular among people suffering from lifestyle diseases in the urban sector. However, in terms of developing local entrepreneurship process optimization considering traditional knowledge and modern scientific inputs together has to be done.

1. Introduction

North-Eastern region of India is not just rich in plant diversity but also known to possess a great treasure of cultural, social and linguistic variability preserved by the tribal people. The region is a treasure of indigenous knowledge systems pertaining to agriculture, food, medicines and natural resources. Wild plants have been selected by rural women for food in the local diet (Dutta and Dutta, 2005). Bamboo shoot is one such delicacy being consumed either as vegetables or in curries or as pickles among the tribal communities of the North-East since ancient times (Kumbhare and Bhargava, 2007; Christine and Wetterwald, 1992; Bhargava et al, 1996). Fermented bamboo shoots are very common in the diet of various tribes (Tamang and Sarkar, 1988; Giri and Janmejy, 2000; Tamang and Sarkar, 1996).

Fermentation is one of the oldest and economical ways of preserving foods. In addition to preservation, fermentation process enhances flavor, increases digestibility and improves nutritional value of food. Fermented foods are not only attractive and palatable in terms of flavor, aroma, texture and appearance but are also rich in nutrients and good for digestion (Steinkraus, 1995). Fermentation of bamboo shoots is carried out in different states of the North-Eastern India. The name of the fermented dish varies with the type of bamboo species, period of fermentation and the fermentation technique. In India the fermentation of bamboo shoots has extensively been carried out in the states of Manipur, Meghalaya, Sikkim, Mizoram etc. by using traditional (indigenous) knowledge. Some of the popular fermented bamboo shoot delicacies include *mesu* (Tamang and Sarkar 1988), *soibum* (Singh et al, 2003a; Giri and Janmejy, 2000), *shoidon* and *soijum* (Tamang and Sarkar 1996). Various dishes locally eaten by various tribes of India are compiled in Table 1. Literature survey reveals that studies on traditional fermentation process used by various tribes have got very little attention of researchers. Also nutraceutical properties of fermented bamboo shoots available in local market have not been attempted. Such investigations would help in improving the traditional fermentation process thereby enhancing the quality of products as well as their market potential. Present paper discusses these aspects based on information collected from the tribal areas in North-East.

2. Understanding the fermentation process

Fermentation involving production of lactic acid is generally safe. In this type of fermentation fermentable sugars are converted to lactic acid by organisms such as *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus cerevisiae*, *Streptococcus thermophilus*, *Streptococcus lactis*, *Lacto- bacillus bulgaticus*, *Lactobacillus acidophilus*, *Lacto- bacillus citrovorum*, *Bifidobacterium bifidus* etc. This category is responsible for processing and preserving vast quantities of human food and insuring its safety. Vegetable foods and vegetable/fish/shrimp mixtures are preserved around the world by lactic acid fermentation. Sauerkraut is a classic example of vegetable fermentation (Steinkraus, 1983). Fresh cabbage is shredded and salt is added. Lactic acid bacteria grow in a sequence. First, *Leuconostoc mesenteroides* grow producing lactic acid, acetic acid and CO₂, which flushes out any residual oxygen making the fermentation anaerobic. Then *Lactobacillus brevis* grow producing more acid. Finally *Lactobacillus plantarum* grow producing more lactic acid and lowering the pH to below 4.0. At this pH and under anaerobic conditions, vegetables will be preserved for long periods (Steinkraus, 1997).

2.1 Laboratory process

Fermentation of bamboo shoot under laboratory conditions has been reported by Sarangthem & Singh, 2003 to optimise the fermentation process. Fresh succulent shoots of *B. balcooa* and *D. strictus* bamboo species were collected during the growing season and processed for making slices. The fresh

shoot slices were then subjected to fermentation by adding the inoculum (thin shoot slices obtained from already fermented samples of bamboo shoots processed by traditional way of fermentation). After inoculation, the samples were kept in an incubator at $35 \pm 2^\circ\text{C}$ for a period of 60 days

2.2 Traditional fermentation

In the traditional method, succulent bamboo shoots are defoliated, chopped and packed into a wooden/bamboo basket lined with banana leaves and left for fermentation for 3-6 months. The steps by which fermentation is carried out are shown in Figure 1. Traditionally a hole is made at the bottom of the basket and a bamboo stick is inserted for draining the sap (Mao and Odyuo, 2007). During the period of fermentation, bamboo culms as cylinders having bamboo shoot slices are kept near water stream in such a manner so that water touches the bottom in order to leach out the toxic compound and thus ensuring it to be safe for consumption (Singh et al 2007). The fermented product on maturity is used in cooking with vegetables like potato, colocasia corms, green peas, pumpkins etc or fried fish. They are also eaten as a curry, pickle or soup in different communities (Jeyram et al, 2009).



Figure 1: Flow chart showing steps of traditional fermentation

Table 1: Fermented bamboo shoot products used by various tribes in India

State	Tribe	Species of bamboo	Local name of fermented product	Reference
Arunachal Pradesh	Apatani	<i>Dendrocalamus giagnteus</i> Munro <i>Phyllostachys assamica</i> Gamble ex Brandis, <i>Bambusa tulda</i> Roxb.	Hikhu, Hiring, Hithyi	Singh et al 2007, Tamang and Tamang, 2009
	Adi	<i>Dendrocalamus hamiltonii</i> Nees and Arnott ex Munro, <i>B. balcoa</i> Roxb., <i>D. giagnteus</i> Munro, <i>Phyllostachys assamica</i> Gamble ex Brandis, <i>Bambusa tulda</i> Roxb.	Ekung, Eyup, Eting	Singh et al, 2007, Tamang and Tamang, 2009
Manipur	Meitei	<i>Dendrocalamus hamiltonii</i> , <i>Dendrocalamus</i> <i>sikkimensis</i> , <i>D. giagnteus</i> , <i>Melocana</i> <i>bambusoide</i> , <i>Bambusa tulda</i> and <i>B. balcoa</i>	Soibum, Soidon	Devi and Singh 1986, Jeyaram et al 2009, Tamang and Tamang, 2009, Singh et al, 2007
Meghalaya	Khasi	<i>Dendrocalamus hamiltonii</i>	Lungseij	Agrahar-Murugkar and Subbulakshmi, 2006, Singh et al, 2007, Tamang and Tamang, 2009
Tripura	Barman	—	Godhak	Singh et al, 2007
Darjeeling hills, Sikkim		<i>Dendrocalamus hamiltonii</i> Nees and Arnott, <i>Bambusa tulda</i> Roxb. and <i>Dendrocalamus</i> <i>sikkimensis</i>	Mesu	Tamang and Sarkar, 1996, Tamang and Tamang, 2009

3. Methodology

3.1 Field visit

To study the consumption pattern of bamboo shoots, a field visit to selected places namely Tinsukia District in Dibrugarh and Jorhat in Assam was made. The study was conducted during the month of July 2010 when bamboo shoot is available in abundance. Information was collected on the variety of bamboo shoot products and the processing and preserving technique used in the tribal homes was understood. Information through direct experience of the authors belonging to Assam (Ms. Puspanjali Das) and Arunachal Pradesh (Ms. Phurpa) was found very valuable in this context.

3.2 Sample collection and processing for analysis

Fermented bamboo shoots of *B. balcoa* species were obtained from the local market in the study area. Sample A is a 6 month old fermented product procured from Arunachal Pradesh. Sample B is a 1 month old fermented product procured from the market place of Shillong. The pictures of both the samples are given in Figure 2. The sample was oven dried at $60 \pm 2^\circ\text{C}$ for 8-10 hours and finely powdered for determination of total phenol content. For pH, titratable acidity, vitamin C and toxicity determination undried sample was used. The samples were analyzed in duplicates.

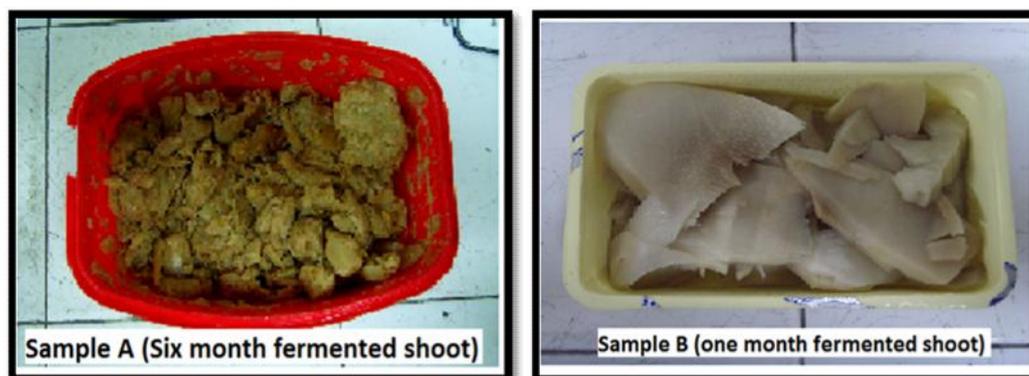


Figure 2: Photographs of fermented Sample A and B

3.2.1 pH and Titratable acidity

A 10 g sample was blended with 20 ml water in a homogenizer, and pH of the slurry was determined using a pH meter (Type HI 2215, Hanna Instruments). Titratable acidity was calculated by titrating the filtrate of the homogenate with 0.1 N sodium hydroxide to an end point of phenolphthalein indicator (0.1% w/v in 95% ethanol (AOAC, 1990))

3.2.2 Vitamin C

Extract 5g of the sample in 10 ml of Acetic-metaphosphoric acid mixture and make up to 100ml. Pipette out 5ml of the supernatant and add 10ml of Acetic-metaphosphoric acid mixture and titrate against the dye 2,6-dichlorophenol indophenol till a light pink colour appears and persists for a second. Similarly titrate the standard solution containing ascorbic acid of 100 $\mu\text{g}/\text{ml}$ concentration (AOAC 1990)

3.2.3 Total Phenol content

One gram of the powdered sample of dried fermented bamboo shoot was extracted with 10 ml methanol in a shaking incubator at 45°C for 2 h. The mixture was centrifuged at 5000 rpm for 10 min and subsequently decanted. The residue was re-extracted as above for 2 h and both supernatants were mixed together. The mixture was concentrated using a rota evaporator and stored at 0°C in freezer

until analyzed for total phenolics. Total phenols were determined by Folin Ciocalteu reagent. A dilute extract (0.5 ml of 1:100 g/ml) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na₂CO₃ (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of gallic acid in methanol : water mixture (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound (Pourmorad et al, 2006)

3.2.4 Ferric reducing power

The ferric reducing power of the bamboo shoot methanolic extract was determined by using potassium ferricyanide–ferric chloride method (Lim et al, 2007). Different dilutions of extracts (corresponding to 1-10 mg/ml of the concentration) were added to 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, after which 2.5 ml trichloroacetic acid (10%) was added. Then 2.5 ml of the mixture was taken and mixed with 2.5 ml water and 0.5 ml 1% FeCl₃. The absorbance at 700 nm was measured after allowing the solution to stand for 30 min. A higher absorbance indicates a higher reducing power.

3.2.5 Cyanide toxicity assay

Cyanogenic Glycoside content was determined using Picrate Paper Kit procured from Dr. J. Howard Bradbury, Division of Botany and Zoology, Australian National University, Australia. Weighed amount (25mg) of the fresh sample was taken and ground using pestle and mortar. The sample was placed in a small flat-bottomed plastic vial. 0.5 ml of the Phosphate buffer (0.1 M) was added. A picrate paper of size 30 mm x 10 mm attached to a plastic backing strip was inserted and the vial was immediately closed with a screw stopper. After about 16-24 hr of incubation at 30°C, the picrate paper was removed and immersed in 5.0 ml water for not less than 30 min and the absorbance was measured at 510 nm (Haque & Bradbury' 2002).

4. Results and discussion

4.1 Field visit

Following important points on the consumption pattern of bamboo shoots as food were noted:

- Bamboo shoots of two species namely *Bambusa balcooa* and *Dendrocalamus hamiltonii* locally named as “Bhaluka” and “Kakoa” respectively were being sold in the local vegetable markets. These are purchased by the local and tribal people.
- It was observed that fermented bamboo shoot is largely being consumed at the home level to make pickles and is also added to meat, fish and vegetable curries to make them more palatable.
- At the home level freshly harvested bamboo shoot is dipped in water for about an hour. The outer sheath is peeled off and the tip is removed. Now the shoot which is tender and white in color is cut into small pieces and grated. Gratings are stored in plastic air tight jars which are then left for fermentation.
- At the commercial level, raw bamboo shoots were being sold by the vegetable vendors. Canned and fermented forms were sold at the Government aided shops.
- As fermentation process proceeds, bamboo shoots become sourer in taste and attain distinctive pungent odor leaving behind the water extract which acts as a preservative thereby increasing the shelf life of the product to months and years.
- Some newer processing practices have been adopted by the younger generation. The conical bamboo basket traditionally used by the tribes has been replaced by the plastic jars. Lining the

cylinder basket with banana leaves is no more into practice. These practices seem to be fading out in cities with more modern and quicker techniques. Now day's local people follow processing steps like dipping shoots in water, removing the tip, slicing into gratings etc. which help in removing the bitterness and thus the toxic compound.

4.2 Chemical analysis, nutraceutical value and toxic content

As mentioned earlier chemical analysis (pH, titratable acidity) nutraceutical value and toxic content was determined in the two fermented samples. Data are given in Table 2 and Fig 3.

4.2.1 pH and titratable acidity

The pH of the 6 month old sample was lower (3.5) as compared to the pH of the 1 month old product (4.2). Acidity was measured on the basis of lactic acid concentration as it is the primary acid produced by lactic acid bacteria during fermentation. As the time of fermentation increases, acidity was found to increase and pH was decreased. Similar pattern was observed in soibum (fermented bamboo shoot from Manipur) by Devi and Singh, (1986) where in the total acid content (% lactic acid) increased tremendously to 0.21% and 0.25% on 3rd and 5th day of fermentation as compared to the raw shoots (0.02%). According to Tamang and Sarkar (1996) the titratable acidity increased from 0.04 to 0.95% upon fermentation resulting in the decline in pH from 6.4 to 3.8.

4.2.2 Vitamin C

Bamboo shoots (*B. tulda* and *D. hamiltoni*) have a higher amount of vitamin C in comparison to the other vegetables like spinach, amaranth, potato and pumpkin except Brassica (Chongtham et al, 2011). Vitamin C content reported by Bhargava et al, (1996) in a few species was 23%. Bhatt et al, (2005) also reported vitamin C content for a number of bamboo species ranging from 3.0 to 12.9%, highest being in *D. hamiltonii* and lowest being in *D. sikkimensis*. Decrease in the vitamin C content upon storage (2.15 %), fermentation (1.09%) and canning (1.8%) was evident as compared to the juvenile shoots (3.28%) in *D. giganteus* as reported by Nirmala et al (2008). A study on pickled garlic also reported loss in ascorbic acid content upon fermentation (Montano et al, 2004).

Table 2: Antioxidant value and Toxic content in fermented shoots

Samples	pH	Titratable acidity (% lactic acid)	Total phenolics (GAE/g dry powder)	Vitamin C (mg/100g fresh wt.)	Cyanoglucosides (ppm)
Sample A	3.5	0.42	137.36	1.18	27.25
Sample B	4.2	0.08	260.94	0.99	30.97

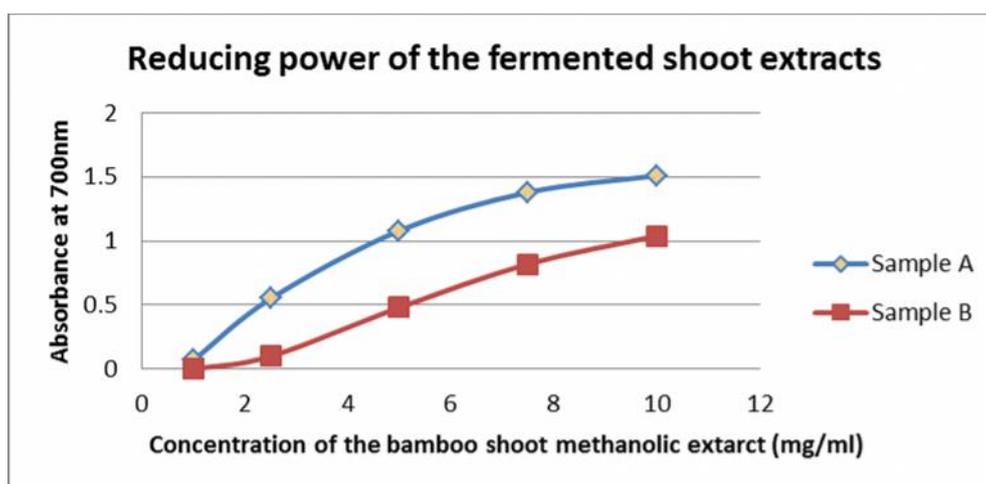


Figure 3: Ferric reducing power (showing antioxidant activity) of the fermented shoot samples

4.2.3 Total phenols

Phenolic compounds, commonly referred to as polyphenols, are secondary metabolites and their distribution is almost ubiquitous (Pereira and others 2009). Eight phenolic compounds namely protocatechuic acid, p-Hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid, syringic acid, p-Coumaric acid and ferulic acid were identified by high-performance liquid chromatography in two species of *P. pubescens* and *P. nigra*. It was determined that the antioxidant capacity was highly correlated with the total phenolic content (Park & Jhon, 2010). In the present study the total phenol content was found to decrease as fermentation increases. The 6 month old sample contains less amount of phenol as compared to the freshly fermented sample. Total phenols in three species of bamboo shoots viz. *Dendrocalamus latiflorus*, *Phyllostachys nigra*, *Bambusa oldhamii* were found to be 31.7, 115, 114 mg per 100 g shoots (Huang et al, 2002). The total phenolic content in *Kaeng kae* and *Kaeng naw mai bai* (northeastern Thai foods) containing 6.9 % and 21.6 % bamboo shoot as the main ingredient was found to be 111.69 ± 1.45 and 60.79 ± 6.57 mg. gallic acid equiv./100g food for *Kaeng kae* and *Kaeng naw mai bai* respectively (Tangkanakul et al 2006)

4.2.4 Antioxidant activity

The presence of reductants such as antioxidant substances causes the reduction of the Fe^{3+} -ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Both the sample were found to possess antioxidant activity as the absorbance was measured to increase with increasing concentration as shown in Figure 3. Few studies report antioxidant potential in raw shoots but antioxidant capacity of fermented shoot still remain unexplored. A recent study reports that methanolic extract of soibum showed significant antioxidant activity ranging from 1.23% to 3.23% when evaluated for antioxidant activity using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (Singh et al, 2011)

4.2.5 Cyanogenic toxicity

Bamboo shoots contain cyanogenic glycoside taxiphyllin, which on hydrolysis yields glucose and hydroxybenzaldehyde cyanohydrin. Benzaldehyde cyanohydrin then decomposes to hydroxybenzaldehyde and hydrogen cyanide. Chronic sub-lethal dietary cyanide has reportedly caused some reproductive effects including lower birth rate, increased number of neonatal deaths, thyroid dysfunction & behavioral defects (FSANZ 2004). Various species of raw bamboo shoots have been reported to contain varying amounts of cyanogenic glycoside content. Cyanogenic glycoside content in *D. giganteus* Munro and *D. hamiltonii* Nees et Arnott have been reported to contain 90-100 mg

HCN/100g fresh weight (Schwarzmaier 1977). Later on, Ferreira et al. (1990) reported that bamboo shoots contain as much as 1000 mg/kg of hydrogen cyanide in the apical part. On the contrary, WHO report (1993) stated that the concentration of cyanide in the immature shoot tip of bamboo was 8000 mg/kg of hydrogen cyanide. A sample of *Dendrocalamus giganteus* contained, on average, 894 mg/kg of hydrogen cyanide (Ferreira et al., 1995). Fermentation was found to be another means to improve the food value by eliminating the cyanogenic glycoside content. The HCN content in the present investigation was found to be as low as 27.25 and 30.97 ppm in samples A and B respectively. In a similar study upon natural fermentation of shoots of *D. giganteus* & *B. Tulda* as the pH drops, the lactic acid bacteria indirectly degrades taxiphyllin into HCN & other components by accumulating acid (Singh and Singh, 1994).

5. Conclusion

In the present study it was observed that bamboo shoot is an integral part of food in the most of the North Eastern communities. It is mostly liked as a fermented dish because of its distinctive odour, flavour and texture and is eaten in the form of pickle or added along with other vegetables. Some newer ways of fermentation have been adopted by the younger generation to save time and thus traditional processing techniques seem to be declining. Traditional ways helped in eliminating the toxic compound which may remain in the newer practices.

Analysis of fermented shoot samples shows that fermentation not only improves the nutritive value of raw shoots but also possess antioxidant activity indicating the nutraceutical potential. It also helped in reducing the toxicity present in raw shoots to the minimal level and thus making it safe for consumption. FAO/WHO Codex Alimentarius has defined a safe limit for human consumption which is 10mg HCN equivalent per kg dry weight. However under the European Union Standards, the maximum permitted levels of HCN are 1 mg/kg in foodstuffs, 1 mg/kg in beverages, with the exception of 50 mg/kg in nougat, marzipan or its substitutes or similar products, 1 mg per percent of alcohol in alcoholic beverages and 5 mg/kg in canned stone fruit. Further intensive research work on this aspect is warranted. Thus fermented bamboo shoot products may find a good market for controlling the life style diseases in urban sector.

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References

- Agrahar-Murugkar, D. and Subbulakshmi, G. (2006). Preparation Techniques and Nutritive Value of Fermented Foods from the Khasi Tribes of Meghalaya. *Ecology of Food and Nutrition*. 45(1):27-38(12).
- AOAC (1990). Official methods of Analysis. 15th ed. Association of Official Analytical Chemists; Washington DC.
- Bhargava, A., Kumbhare, V., Srivastava, A., Sahai, A. (1996). Bamboo parts and seeds for Additional source of nutrition. *J. Food Sci. Technol.* 33(2):145-146.
- Bhatt, B.P., Singh, K. and Singh, A. (2005). Nutritional values of some commercial edible bamboo species of the north Eastern Himalayan Region, India. *Journal of Bamboo and Rattan*. 4 (2):111-124.
- Cho, Y.S.; Yeum, K.J.; Chen, C.Y.; Beretta G.; Tang, G.; Krinsky, N.I.; Yoon S.; Lee-Kim Y.C.; Blumberg J.B. and Russell M.R. 2007. Phytonutrients affecting hydrophilic and lipophilic antioxidant activities in fruits, vegetables and legumes. *J Sci Food Agric*, 87,1096–1107.

- Chongtham N, Bisht M.S., Haorongbam S. 2011. Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and Food Safety* 10 (3):153–168.
- Christine, R., Wetterwald, M.F. (1992). Bamboos. Timber Press Inc., Oregon, USA.
- Devi, S.P., and Singh, H.T. (1986). Studies on the chemical and nutritional changes of Bamboo Shoots during fermentation. *J. Food Sci. Technol.* 23:338-339.
- Dutta BK, Dutta PK. Potential of ethnobotanical studies in North East India: An overview. *Indian Journal of Traditional Knowledge* 2005 , 4:7-14.
- F. Pourmorad1, S. J. Hosseinimehr1, N. Shahabimajd. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology* Vol. 5 (11), pp. 1142-1145, 2 June 2006
- Food Standards Australia New Zealand (FSANZ). (2003). Advice on the preparation of cassava and bamboo shoots. Draft assessment report Proposal P257. http://www.foodstandards.govt.nz/srcfiles/P257_DAR.pdf. Accessed on 25/05/09.
- Giri, S.S., and Janmejay, L.S. (2000). Effect of Bamboo shoot Fermentation and Aging on Nutritional and Sensory Qualities of *Soibum*. *J. Food Sci. Technol.* 37 (4):423- 426.
- Haque, M.R. and Bradbury, J.H. (2002). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chemistry.* 77:107–114
- Huang, L.C.; Lee Y.L.; Huang B.L.; Kuo1 C.I., and Shaw1 J.F. 2002. High polyphenol oxidase activity and low titratable acidity in browning Bamboo tissue culture. *In Vitro Cell. Dev. Biol.—Plant*, 38, 358–365.
- Jeyaram K., Singh Th A., Romi W., Devi AR., Singh W M., Dayanidhi H., Singh N R and Tamang J P. (2009). Traditional Fermented foods of Manipur. *Indian Journal of Traditional Knowledge.* 8(1): 115-121.
- Kumbhare, V., and Bhargava, A. (2007). Effect of processing on nutritional value of central Indian Bamboo Shoots. Part I. *J Food Sci Technol.* 44(1):29-31.
- Lim YY, Lim TT and Tee JJ. Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry Volume 103, Issue 3, 2007, Pages 1003-1008*
- Mao A A. and Odyuo N. 2007. Traditional Fermented foods of the Naga tribes of Northeastern, India. *Indian Journal of Traditional Knowledge.* Vol. 6(1), pp 37(1).
- Montano A, Casado FJ, Castro AD, Antonio HS, and Rejano L. Vitamin Content and Amino Acid Composition of Pickled Garlic Processed with and without Fermentation. *J. Agric. Food Chem.* 2004, 52, 7324–7330
- Nirmala, C., Sharma, M. L., & David, E. (2008). A comparative study of nutrient components of freshly harvested, fermented and canned bamboo shoots of *Dendrocalamus giganteus* Munro. *The Journal of the American Bamboo Society.* 1010 21(1), 33-39.
- Park EJ, Jhon DY. 2010. The antioxidant, angiotensin converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. *Food Sci Techn* 43:655–9.
- Sarangthem, K, Singh, T.N., and Thongam, W. (2003). Transformation of fermented Bamboo (*Dendrocalamus hamiltonii*) shoots into Phytosterols by microorganisms. *J. Food Sci. Technol.* 40 (6):622-625.
- Satya, S., Singhal, S., Prabhu, G., Bal, L.M., and Sudhakar, P. (2009). Exploring the Nutraceutical potential and Food Safety Aspect of Bamboo shoot of Some Indian Species. Proceedings. World Bamboo Congress. 16-19 September, Bangkok.
- Singh A., Singh R.K., Sureja A K. (2007). Cultural significance and diversities of ethnic foods of Northeast India. *Indian Journal of Traditional Knowledge.* 6(1): 79-94.
- Singh S.A, Singh H.D, Nongmaithem R, Bora T.C., and Singh N.R. 2011. Comparative Study of Chemical Properties of Soibum- A Traditional Fermented Bamboo Shoot Product and Its Biological Investigation. *International Journal of Bioscience, Biochemistry and Bioinformatics,* 1, (2): 114-118.
- Singh, R.K.P., Satapathy, K.K. and Singh, K.S. (2003a). Common Fermented food products of Manipur. *Indian Journal of Hill Frmng.* 16(1&2):113-115.
- Singh, S.G. and Singh, L.J. (1994). Release of HCN in Soibum fermentation. *J. Phytol Res.* 7(2):169-170.

- Steinkraus K.H. 1983. Lactic acid fermentation in the production of foods from vegetables, cereals and legumes. *Antonie van Leeuwenhoek* 49:337-348.
- Steinkraus K.H. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. *Food control* 8 (S/6):311-317.
- Steinkraus, K.H. (1995). Handbook of indigenous fermented foods. 2nd Edition. CRC Press (USA).
- Tamang B and Tamang J. P. (2009). Traditional Knowledge of Biopreservation of perishable Vegetable and Bamboo Shoots in Northeast India as food resources. *Indian Journal of Traditional Knowledge*. 8(1): 89-95.
- Tamang, J. P., Sarkar, P.K. (1988). Traditional Fermented foods and beverages of Darjeeling and Sikkim- A Review. *J Sci Food Agric*. 44:375-385.
- Tamang, J. P., Sarkar, P.K. (1996). Microbiology of mesu, a traditional fermented bamboo shoot product. *International Journal of Food Microbiology*. 29(1):49-58.
- Tangkanakul, P.; Trakoontivakorn, G.; Auttaviboonkul, P.; Niyomvit, B. and Wongkrajang, K. 2006. Antioxidant Activity of Northern and Northeastern Thai Foods Containing Indigenous Vegetables. *Kasetsart J. (Nat. Sci.)* 40, 47 – 58.

Session 5. Building material

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Racking performance of traditional and non-traditional engineered bamboo shear walls

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Abstract

Guadua Angustifolia Kunt (G.A.K.) is a bamboo species that has been considered an alternative material for construction in some of the Latin-American countries. In Colombia, specifically in the coffee region, G.A.K. has been used mainly for residential buildings, having *bahareque* (Split bamboo and mortar connected to a *guadua* frame) as the preferred structural system for this type of structures. This system has shown an adequate seismic behavior during earthquakes like the one in *Armenia* (1999), (EERI, 2000). Nowadays, there is not a real understanding of the structural behavior of this kind of bamboo shear walls. For this reason, a study on the seismic performance of engineered *bahareque* shear walls and two kinds of non-conventional G.A.K. shear walls is conducted. Throughout this article, the preliminary results of this study are presented, based on monotonic tests only. So far, 13 tests have been run on the different types of shear walls. In addition, 6 static tests were performed on the connection of the shear walls to the foundation, to understand the capacity and the behavior of this type of joint. The results show that the structural behavior of engineered *bahareque* shear walls is both adequate and better than the one observed on the two kinds of non-conventional shear walls. In spite of that, some changes on the constructive details could improve the structural behavior of these two kinds of shear walls. As for the connection of the shear walls to the foundation, it is observable that the capacity of the joint is related to the resistance of cement mortar. Also two types of failure, identified in this study, affect directly the connection behavior.

Keywords

bamboo, guadua, shear walls, structural behavior.

Introduction

Nowadays, it has been estimated that in Colombia, the housing deficit reaches 3.800.000 homes (DANE 2005); this represents 36% of all the families in Colombia. Due to this problem, it is necessary to find new constructive systems with alternative materials that show an economic advantage over traditional materials used in housing construction like masonry or concrete, even more, in areas where these traditional materials are difficult to find. Moreover, it is important to have in mind that Colombia is a country that has a high level of seismic risk. Recent earthquakes such as the ones in *Popayan* (1984), *Armenia* (1999) and *Quetame* (2008) have caused important economic damages and a large number of deaths.

Guadua Angustifolia Kunt (G.A.K.) is a bamboo species that has been considered an alternative earthquake-resistant material for structural purposes in countries like Colombia, Mexico, Costa Rica, Peru and Ecuador. In Colombia, especially in the coffee zone, G.A.K. has been used in one and two story dwellings. *Bahareque* is the most used structural system for this type of buildings; this is a shear wall-based system that consists in *guadua* frames covered with “*esterilla*” (split *guadua* culm) panels and a steel mesh with mortar topping. This type of system has proven to have a good seismic behavior due to the few damages seen in these buildings after the Armenia earthquake (1999), reported by the Earthquake Engineering Research Institute (EERI 2000) from the United States.

Besides its good seismic behavior (López et al. 2000; González et al. 2005), G.A.K. is a renewable and sustainable material, given its high growth rate (3-4 years old) (Correal et al. 2010). This material has low production costs and is eco-friendly because of the little transformation required to be used in construction industry. Furthermore, previous researches have shown that it has a high resistance-weight ratio. All of these characteristics turn G.A.K. into an alternative, interesting material for construction. Nevertheless, nowadays, there is lack of analytical understanding of the structural behavior of this material, and because of this issue, there are no clear and concerted design methodologies that increase liability on this material and its use. For this reason, the Investigation in Civil Engineering Works and Materials Center (CIMOC) of the *Universidad de Los Andes* in Bogotá, Colombia, has been conducting an investigation project to validate the structural behavior of G.A.K. in its natural hollow-round form. Part of this investigation consists in analyzing and comprehending the seismic behavior of *guadua* shear walls.

This article presents the preliminary results of the study of different types of *guadua* shear walls. These results are focused mainly on monotonic lateral load tests. Main parameters of design such as maximum lateral load capacity, elastic stiffness and ductility of three different types of shear walls have been analyzed. The three different shear walls are: contemporary *bahareque* (braced *guadua* shear wall with a *guadua* diagonal), braced *guadua* shear wall with bars and tensors and braced *guadua* shear wall with plywood. In the first system, the effect of the *guadua* diagonal on the lateral load capacity and on the stiffness of the shear wall is analyzed. Additionally, the structural behavior of the three types of shear walls is compared. Finally, the results of the study of the shear wall-foundation connection are presented, which is essential on the seismic behavior of the shear walls system.

Materials

Guadua

Bamboo, as wheat and rice, is a woody perennial grass that belongs to the Poaceae family. In the world there are around 1.100 bamboo species, 451 of them are located in tropical America (Castaño et al. 2004). G.A.K. is a gigantic bamboo specie, being the biggest one in America and third biggest in the world. G.A.K. is composed of six different parts: rhizome, cepa (bottom), basa (medium), sobrebasa (top), varillón and copo. The stem or culm is the visible part of the *guadua*, it has a conical cylinder form, which is divided by nodes, the distance between the nodes varies along the culm and the cavity between two nodes is known as internode. The diameter and thickness of the *guadua* also vary along the culm, being smaller in the higher parts. Traditionally, the parts of *guadua* that are used in construction are basa and sobrebasa due to the fact that the taper of the culm is smaller in these parts, allowing to obtain geometrically regular structural elements.

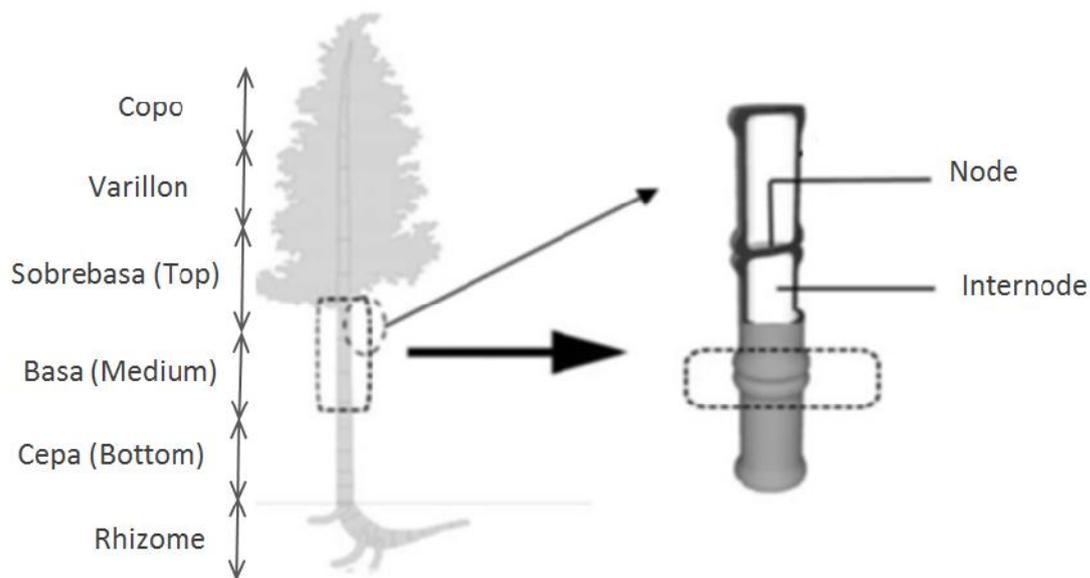


Figure 1. *Guadua* parts

In order to construct the shear walls and the specimens of shear walls-foundation connections, dry and immunized 3 and 4 year old G.A.K., cultivated in the city of *Caicedonia, Valle* (Colombia), located at 1400 m.a.s.l., was used. Specifically, sobrebasa was used (Figure 1) with diameters between 10 and 12 cm. The maximum taper of the diameter of any structural element of the shear wall allowed was 1 cm. The cuts were made at a maximum distance of 5 cm from the nodes.

Shear Walls

Shear walls 2.40 m tall and 1.2 m wide were built, for which frames made of vertical, horizontal and in some cases diagonal *guadua* elements were fabricated. Using traditional methods of construction of connections, cuts at the end of the elements were made with a cylindrical drill (“boca pescado” joint) and using threaded bars of 3/8 inches the elements were connected (Figure 2). Additionally, the internode of each element that was part of a joint was filled with mortar with a fluidity of 153% and an average strength f_c of 12 MPa. On all the connections between the shear walls and the foundation, corrugated bars of 1/2 inches of diameter and f_y of 420 MPa were used. This kind of connection will be explained with more detail further in this article.

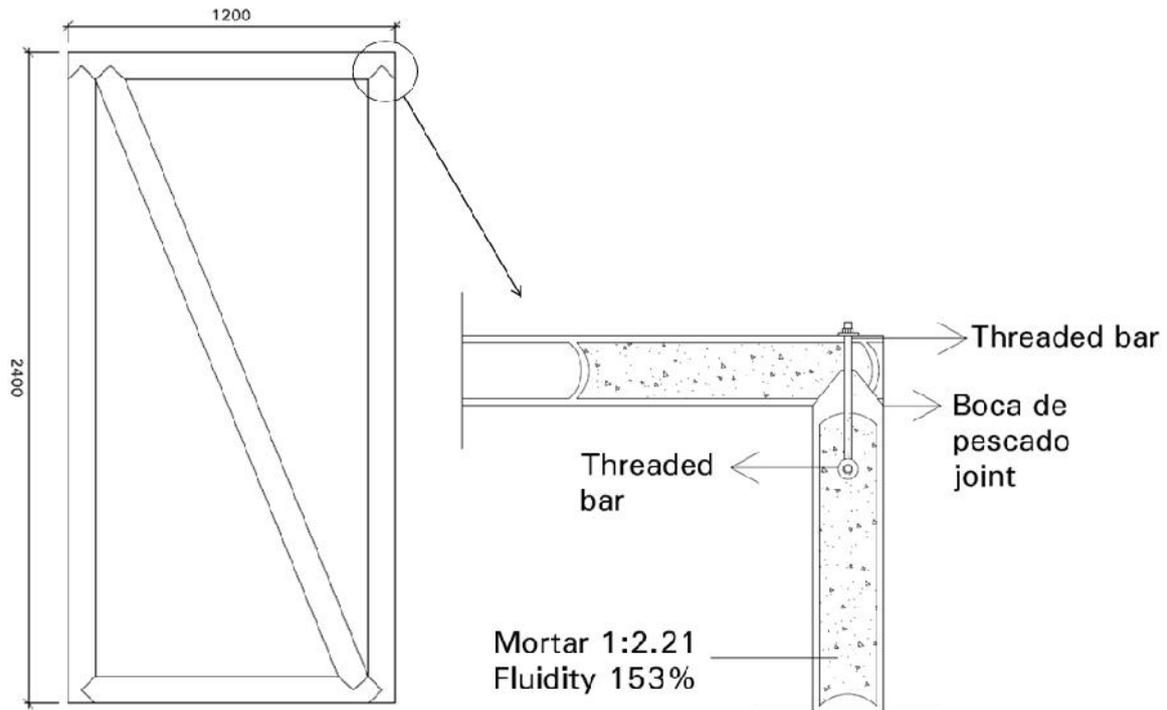


Figure 2. Guadua frame with diagonal and boca de pescado joint (dimensions in mm)

Contemporary *Bahareque* Shear Walls (type Imv)

This type of shear wall is composed of a *guadua* frame with a *guadua* diagonal. Wood ribbons are connected to this frame using screws on each end of the ribbon. These ribbons are placed horizontally every 50 cm and around the entire frame. Using nails and wire, a steel mesh is connected to the wood ribbons. Over the steel mesh, a layer of mortar is applied on both sides. Figure 3 presents a sketch of the composition of this type of shear walls.

To understand the effect of the *guadua* diagonal on the structural behavior of the shear wall, *guadua* frames with *guadua* diagonal (M-I) and contemporary *bahareque* shear walls, which had a vertical element in the frame (m-mv) instead of the *guadua* diagonal, were built and tested. Figure 3 shows the details of these specimens.

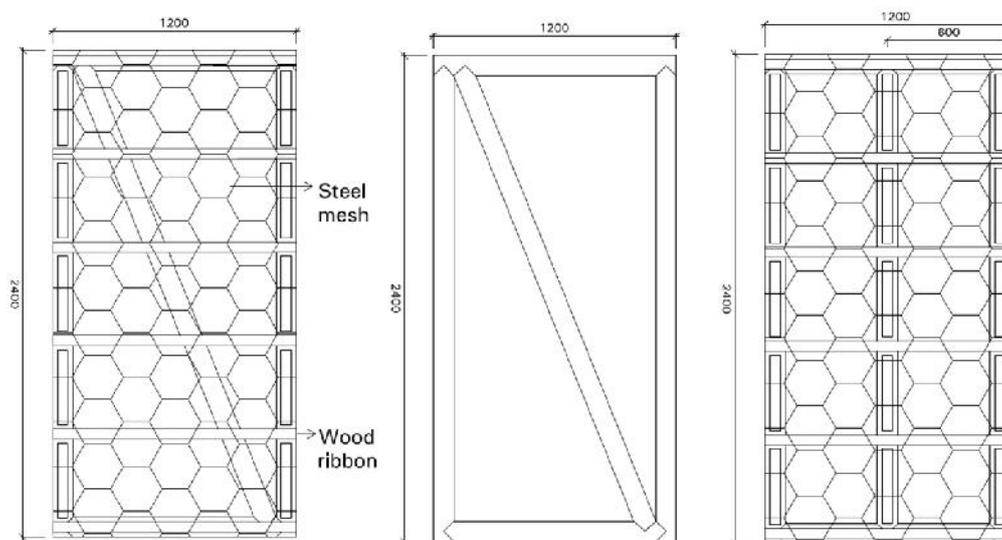


Figure 3. Contemporary bahareque shear wall (dimensions in mm)

Non-conventional Shear Walls

In order to explore other alternatives of bracing *guadua* shear walls, two different systems of braces were tested: braced *guadua* shear wall with bars and tensors (M-II) and braced *guadua* shear wall with plywood (M-III).

Braced *guadua* shear wall with steel bars and tensors is a G.A.K. frame with 4 steel angles, one in each corner, that connect the tensors and the corrugated bars (1/2 inches of diameter and f_y of 420 MPa) to the frame. The steel angles are made of A36 steel and their thickness is 1/4 inches

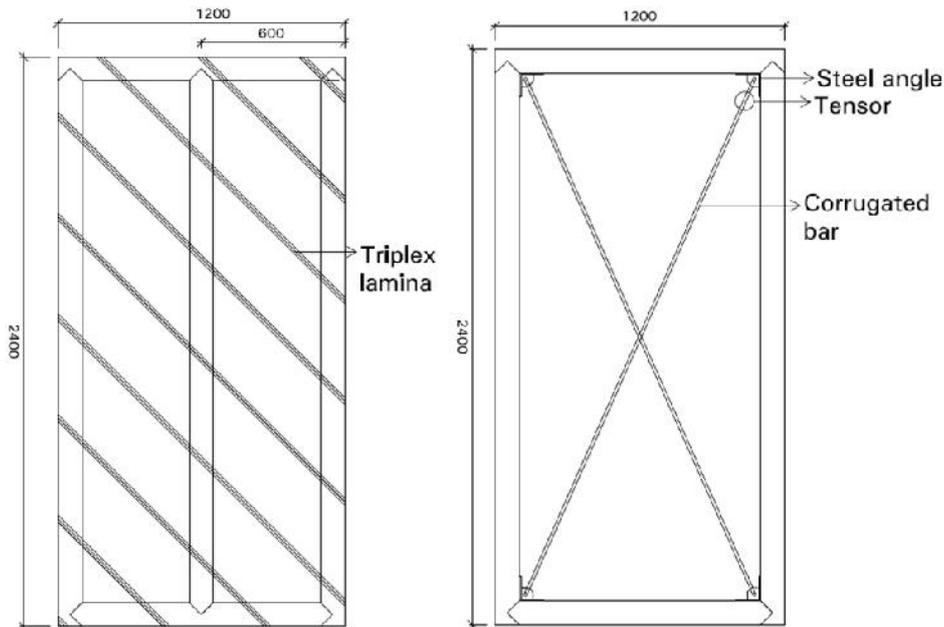


Figure 4. Non-conventional shear walls (dimensions in mm)

Braced *guadua* shear wall with plywood consists of a G.A.K. frame with a vertical *guadua* element in the middle. The triplex lamina is connected to the frame using screws that are placed on every node of the *guadua* elements, which corresponds to a 30 cm screw spacing. A sketch of a plywood shear wall is presented in Figure 4. (Figure 4)

Shear Wall-Foundation Connection

This connection consists in a corrugated bar of 1/2 inches of diameter embedded in mortar. The development length of the bar is between 0.8 m and 1 m, and this distance indicates how many internodes (2 or 3) should be filled with mortar depending on their length. Figure 5 shows this type of connection.

To be able to test this type of joint, specimens consisting in a culm with 3 internodes, two of which were filled with mortar, were constructed, as shown in Figure 5. In order to study the effect of mortar strength on the load capacity of the connection, two different mortar mixes to fill the specimens were prepared, one of the mortar mixes with low strength (around 3 MPa) and the other one with higher strength (around 13 MPa). Six cubes of each mortar mix were tested to verify their strength.

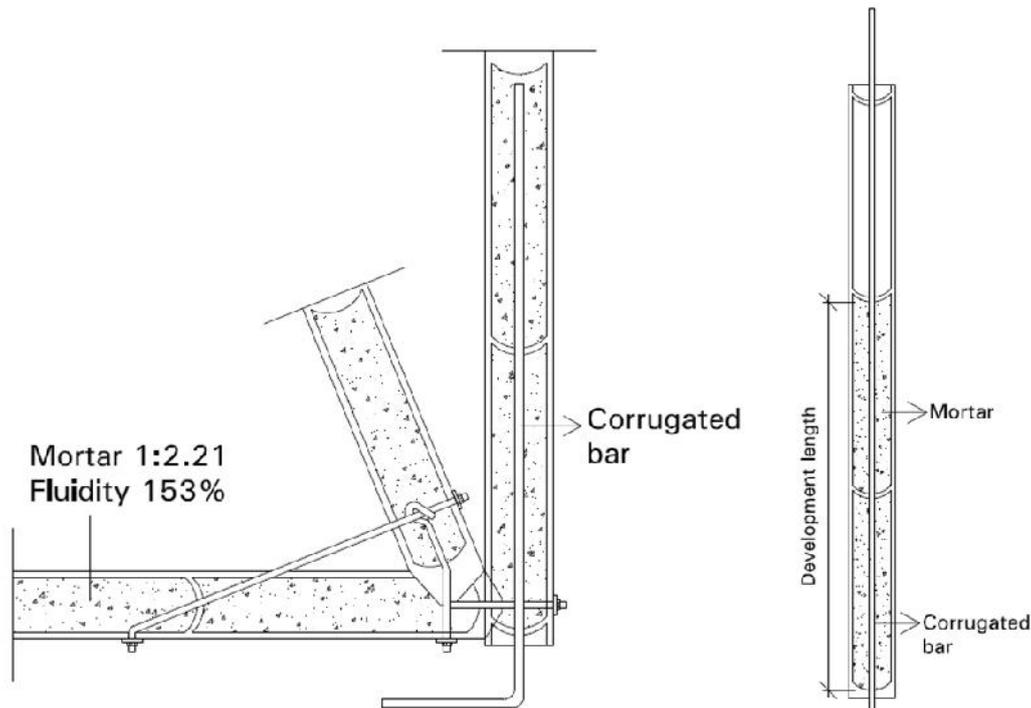


Figure 5. Shear wall-foundation connection and specimen connection

Assembly and Test Procedure

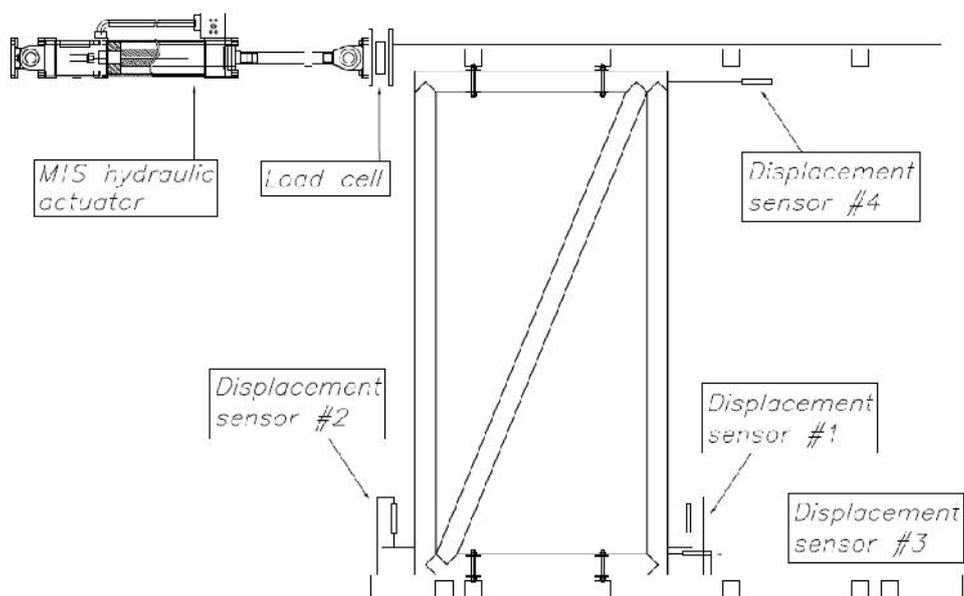
Shear Walls

Table 1 presents the testing schedule. The name corresponding to the type of shear walls is composed of letters and numbers that specify the type of brace and, for those with a diagonal, whether the diagonal worked under tension or compression load during the monotonic test. The type of brace are: M-I for *guadua* diagonal brace, M-Imv for *guadua* diagonal combined with the steel mesh and mortar topping, M-mv for the steel mesh and mortar topping, M-II for the corrugated bars with tensors shear walls and M-III for the plywood shear walls.

The tests were performed following the specifications of the ASTM E 564 (2006). A hydraulic actuator MTS with displacement control (15 mm/min) was used. All of the tests were conducted in the structural models laboratory of the *Universidad de Los Andes*, in Bogotá, Colombia. The typical assembly of the shear walls is shown in Figure 6. The shear walls are connected to a rectangular steel tube which is also connected to the lab strong floor. The instrumentation consists in four displacement sensors and a load cell. Displacement sensors #1 and #2 measure the lift of the shear wall. Displacement sensor #3 measures the lateral displacement of the shear wall on the base, and #4 measures the horizontal displacement of the shear wall where the load is applied. Using a special steel device, the lateral displacement of shear walls was restricted as shown in Figure 7.

Table 1. Shear wall test program

Shear wall type	Lateral load brace	Number of test
DT M-I	<i>Guadua</i> diagonal under tension load	2
DC M-I	<i>Guadua</i> diagonal under compression load	2
DT M-Imv	<i>Guadua</i> diagonal under tension load + steel mesh with mortar topping	1
DC M-Imv	<i>Guadua</i> diagonal under compression load + steel mesh with mortar	1
M-mv	Steel mesh and mortar	3
M-II	Corrugated bars diagonals	2
M-III	Plywood	2

**Figure 6. Assembly and instrumentation of shear wall test****Figure 7. Steel device to restrict lateral shear wall displacement**

Shear Wall-Foundation Connection

Six static tests were performed. For these tests a universal MTS machine with a load cell was used. The tests were performed with displacement control (15 mm/min). Figure 8 shows a sketch of the test assembly.

On all the specimens, two internodes were filled, reason why the development length would vary according to the length of the specimen internodes.

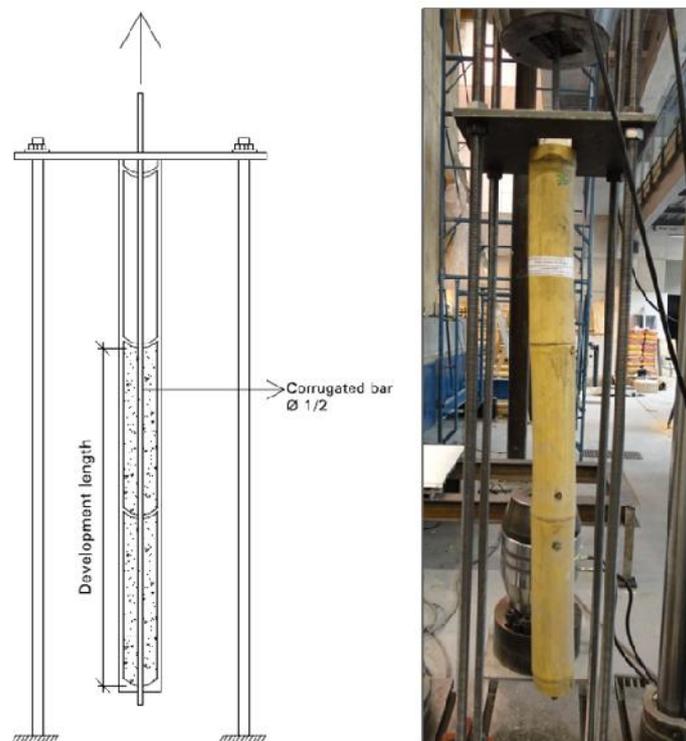


Figure 8. Assembly and instrumentation of shear wall-foundation connection test

Experimental results

Shear Walls

The parameters calculated in the monotonic tests of the shear walls were: maximum lateral load capacity with its respective story drift, stiffness in the elastic range and displacement ductility.

The stiffness and ductility were calculated from the Equivalent Energy Elastic-Plastic curve (EEEP), as indicated in the ASTM E 2126 (2009). This curve is an ideal elastic-plastic curve circumscribing an area equal to the area enclosed by the experimental load-displacement curve. Table 2 presents the average values of the calculated parameters for each type of shear wall.

Shear Wall-Foundation Connection

Table 3 presents the values of maximum load in each joint, the average value of the external diameter of the *guadua*, the development length and the average stress value f_c of the mortar mix of each specimen.

Table 2. Experimental results of shear wall tests

Shear wall type	Maximum lateral load [kN]	Story drift at P_{max} [%]	Stiffness [kN/mm]	Ductility
M-I DT	4.2	6.2%	0.13	---
M-I DC	9.9	5.5%	0.32	---
M-Imv DT	12.3	2.0%	1.15	9.1
M-Imv DC	13.0	2.9%	0.93	7.2
M-mv	10.9	2.8%	0.65	6.8
M-II	7.3	1.6%	0.41	2.9
M-III	7.7	3.5%	0.28	4.3

Table 3. Experimental results of shear wall-foundation connection tests

Specimen	Diameter [mm]	Development length [mm]	Max. load [kN]	Mortar mix	f'_c [MPa]
1-E	88	848	14.8		
2-E	110	993	20.9	I	2.85
3-E	116	935	21.2		
4-E	94	801	29.8		
5-E	102	794	40.7	II	11.34
6-E	102	975	50.2		

Results Analysis

Shear Walls

Effect of the Diagonal on the Contemporary *Bahareque* System

Table 2 shows how the frame with *guadua* diagonal (M-I) presents a better structural behavior, in terms of lateral load capacity and stiffness, when the *guadua* diagonal works under compression loads than when it works under tension loads. This occurs because the connection of the diagonal to the frame is fragile under tension loads due to the low capacity of *guadua* to resist tension stresses perpendicular to the grain.

Figure 9 presents the average behavior curves of three types of shear walls: contemporary *bahareque* shear walls (with *guadua* diagonal under tension and compression loads) (M-Imv), the *bahareque* shear wall that has a vertical *guadua* element in the middle of the frame (M-mv) instead of the *guadua* diagonal, and the *guadua* frame with a *guadua* diagonal under compression (M-I).

Based on Figure 9, the structural behavior of contemporary *bahareque* is symmetric and does not depend on the load direction applied to the diagonal element. When comparing the behavior curve of the *guadua* frame with diagonal, to the one of contemporary *bahareque*, it is clear that the steel mesh and the mortar topping increase the system stiffness in 69% and the maximum lateral load capacity in a 40%, with a story drift of approximately 2.5%.

On the other hand, the mv shear wall presents a good structural behavior, close to the contemporary *bahareque*. This proves that it would be possible to leave aside the *guadua* diagonal of this system,

allowing the lateral load to be resisted by the screws (connection between the frame and the sheathing: steel mesh and mortar topping).

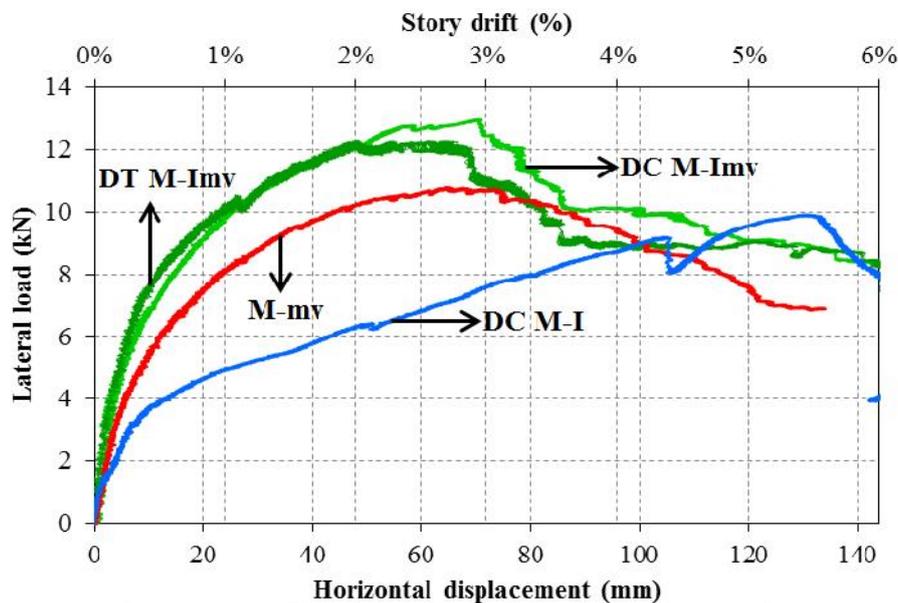


Figure 9. Structural behavior curves of shear walls I, Imv and mv

Based on Figure 9, the structural behavior of contemporary *bahareque* is symmetric and does not depend on the load direction applied to the diagonal element. When comparing the behavior curve of the *guadua* frame with diagonal, to the one of contemporary *bahareque*, it is clear that the steel mesh and the mortar topping increase the system stiffness in 69% and the maximum lateral load capacity in a 40%, with a story drift of approximately 2.5%.

On the other hand, the mv shear wall presents a good structural behavior, close to the contemporary *bahareque*. This proves that it would be possible to leave aside the *guadua* diagonal of this system, allowing the lateral load to be resisted by the screws (connection between the frame and the sheathing: steel mesh and mortar topping).

Structural Behavior of All Types of Shear Walls

Figure 10 presents the average behavior curves of all types of shear walls. It shows that contemporary *bahareque* system is the type of shear wall with the best structural behavior in terms of maximum lateral load capacity, stiffness and displacement ductility (Table 2).

Bahareque shear wall without a *guadua* diagonal (M-mv) presents a maximum lateral load capacity 14% below the one of the contemporary *bahareque* (with a *guadua* diagonal), and a stiffness 36% lower. Nevertheless, its behavior is adequate.

The braced *Guadua* shear wall with plywood (M-III), compared to the previous two shear walls presents a low lateral load capacity, stiffness and ductility. The failure mode observed in this type of shear walls was the tearing of the plywood due to the screw, as shown in Figure 11.

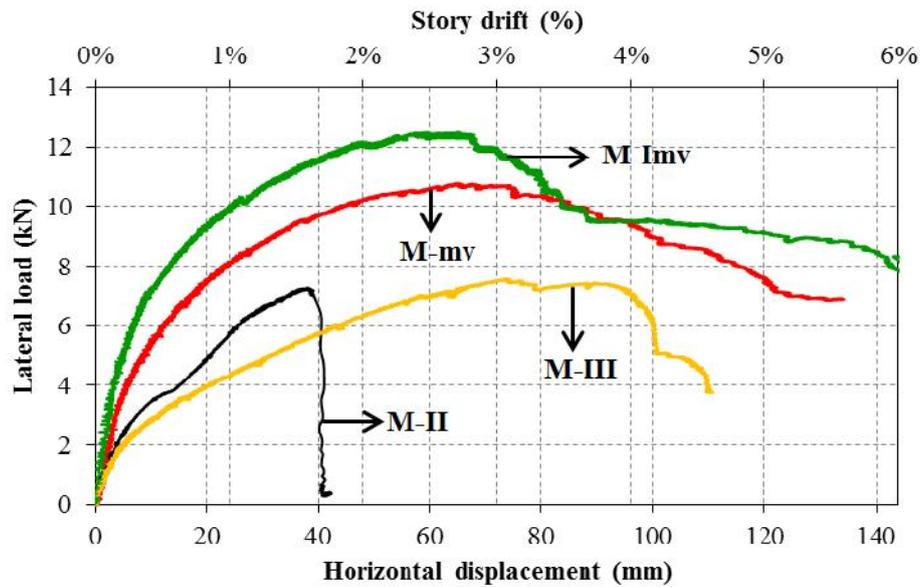


Figure 10. Structural behavior curves of guadua shear walls



Figure 11. Failure mode of shear wall III

Given this failure mode, it is possible to improve the structural behavior of this type of shear wall, by increasing the number of screws that connect the plywood to the frame. In wooden shear walls with plywood, the minimum recommended nail spacing is 15 cm, which is half of the one used in *guadua* shear walls.

The braced *guadua* shear wall with bars (M-II) presents a fragile behavior curve, which is reflected on the low ductility value presented on Table 2. Nevertheless, its maximum lateral load capacity is quite close to the one of the braced *guadua* shear wall with plywood and presents twice the stiffness. This fragile behavior is directly related to the failure mode of the shear wall. This type of shear wall failed because the tensor broke suddenly in a fragile way (Figure 12) and it did not allow the shear wall to develop its whole potential. To improve this constructive detail, higher quality tensors may be used, but it could become an important increase in the shear wall cost. For this reason, a different solution has been considered in which tensors would not be needed, although it is not yet entirely designed.

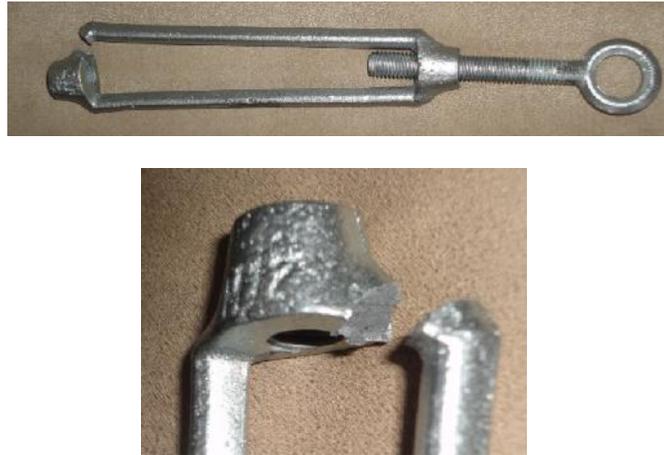


Figure 12. Failure mode of shear wall II

Shear Wall-Foundation Connection

Table 3 presents the average load capacity of the connections for every type of mortar mix. These values show that the influence of the mortar strength f'_c on the load capacity of the joint is crucial. Connections where 11.34 MPa strength mortar was used, showed an average load capacity twice the one in the connection with 2.85 MPa strength mortar. Nevertheless, the load capacity values of the connections for each mortar mix are scattered (Table 3), due to the variable geometry of the specimens (diameter and length internode of the *guadua*) which is difficult to control, being a raw material.

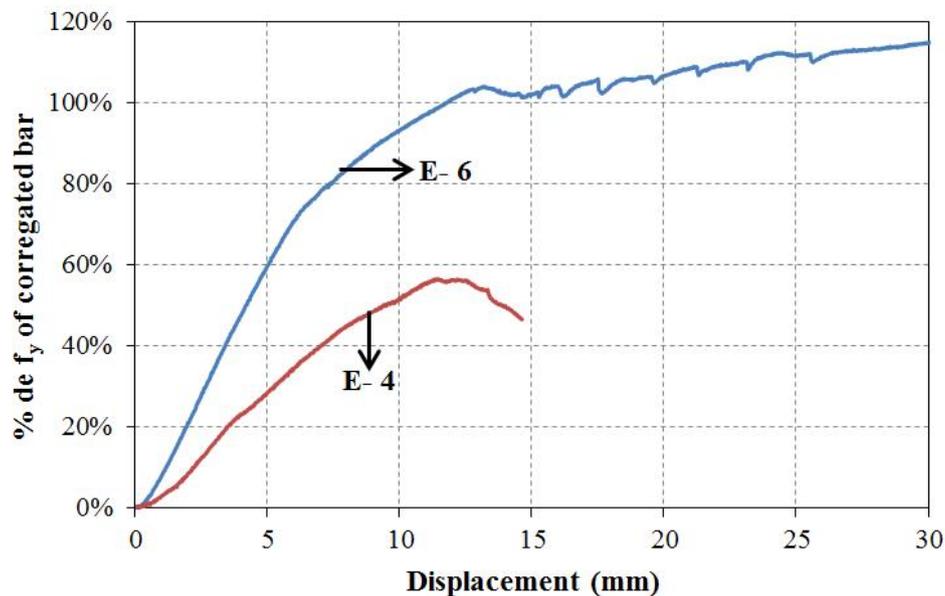


Figure 13. Structural behavior curves of shear wall-foundation connection

Besides the load capacity, the joint behavior is important. The ideal connection is one that allows to develop all the capacity of the bar, taking it further than its yielding stress. That way the failure mode in the joint is controlled by the ductile failure of the bar, instead of the fragile failure of the mortar. Figure 13 shows the behavior curve of two specimens built with 11.34 MPa strength mortar. The difference in terms of their load capacity is due to the diameter of the *guadua* (bigger area of mortar) and to the development length of the bar, presenting an elastic-plastic behavior with an important

ductility, as the specimen E-4 is able to develop only 56% of the capacity of the bar and its behavior is fragile.

Conclusions

The important conclusions of this preliminary study are:

1. Frames with a *guadua* diagonal (M-I) present a better structural behavior when the diagonal element works under compression loads rather than under tension loads. Nevertheless, once this frame is sheathed with a steel mesh and mortar topping (M-Imv), the behavior of the shear wall becomes symmetric for both tension and compression, and increases its stiffness in 69% and the lateral load capacity in 14%, with an approximate story drift of 2.5%, regardless the load direction in the diagonal element.
2. Due to the fact that *bahareque* shear wall with no diagonal presented a good structural behavior, it was proven that the screws (connection between the frame and the sheathing: steel mesh and mortar topping), are able to resist the lateral load and develop a ductile behavior, without the need of a diagonal element as part of the system.
3. The braced *guadua* shear wall with plywood can improve its structural behavior if the screw spacing shortens. Moreover, it is possible that the lateral load capacity of this system is controlled by fasteners spacing.
4. The braced *guadua* shear wall with bars and tensors showed the worst structural behavior when compared to all the other types of shear walls, due to the sudden fragile failure of the tensor. Nevertheless the curve prior to the failure showed good behavior, being clear that the stiffness is higher than the one of the braced *guadua* shear wall with plywood, and reaches its lateral load capacity.
5. The shear wall-foundation connection should develop the yielding capacity of the bar, to accomplish a ductile behavior. Otherwise, the mortar failure leads to a fragile behavior of the shear wall, which turns the structure into an insecure and dangerous one upon seismic event.
6. The influence of the mortar strength on the load capacity of the connection must be studied in greater depth. Moreover, the effects of the diameter of the *guadua* and the development length on the capacity of the joint, to be able to propose minimum dimensions that assure a good behavior of the connections, must be considered for further study.
7. Out of all the types of shear walls, contemporary *bahareque* presented a higher lateral load capacity, stiffness and ductility. Nevertheless, it is necessary to improve constructive details of each of the other shear walls systems proposed, in order to have a better understanding of their potential.

Acknowledgements

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References

- AIS Asociación Colombiana de Ingeniería Sísmica (2010). Reglamento Colombiano de Construcción Sismo-Resistente NSR-10.
- AIS Asociación Colombiana de Ingeniería Sísmica, FOREC Fondo para la Reconstrucción y el Desarrollo del Eje Cafetero (2002). Estudio de Vulnerabilidad Sísmica, Rehabilitación y Refuerzo de Casas de Bahareque.
- AIS Asociación Colombiana de Ingeniería Sísmica, FOREC Fondo para la Reconstrucción y el Desarrollo del Eje Cafetero (2001). Manual de Construcción Sismo Resistente de Viviendas de Bahareque Encementado.
- ASTM International (2006). Standard Practice for Static Load Test for Shear Resistance of Framed Walls for Buildings, ASTM E564-06.
- ASTM International (2009). Standard Test Methods for Cyclic (Reversed) Load Test for Shear Resistance of Vertical Elements of the Lateral Load Resisting Systems for Buildings, ASTM E2126.
- Castaño F. and Moreno R.D. (2004). Guadua para todos cultivo y aprovechamiento.
- Correal, J.F. and Arbeláez J. (2010). Influence of age and height position on Colombian Guadua Angustifolia Bamboo mechanical properties. *Maderas ciencia y tecnología*, 12(10), 105-113.
- DANE Departamento Administrativo Nacional de Estadística (2005). *Colombia. Censo General 2005. Muestra Cocensal. Déficit de Vivienda* [online]. Retrieved on June 15, 2010 from: http://www.dane.gov.co/index.php?option=com_content&view=article&id=473&Itemid=66.
- EERI Earthquake Engineering Research Institute (2000). El Quindío, Colombia, South America earthquake, January 25, 1999.
- González, G. and Gutiérrez, J. (2005). Structural Performance of bamboo bahareque walls under cyclic load. *Journal of Bamboo and Rattan*, 4(4), 353-368.
- López, L.F. and Silva M.F. (2000). Comportamiento Sismo Resistente de Estructuras en Bahareque. Proyecto de grado, Universidad Nacional de Colombia, Departamento de Ingeniería Civil.

Experimental study of Glued Laminated Guadua bamboo panel as an alternative shear wall sheathing material

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Abstract

Taking into account the increasing forest demand for wood, there is a global need to find alternative energy-efficient, renewable and eco-friendly construction materials. Giant bamboo like *Guadua angustifolia kunt* emerges as an interesting construction material, since past study conducted at the Universidad de los Andes in Bogotá-Colombia reported that Glued Laminated Guadua bamboo (GLG) has mechanical properties comparable to those of the best structural timbers in Colombia. Potential applications of GLG include not only laminated beams and columns, but also structural panels to be used as a sheathing material for wood frame shear walls.

A comprehensive experimental study has been performed on GLG sheathed shear walls in order to find an alternative sheathing material for woodframe buildings. A series of tests were conducted on full-size shear wall specimens in order to study the influence of the wall aspect ratio and the edge nail spacing on the shear wall performance. Based on cyclic tests on shear walls, it was found that the stiffness and maximum load carrying capacity of the wall increases as edge nail spacing decreases. In contrast, the displacement ductility capacity decreases, since the rotation of the panels is restricted when the edge nail spacing is reduced. Experimental results also revealed that stiffness, maximum load capacity, and ductility of the GLG sheathed shear walls are not affected by the aspect ratio of the wall. The final stage of the present study included dynamic shake-table tests on full-size one and two-story housing units using GLG sheathed shear walls. Results showed that the units had similar performance characteristics to those of OSB and Plywood sheathed shear walls, and it was concluded that wood-GLG combination could be a viable construction alternative from a structural point of view.

Keywords

Laminated bamboo, sheathing material, shear wall, bamboo panels.

Introduction

Some species of bamboo have been used as a structural material for building since these have shown excellent physical and mechanical properties. *Guadua Angustifolia kunt* is a South American Bamboo species that not only exhibits suitable physical and mechanical properties for construction of structures, but also has fast growing rate since the mature age is reached between 3 to 4 years old (Correal et al. 2010). In order to overcome the cross section limitation of the round bamboo, Glued Laminated Guadua (GLG) bamboo products have been developed. Preliminary research show that GLG has very good mechanical properties when compared to the best structural timbers in Colombia (López et al. 2009), which make GLG an alternative, renewable and sustainable material besides wood. A possible application of GLG in wood frame buildings could be in the form of structural sheathing used in the construction of shear walls, which are typically made of Oriented Strand Board (OSB) or plywood.



Figure 1. Glued Laminated Guadua (GLG) panel

Structural panels' sheathing is the key element in the lateral force resisting system of woodframe structures, since it provides the lateral stiffness and strength of Light-Frame Systems subjected to earthquake or wind forces. Extensive experimental research has demonstrated that the performance of wood frame shear walls is essentially governed by the behavior of sheathing-to-framing connections (nail diameter and spacing, type of sheathing material) and shear wall aspect ratio. Nonetheless, wood Light-Frame Systems have been used extensively in North American and Europe, and alternative materials like bamboo could be an interesting possibility to explore in order to reduce the pressure on the forest.

The Universidad de los Andes at Bogotá, Colombia, conducted a comprehensive study of the structural potential of Glued Laminated Guadua *Angustifolia kunt* Bamboo (GLG) as a construction material. As part of this research, monotonic and cyclic lateral load tests were conducted on shear walls sheathed with GLG panels with aspect ratios of 1:1 and 2:1 and three different sheathing nail schedules. In order to evaluate the performance of GLG panels within an actual structural system, a series of shake table tests were conducted to simulate earthquake-loading conditions on a 2-story woodframe housing module with shear walls sheathed with GLG panel.

Shear Wall Test

Variables Studied

Aspect ratios (height: length) of 1:1 and 2:1 were studied. A typical shear wall segment is sheathed with a panel of 2.4 m x 1.2 m (8 ft x 4 ft), which is the minimum aspect ratio (2:1) permitted by USA design codes at high seismic zone (American Forest and Paper Association 2008). The aspect ratio of

1:1 corresponds to standard dimensions established by ASTM E-72 (ASTM International 2010) and adopted by APA (The Engineered Wood Association) in order to obtain design capacities of wood shear walls in the United States. Moreover, the aspect ratio of 1:1 has been used in most of the experimental research done in shear walls. Traditionally, design practice of shear walls has considered that the capacity of shear walls with aspect ratio less than 2:1 has a direct proportion with the length of the wall, since failure mode is similar between shear walls with aspect ratios less or equal to 2:1 (Salenikovich et al. 2003a, 2003b).

Edge nail spacing of 152 mm (6 in), 76 mm (3in) and 51 mm (2in) were considered on this study. Nail spacing on the panel fields was 304.8 mm (12 in). These nails spacing correspond to typical spacing used in construction of shear walls on wood Light-Frame Systems.

Materials

Glued laminated Guadua panels were fabricated by Colguadua Ltda. GLG panels have three 3-mm layers arranged in a cross-ply configuration which give a total thickness of 9 mm (3/8 in). The overall dimensions were 1.2 m by 2.4 m (4 ft x8 ft) with average specific gravity of 0.72. In addition, OSB and Plywood panels of 1.2 m x 2.4 m x 9 mm were tested for comparison purposes. Common nail fabricated by Caballo Ltda. with 63.5 mm (2.5 in) in length, 3.05 mm (0.12 in) in diameter (2 1/2" x 11 denomination) were used. These nails are equivalent to 8d common nails used in the construction of wood shear walls. All wood frame and studs were built using Chilean Radiata Pine framing members (MSD 2 in x 4 in) produced by the Chilean company ARAUCO, with an average specific gravity of 0.42, a similar to the specific gravity of Spruce-Pine Fir typically used in the construction of wood shear walls in USA.

Test Program

Table 1 presents the test program of the shear walls with the variables studied (aspect ratio, edge nail spacing, (S_{en}), loading type (monotonic or cyclic) and number of tests.

Table 1 – Shear wall test program

Set	Aspect Ratio (AR)	S_{en} mm (in.)	Loading type	Number of tests
1	1:1	152 (6)	Monotonic	1
			Cyclic	2
2	1:1	76 (3)	Monotonic	1
			Cyclic	2
3	1:1	51 (2)	Monotonic	1
			Cyclic	2
4	2:1	152 (6)	Monotonic	1
			Cyclic	2
5	2:1	76 (3)	Monotonic	1
			Cyclic	2
6	2:1	51 (2)	Monotonic	1
			Cyclic	2
7	1:1 (OSB)	152 (6)	Monotonic	1
			Cyclic	2
8	1:1 (Plywood)	152 (6)	Monotonic	1
			Cyclic	2

All shear walls were constructed at the Large Scale Structural Laboratory at Universidad de Los Andes. Shear walls test setup is shown in Figure 2. The lateral load was applied through a structural steel tube connected to servo-controlled MTS hydraulic actuator with a 152 mm (6 in.) stroke. The structural tube was attached with bolts to the top plate of the shear wall. The top of the shear wall was restrained laterally in order to avoid failure perpendicular to the plane. The bottom of the shear wall was fixed to the strong floor of the lab in order to have a cantilever shear wall test setup. A total of 7 instruments were used to record lateral load, lateral displacement at top and bottom of the wall, vertical displacement at the bottom and strain deformation at hold-downs.

Monotonic test were performed following the recommendations in ASTM E564 with displacement velocity control of 15mm/min (0.59 in/min). Even though this type of test gave a good approximation of the overall behavior of the shear walls, this test was primarily used to define the displacement amplitudes of the cyclic test based on ASTM E2126 (ASTM International 2009). Monotonic and cyclic test were conducted until a story drift of 6.5% was reached, equivalent to a top displacement of 152 mm (6 in). Figure 3 shows the test protocol of monotonic and cyclic tests.

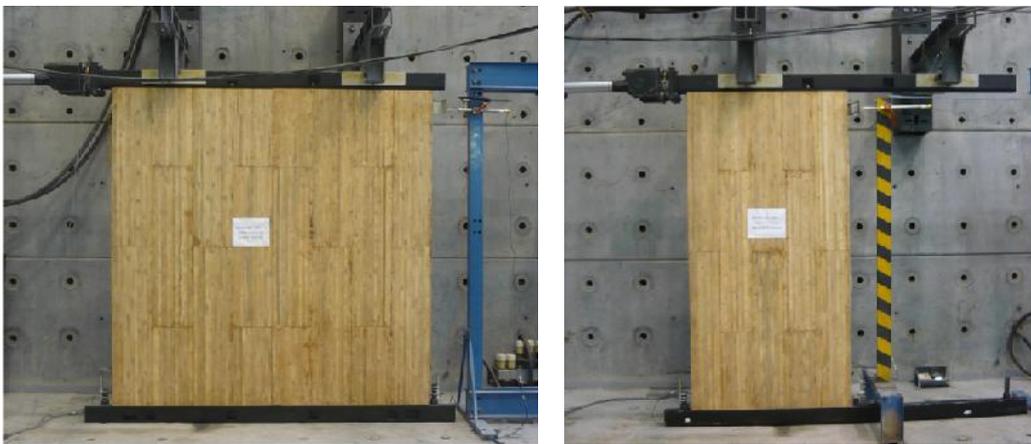


Figure 2 – Shear walls test setup. Left: 1:1 aspect ratio wall. Right: 2:1 aspect ratio wall.

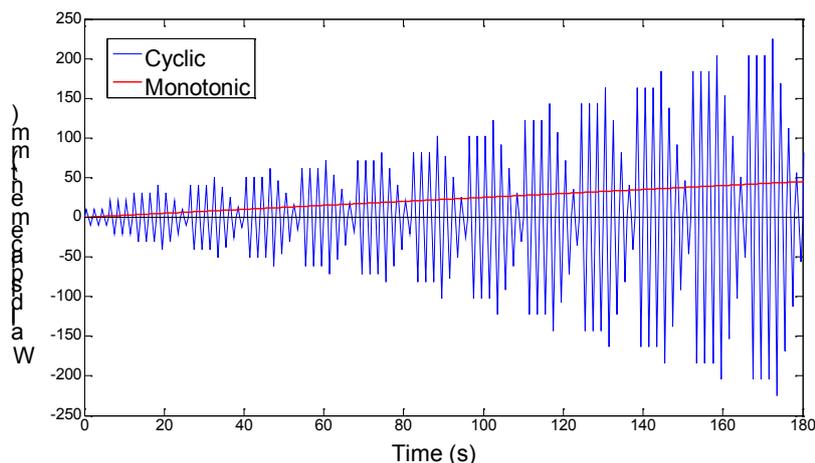


Figure 3. Wall displacement history used in monotonic and cyclic tests

Test Results

Figure 4 shows a typical load-deflection curve of the shear walls subjected to cyclic load. An envelope curve was obtained based on the maximum force and the corresponding displacement point at each cycle. Based on the envelope curve, an idealized elastic-plastic model was developed using equivalent energy method. Figure 4 shows the Equivalent Energy Elastic-Plastic Curve (EEEE) which was used to determine the elastic stiffness and ductility displacement capacity.

Figure 5 presents the average values of lateral capacity (V_{max}), elastic stiffness (K_e) and ductility displacement capacity (μ) obtained from EEEP curve. In addition, on Figure 5 is shown V_{max} for a typical OSB/Plywood sheathed shear wall, for comparison purposes. These values were normalized with respect to the corresponding GLG maximum values for a specific nail spacing (6 in) and are showed as a function of the inverse of the nail spacing ($1/S_{en}$). Figure 5 shows that K_e and V_{max} increased as nail spacing decreased, whereas the ductility displacement capacity decreased as nail spacing decreased. Based on the experimental results (Figure 5), shear walls with GLG panels showed the same $V_{max} - 1/S_{en}$ behavior compared to shear walls with OSB/Plywood panels. Moreover, GLG panels had an equivalent or superior lateral load capacity than OSB/Plywood panels reported in the International Building Code. Also, shear walls with GLG panels presented less damage compared to shear walls with OSB/Plywood panels.

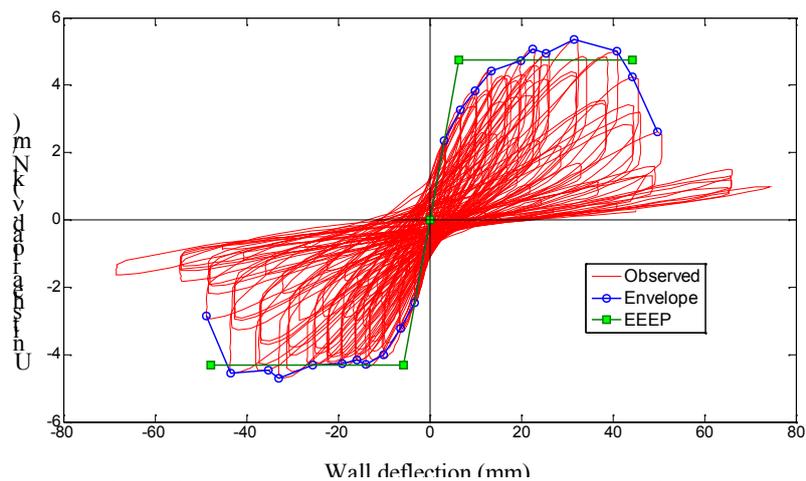


Figure 4. Typical load-displacement response observed in cyclic tests

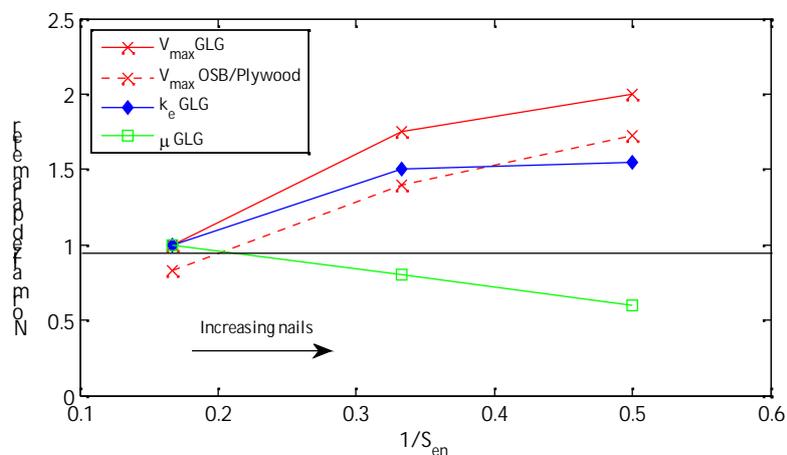


Figure 5. Parameter variation with respect to nail spacing, normalized by the larger spacing

Figure 6 shows the displacement ductility capacity (μ) for aspect ratios of 1:1 and 2:1. It was found that μ was not affected by the aspect ratio, corresponding to the behavior observed in previous research conducted in wood shear walls (Salenikovich et al. 2003a, 2003b). Typical damage of GLG, OSB and Plywood panels are showed in Figure 7. Based on observed damage, it will be possible to re-use GLG panels after a strong earthquake by re-nailing the panel at the middle of the old nails positions, since the GLG panels' failure occurred by nail withdrawal (with few or no damage to the panel, as shown in Figure 7 a), while the failure in OSB/Plywood panels was observed as tearing (Figure 7 b) and pull-through (Figure 7 c), concentrated on the panel edge.

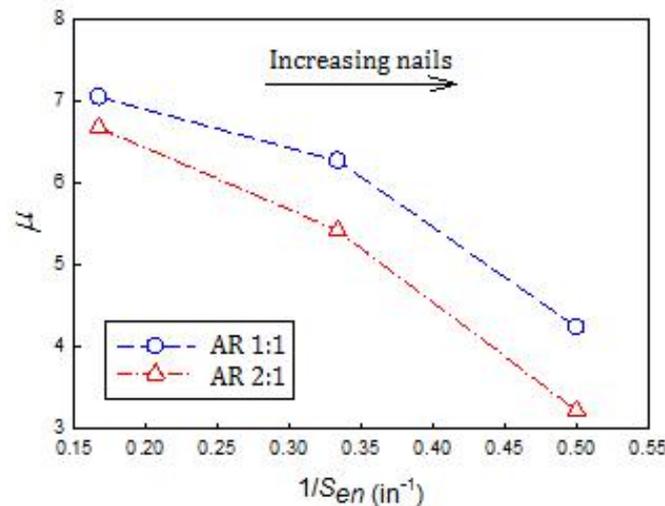


Figure 6. Displacement ductility variation with respect to edge nail spacing



Figure 7 – Observed damage in the sheathing connection zone. Left: GLG panel; middle: OSB panel; right: Plywood panel

Shake Table Test

Description of the Model, Instrumentation and Test Procedure

Shake table tests are a useful experimental tool since it is the best way to understand the seismic behavior of a structural system. In addition, this type of test gives useful information about the performance of a component in a structural system. One story, two stories and two stories with finishing models were studied in this research. Figure 8 shows the overall dimensions of the three models. In general, the structural system of the models consisted of wood shear walls with GLG panels and wood diaphragms with GLG sheathing. The one story model was designed to support the second story after the first model was tested. The dimensions were selected based on the maximum dimensions allowed by the shake table platform. All the structural components of the models were full scale.

The plant view dimensions of the models were 3.75m x 3.75m, with first story height of 2.6 m and second story height of 3.35 m. The shear walls were built with GLG panels of 1.2 m by 2.4 m by 9 mm. The GLG panels were nailed to the wood framing (2 in x 4 in, radiata pine) by 3mm in diameter and 64mm in length common nails at 152mm edge nail spacing and 300 mm intermediate nail spacing. All shear walls had Simpson hold-down type HTT-5 at each end in order to resist overturning moment.



Figure 8 – Shake table test building modules. Left: 1 story module; middle: 2 story module; right: 2 story module + wall finish

Since the shake table tested was uniaxial, finishing for the third model was installed only at the façade of the north and south shear walls (Figure 8 c). The exterior finishing consisted on a steel mesh with 20 mm stucco cover, whereas the interior finishing was 13mm drywall attached to the wood framing by 6in x 1-1/4 in screws with 150 mm edge spacing and 300 mm intermediate spacing.

A total of 40 instruments were installed in order to record displacement, acceleration, and strain at specific locations in the models. All the models were subjected to a sequence of ground motions with increasing intensity, considered to be representative of those expected on a high seismic hazard zone in Colombia. El Centro (California, 1940), Quindío (Colombia, 1999), Northridge (California, 1994) and Kobe (Japan, 1994) comprised the earthquake record selected for this research. Free vibration tests were also conducted between all of the test phases to monitor changes in the fundamental vibration period of the structure.

Observed Results

No substantial damage was observed on shear walls and bare woodframe structure, whereas considerable cracking of exterior stucco and interior gypsum wallboard were observed when the structure was subjected to a 2% exceedance probability in 50 years event. Most of the damage at the interior and exterior finishing was observed at the corners of the windows and at the joints between structural and non-structural walls (Figure 9). The test structure exhibited a pinched global hysteretic behavior (base shear vs. roof relative displacement) in all test phases, similar to that observed for the isolated shear wall tests. Free vibration tests showed that the vibration period decreased when wall finishing materials were added, suggesting an increase in stiffness. However, as the full amplitude of the ground motion was built up, wall finishes were incrementally damaged and the fundamental vibration period of the structure approached the vibration period without wall finishing materials. In general, good behavior of lateral force resisting system of the test structure was observed in all test phases, and significant overstrength of the test structure was observed compared to that considering only the contribution of the structural shear walls.



Figure 9 – Cracking pattern in exterior stucco after shake table tests

Conclusions

Based on the experimental work conducted within this research, the following conclusions can be drawn:

1. Shear walls sheathed with Glued Laminated Guadua have a similar load-displacement behavior to shear walls sheathed with OSB and Plywood panels. Shear walls with GLG panels are affected by the edge nail spacing in a similar way as OSB and Plywood.
2. The stiffness and maximum load carrying capacity of the wall increases as edge nail spacing decreases, whereas the displacement ductility capacity decreases as edge nail spacing decreases. Ductility of the GLG sheathed shear walls is not affected by the aspect ratio of the wall.
3. Limited damage was observed on shear walls sheathed with GLG panels after strong earthquake records simulated by shake table tests. Considerable but repairable cracking of exterior stucco and interior gypsum wallboard were observed after design earthquake with a PGA of 0.3g expected on a high seismic hazard zone in Colombia.

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References

American Forest and Paper Association. 2008. ANSI&AFPA-SPWS-2008, Special Design Provisions for Wind and Seismic. Standard with Commentary.

- ASTM International. 2009. Standard Test Methods for Cyclic (Reversed) Load Test for Shear Resistance of Vertical Elements of the Lateral Load Resisting Systems for Buildings, ASTM E2126.
- ASTM International. 2010. Standard Test Methods of Conducting Strength Tests of Panels for Building Construction, ASTM E 72-10.
- Correal, J.F.; Arbeláez J. 2010. Influence of age and height position on Colombian Guadua Angustifolia Bamboo mechanical properties. *Maderas ciencia y tecnología*, 12(10), 105-113.
- López L.F.; Correal J.F. 2009. Estudio exploratorio de los laminados de bambú Guadua *angustifolia* como material estructural. *Maderas ciencia y tecnología*, 11(3), 171-182.
- Salenikovich, A.; Dolan, J.D. 2003a. The racking performance of shear walls with various aspect ratios. part 1: monotonic tests of fully anchored walls. *Forest Products Journal*, 53(10), 65-73.
- Salenikovich, A.; Dolan, J.D. 2003b. The racking performance of shear walls with various aspect ratios. part 2: cyclic tests of fully anchored walls. *Forest Products Journal*, 53(11, 12), 37-45.

Development of Lashing-Based Bamboo Joints

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Abstract

Bamboo has very high tensile strength, but employing its strength in real construction application is not easy because of the lack of joining system that is able to accommodate it. The difficulties in bamboo joinery are generated by the characters of bamboo itself. The round shape and cavities inside the bamboo are some of them, nevertheless they contain a huge potency when lashing joint is utilized. Lashing is one of the most ancient joining systems in bamboo construction, widely used in traditional and vernacular building, as well in scaffolding. Some remaining problems make this kind of joint difficult to accept in modern bamboo construction. It is mainly because of the difficulty in determining its strength. The development of lashing-based bamboo joint is endeavored to revive lashing bamboo joint, regardless different construction material used than those done in olden time. Steel wire is employed for better prediction of the strength and better durability.

From many proposed alternatives, lashing bamboo joint with eye-bolt was chosen to test in a tension apparatus. Each of three samples consisted of two similar joints. To predict the strength and the failure of the joint, pre-calculations and a test of circumferential compression perpendicular to the fiber was conducted previously.

The joining system performed very well. The average tension strength of three samples or six joints was 34kN. It almost passed the ultimate strength of the used 5 mm steel wire of 34.8kN, although it was far below the ultimate tension strength of the whole section of the bamboo. The failure showed the important role of the ring to protect the hole as the way of wire entering the cavity of the bamboo. The result led the possibility of multiplying the same lashing technique in a joint to multiply the strength.

Keywords

bamboo, joint, lashing, tension

1 Introduction

Lashing joint is very familiar in bamboo construction. There are at least two reasons that cause bamboo and lashing inseparable in traditional or vernacular bamboo construction. Lashing is naturally the easiest technique to joint round shape material like bamboo. The rope touch perfectly fit with the half or full round surface, and the friction between them is distributed equally. It is different with square shape, in which friction concentrates at the edge and less friction on the flat surface.

Another reason of a close relation between lashing and traditional bamboo construction is the capability of bamboo itself to be employed as rope. It means that a structure can be built with the use of bamboo without any other material. Even in Indonesia a native bamboo of *Gigantochloa apus* is called as *bambu tali* in Indonesian term, which mean 'bamboo rope'. It is because the bamboo is very flexible and commonly utilized for rope making when it is fresh after harvesting.

The techniques of lashing joint are described in many literatures (Janssen 1981; Dunkelberg 1985; Hidalgo-Lopez 2003) mainly based on traditional application. Common traditional ropes are coco-palm fiber, rattan, bamboo, bast, etc. The use of lashing in modern construction are limited for scaffolding and some temporary artworks. The rope is made of plastic or rubber-based material.

Some disadvantages of lashing were explained by Arce (1993). Although using natural or artificial rope to tie some bamboos together does not make any damages to the poles, there are some restrictions to these joints to transfer load. Some of natural ropes used for this purpose are not strong enough and there is a lack of friction between rope and bamboo skin. The shrinkage of the bamboo due to drying of green or wet bamboo will reduce the diameter of the bamboo and thus it reduces the bond between bamboo skin and rope. Some trials at the University of Technology of Eindhoven showed though that the amount of tying is not sufficient to avoid slipping of the culms in the case of bamboo. Sometimes the wires cause early cracking by crushing of the ends. Another problem of lashing joint is the difficulties to determine the strength and the security of the connection in a long term especially by using rope from natural fiber.

Despite of some problems mentioned above, this research tries to develop this kind of joint and to revive lashing method for some particular purposes. Rarely used in modern bamboo construction except for scaffolding or temporary construction, lashing joints exploit the effectiveness of friction between the surface of rope and round shape skin of bamboo, compared to square shape material. Other advantages of rope-based connection are as follow:

- Using the outer side of bamboo pole means also benefiting the strongest part of bamboo because the fiber content increases from inner to outer part. Although outer layer of bamboo has more strength due to its higher content of fiber, attaching connector on the outside is difficult to do because bamboo has hard and slippery skin and round shape. The most effective way of attaching connector on the outside is lashing.
- In contrary with the statement of Arce above, compared with most other joints, lashing will protect the bamboo from cracking much better. Therefore it is very common to place an enlacing-rope in the end of the bamboo or close to the joint just to prevent cracking.
- Lashing joints are in general the easiest connection in bamboo construction.

2 Development of Lashing Joint

Based on author's classification (Widyowijatnoko and Trautz 2011), bamboo joints are classified into 6 groups as follow:

1. Group 1 – Transferring compression through whole section
2. Group 2 – Transferring force through friction from inside
3. Group 3 – Transferring force through friction from outside
4. Group 4 – Transferring force through shear from element perpendicular connected from inside (4A) and outside (4B)
5. Group 5 – Transferring force perpendicular to the fiber (bending stress)
6. Group 6 – Transferring force perpendicular to the fiber to the center of the pole

Traditional lashing bamboo joints are classified mainly in Group 3 which transfer force (only tension) through friction from outside. It is very common to be combined with principle of Group 4 which transfer force through shear from element perpendicular. The perpendicular element or dowel prevents movement of the rope or the rope transfer tension to the dowel which continue to the bamboo through shear. Usually, to transfer compression principle of Group 1 which transfer compression through whole section is added.

These new developments of rope-based connections are considered as combination of many principles, but mostly use the principle of Group 6, which transfers compression force perpendicular to the fiber to the center of the pole. They are different with traditional lashing joints which are mostly in Group 3. The rope fastens the bamboo on the outer skin by twining it around the bamboo and slipping inside through small holes in the wall to the cavity within the bamboo. Then it comes out in the end of the pole or being connected to a connector inside. It causes a mechanism: tension force parallel to the fiber through the rope is converted to circumferential compression force perpendicular to the fiber to the center of bamboo section. The stronger tension force, the stronger squeezing force to the bamboo will generate bigger friction on the outer skin. And because bamboo has a round section, the circumferential compressive stress perpendicular to the fiber will be distributed almost equally.

In regard to bamboo as low cost building material, utilizing an open source of connectors is very important to maintain the whole structure inexpensive. In this development of lashing joint this principle will be kept as the first option.

2.1 Lashing Configuration

The concept of lashing joints with a certain mechanism under tension as described above can be implemented in various ways, and three of those are shown in Figure 1. One or two rope can be utilized in different ways. It is also important to put at least one node between the holes and the loaded edge. To prevent the rope slice the bamboo or tear the holes easily, a ring should be placed on each hole to hold the rope from moving in opposite direction, except the one with the configuration of a kind of lasso technique with eye splice in the end of the rope (Figure 1, Joint A).

To provide a better angle avoiding severe buckling on the edges of the holes when the ropes slip inside, some configurations of lashing are proposed by twining the rope not in perpendicular direction

to the fiber but in a certain angle (Figure 2, Joint D and E). In this manner the minimum bending radius of the rope can be provided easier.

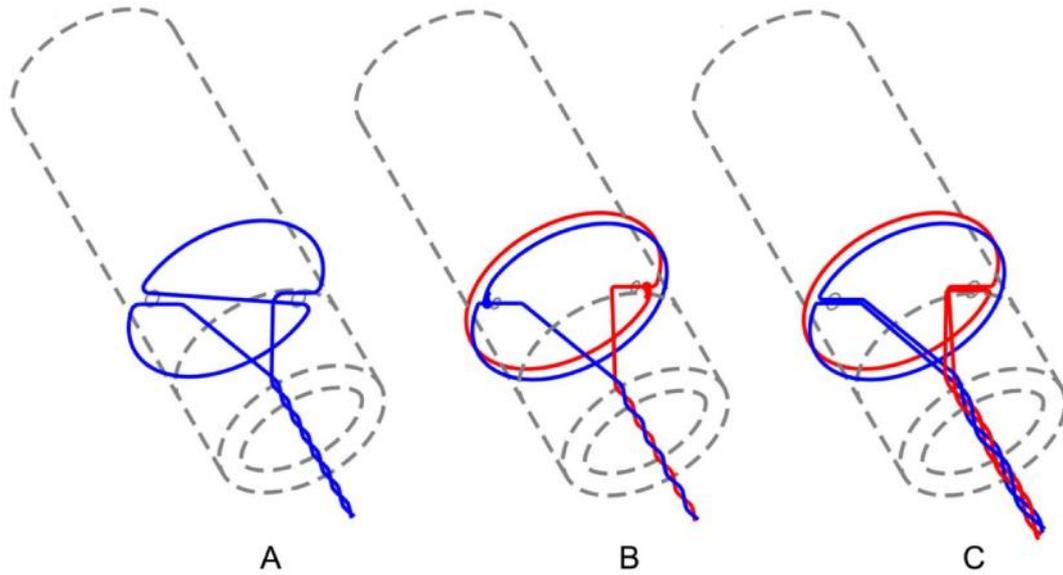


Figure 1. The conceptual lashing configuration: using one rope with two outputs (left), using two ropes with two outputs (middle), using two ropes with four outputs (right)

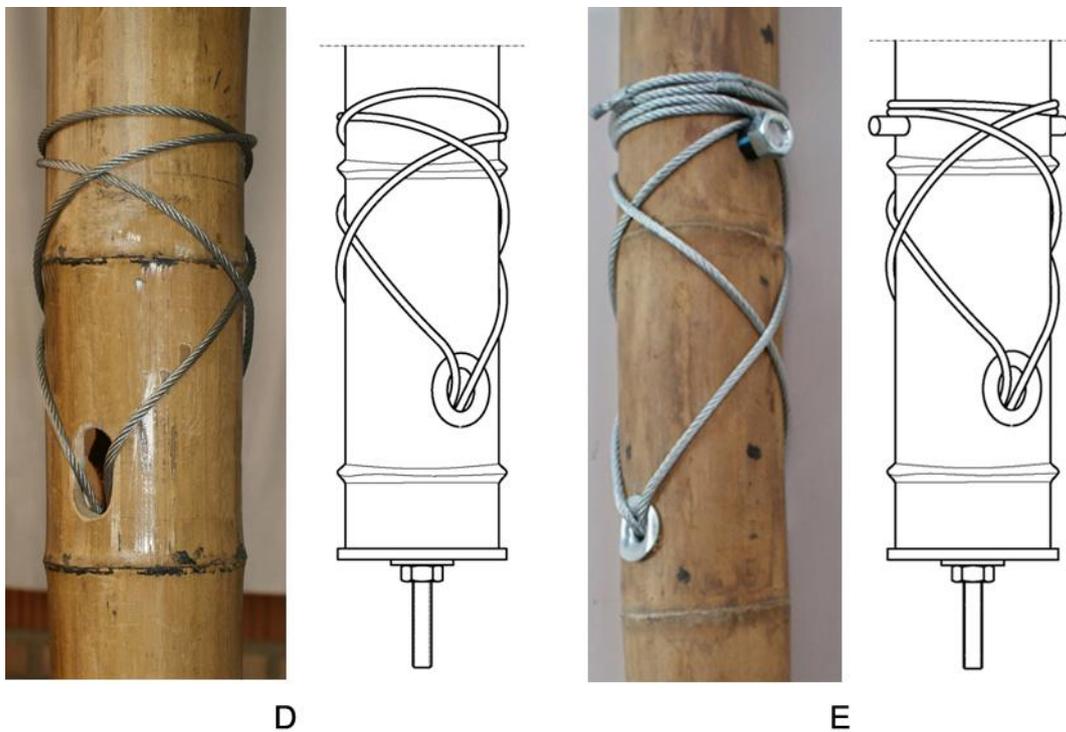


Figure 2. Lashing configuration to avoid severe bending of the rope in the edge of the hole without (left) and with dowel (right)

In Figure 2, the rope is twined around the pole in one internode near a node and slip inside two holes in another internode close to a node. In Joint D, the contour of bulge in the node and cleft in the internode is employed to maintain the position of the winding rope although the stability of its position under high tension force remains questionable. There is a contrast difference of the bulge and cleft contour of nodes and internodes in some species of bamboo, such as *Guadua angustifolia*, which is suitable for this purpose. But some others bamboo, such as *Gigantochloa atroviolacea*, have slightly smooth surface without significant contour difference and thus a dowel should be used to maintain the position of the winding rope, as shown in Joint E.

Another possibility can be seen in Figure 3 by twining the rope around the pole one turn and stabilizing the position of the rope with some small dowels. This option reduces the length of used rope and distributes shear and compression force to a wider surface.

Lashing configuration covers some techniques of lashing to connect rope with bamboo. To connect the rope or bamboo with other element, for example with spherical node in space structure, there are some proposed designs as described in following sub-chapter.



Figure 3. Lashing configuration using small helical dowels to maintain the position of the ropes

2.2 Lashing Joints with Twisting as Pre-Tensioning

Twisting or spinning the rope to tighten the connection is very common in traditional or vernacular lashing joints. This technique is widely used to construct scaffolding in Hong Kong by tightening plastic strips as connector or in Indonesia by using coco-palm fiber. Twisting reduces the length and increases inner tension force and thus tightens the connection. The weakest point in the rope is at the edge of the twisted and the untwisted part, because at this point the rope is severely bent.

This type of joint is proposed for tension only, but it can be developed to have tension and compression strength using principle of compression transfer through the whole section of the bamboo, as shown in Figure 4. The ropes are inserted through a hole in a plate and then connected to a

draw stick. When it is turned, it will twist the ropes and reduce the length of the ropes. Furthermore, a pre-tensioning mechanism is conducted in the joint.

The compression will be transferred from the plate to the whole section of bamboo, and tension will be transferred from the plate to the drawing stick and then to the ropes and continued to the outer part of the bamboo.

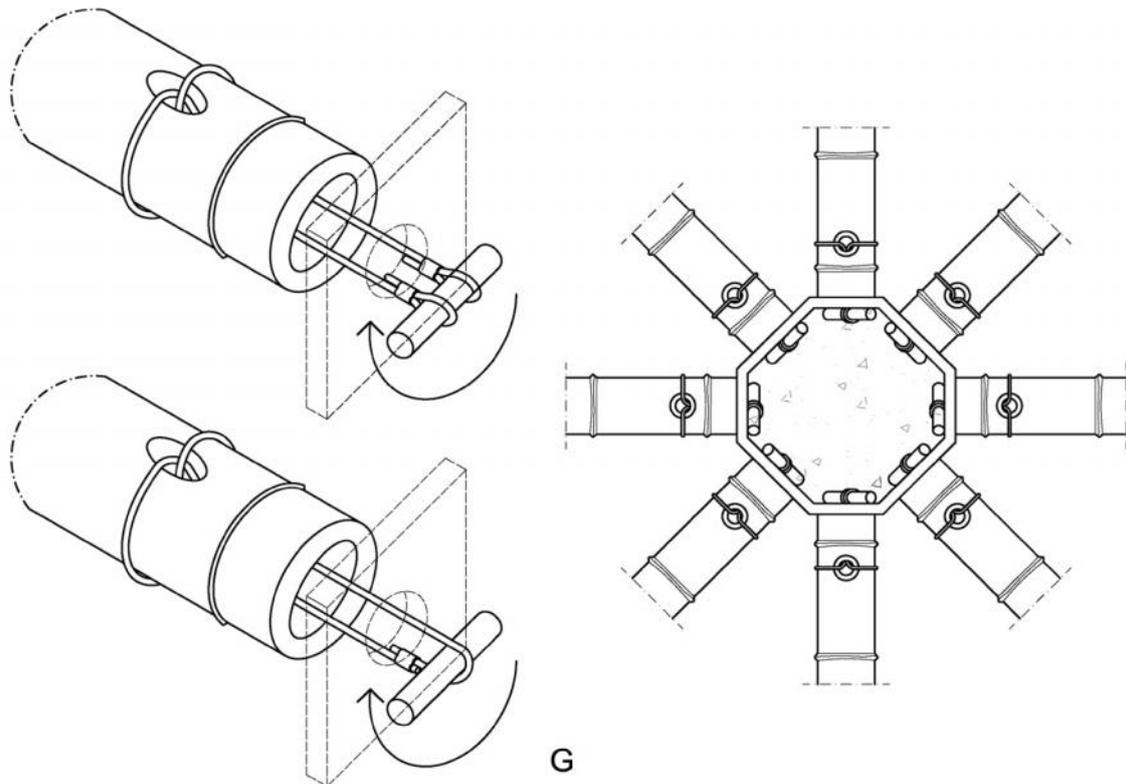


Figure 4. The idea of joining many bamboo poles in one point with the help of draw sticks and plates

2.3 Lashing Joints with Eye-Bolt

The development of lashing joint with eye-bolt is based on the fact that twisting the rope as post tensioning is difficult to do in some cases and consequently reduces its strength. Although using the same lashing configuration and the same principles, the difference of this development is in the use of eye-nut to connect wire rope and threaded rod. Eye-nut and rod can be replaced by eye-bolt, although generally the last has limited length. Eye-nut itself can be replaced by any means which have ring or hook to hold the rope in one side and threaded hole (female thread) to fasten threaded rod (male thread) in another side.

The detail of joints with eye-nut can be seen in Figure 5. The position of the eye-nut is more stable in Joint H and J than Joint I. But Joint L can be assembled with shorter rope. To assemble Joint K and M, longer rope is needed because the rope has to be slipped into the holes and come out of the cavity in the end of bamboo to be connected with eye-nut in the outside. After that, the eye-nut and rod is slipped into the cavity into its planned position and the rope can be tightened and be cut. That is why much longer rope is needed for assembling. Joint L can be assembled with shorter rope because it can be done just by inserting the rope through the holes in the bamboo and eye-nut.

The angle composed of the rope bended by eye-nut plays very important role, because the force transferred from the rod to the rope and later converted to circumferential compression perpendicular to the fiber depends on this. From this point of view the exact angle of the rope in Joint B is easier to make, compared to the other options.

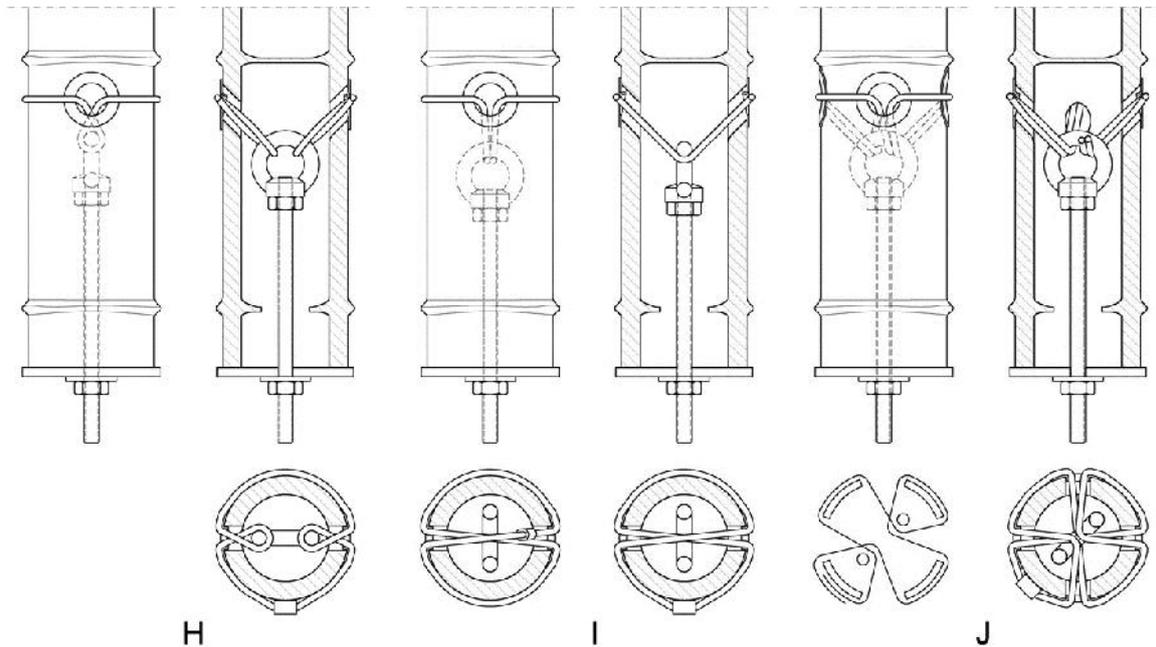


Figure 5. Alternatives of joint with eye-nut and rod or eye-bolt, using two holes (H&I) and four holes (J)

3 Experimental Research on Lashing Joints

This part focused on the development of rope-based joint by using experimental research. The main aim of this experimental research is to determine the tension strength of proposed joint. The other important goal is to know the pattern of the failure especially in a ductile or inelastic manner. There were two laboratory tests in this experimental research as follow in chronological order:

- Circumferential compression test on bamboo tube
- Tension test on the joint with eye-bolt (Joint H, Figure 7)

First test was preliminary test for calculation of second test. The detail of the laboratory tests will be described in the following sub-chapters.

3.1 Circumferential Compression Test on Bamboo Tube

Circumferential compression test on bamboo tube was considered as part of pre-calculation stage to predict the strength of the proposed joint. In the development of this new lashing joint this kind of loading happens in the joint when the rod is pulled, but the value of this strength cannot be found in any literatures.

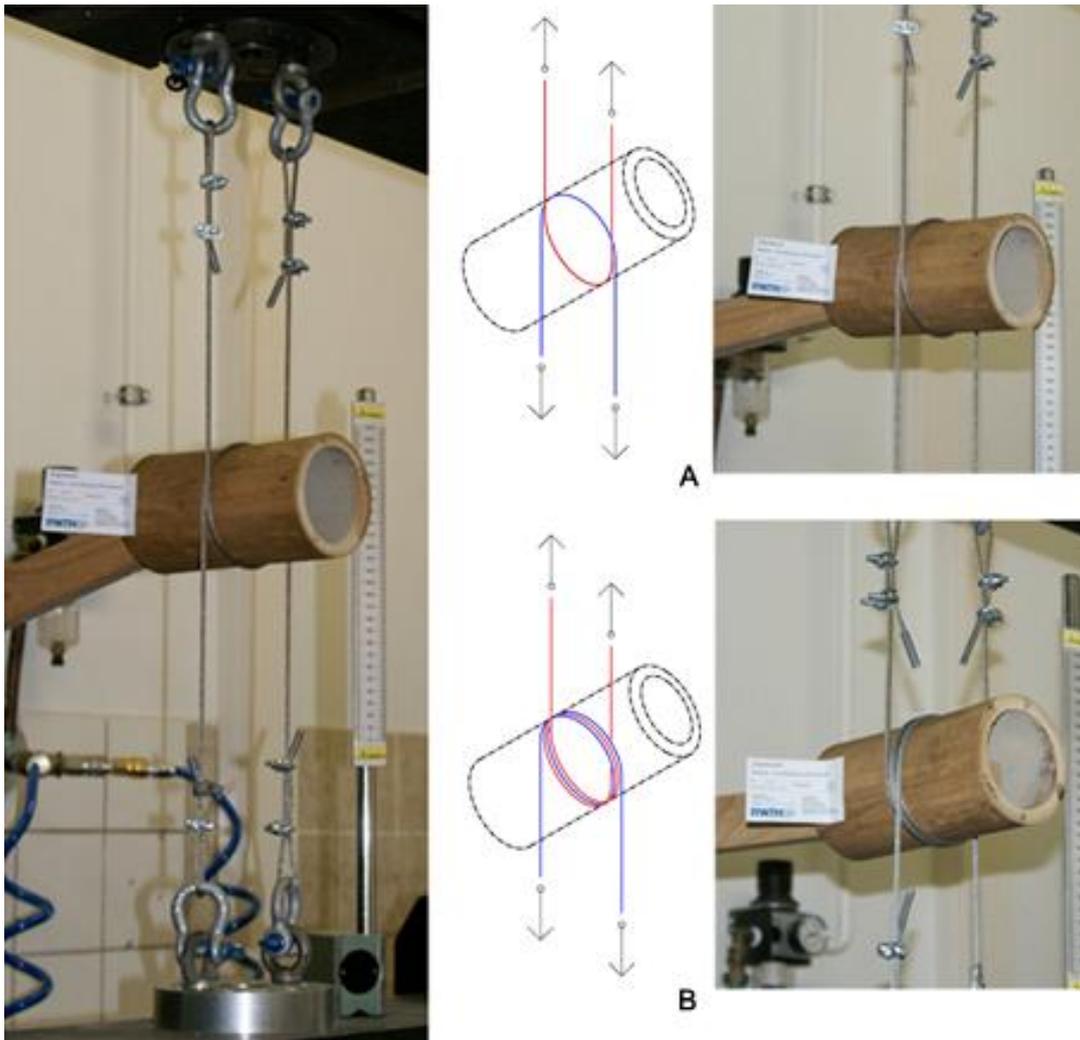


Figure 6. Setting of the sample and test equipment (left), two types of winding, each of two ropes enlanced half round (A, above) and one and half round (B, bottom)

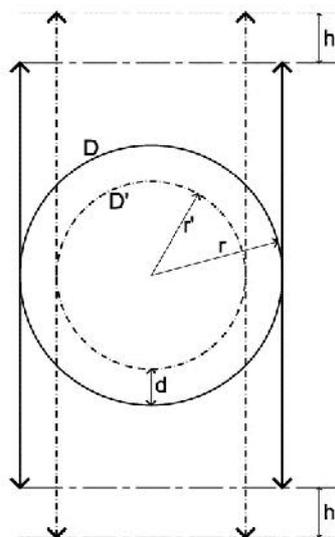


Figure 7. Deformation of bamboo tube, its original diameter (D) and after circumferential deformation (D')

Another aim of this test is to determine whether more winding rope will increase the strength against such load or not. The formulated hypothesis was that more winding rope would strengthen the joint because the compression would be distributed in a wider surface.

3.1.1 Methods and Materials

This test compressed bamboo tube perpendicular to fiber to its center, the loading mechanism that was predicted to happen in the joint. To have a relatively similar mechanism, a pair of steel wire (dia. 4 mm) was twined around the tube in opposite direction and pulled farther apart (Figure 6) by the test machine Instron 1185 Series, with maximal load capacity of 100 kN.

There were two types of test with two different types of winding and with three samples in each test. The first lashing used two ropes, each enlacing half of the bamboo profile or one hemispherical-winding in total. The second lashing also used two ropes but each enlaced 1 and half time enlacing or in total it demonstrated three hemispherical-winding. Both can be seen in Figure 6. All windings were placed in the middle of the samples.

In this test the movement of lower table relative to upper plank of the machine ($\Delta L=2h$, see Figure 7) was recorded directly to the computer. But it did not directly show the deformation of the tube in circumferential direction (d). To measure the circumferential deformation, an Equation 1 was used for Test A and Equation 2 was used for Test B.

For Test A with one hemispherical-winding applied:

$$h = \frac{\pi \cdot d}{2}$$

If the movement of lower table relative to the upper plank of the machine was ΔL ,

$$d = 0.3183 \Delta L \dots \dots \dots (1)$$

For Test B with three hemispherical-winding applied:

$$h = \frac{3\pi d}{2}$$

$$d = 0.1061 \Delta L \dots \dots \dots (2)$$

Each of the two compression tests used three short tubes of bamboo as samples. The tubes were taken from internode part, 18 cm long. It was the longest tube without node which could be produced from shortest internode. All 6 tubes came from a same bamboo pole of *Guadua angustifolia* and from consecutive 6 internodes.

To minimize the effect of different positions of internode in a pole to the results of two different tests, each test used three tubes in an alternating position. Test A used tubes number 1, 3, 5 and Test B used tubes number 2, 4, 6. Numbering 1 to 6 started from upper part of a bamboo pole.

The dimensions of the samples are described in Table 1 below:

Table 1: Dimensions of samples

	Specimen Number	Upper/ Lower Part	Diameter (mm)	Wall Thickness (mm)
Test A	1	Upper	95	10
		Lower	96	10
	3	Upper	95	10
		Lower	98	13
	5	Upper	98	12
		Lower	100	14
Test B	2	Upper	95	9
		Lower	97	10
	4	Upper	98	11
		Lower	99	11
	6	Upper	98	15
		Lower	100	14

3.1.2 Result

Both tests showed similar failure over six tests performed although more compression force was induced to Test A. The failures started with cracking in the end of the tubes and it continued along by the increasing of the load (Figure 8). The fibers in the inner part of the bamboo right in the middle in the same place of winding on outer part were cut off.

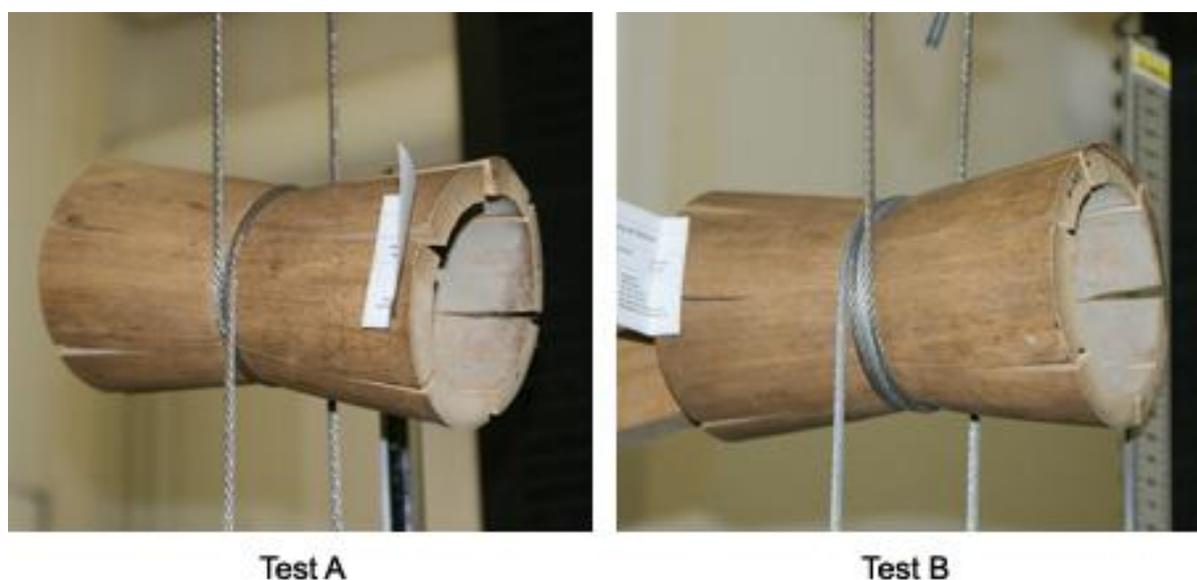


Figure 8. The similar failure of cracking in the ends of the tube of both tests

Bamboo tubes with one hemispherical-winding performed higher compression strength than those with three hemispherical-winding as shown in Figure 9 and Figure 10. Initial crack or splitting started at the load range from around 15.6 kN (Sample 1) to 17.0 kN (Sample 5) in Test A. An error occurred in the machine as it stopped suddenly when the load reached 2.0 kN (see also Figure 9).

The result of Test B can be seen in Figure 10. Initial crack or splitting started at the load range around 6.4 kN (Sample 4) to 8.0 kN (Sample 2). Two errors happened at the test machine during the test of Sample 2 when it suddenly lost the power (see also Figure 10).

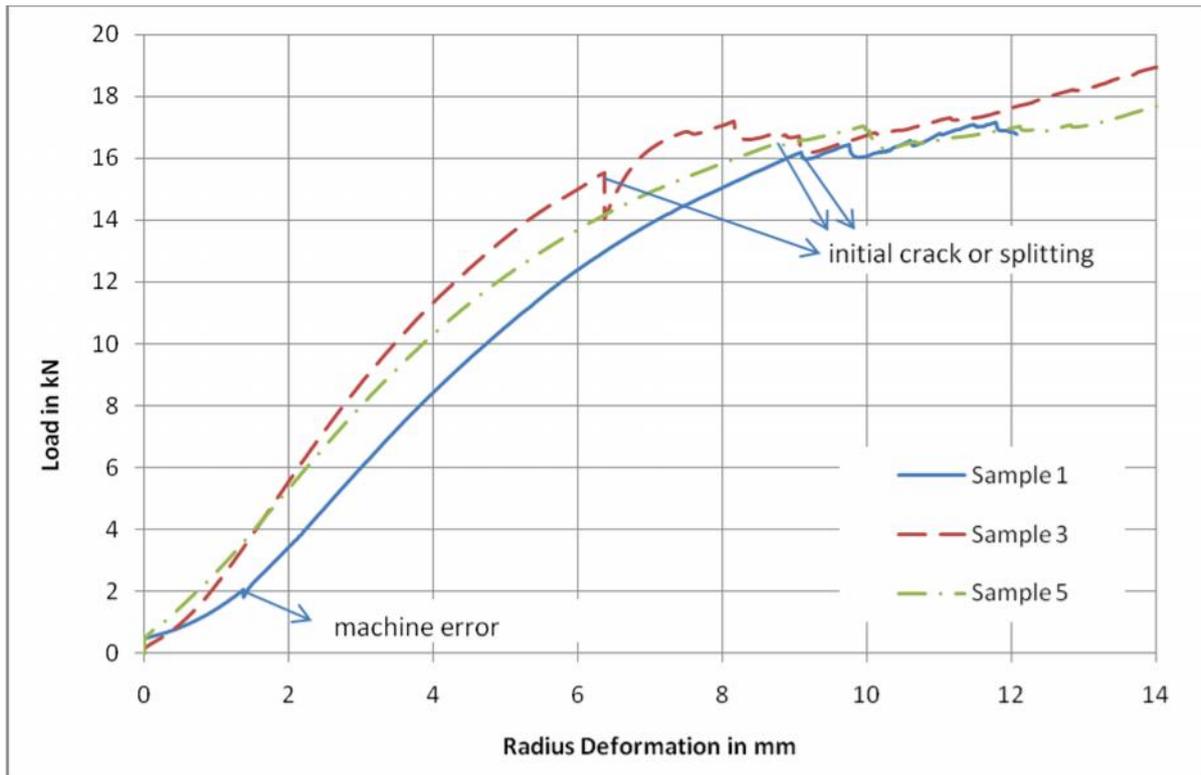


Figure 9. Circumferential compression test results of Test A (Sample 1, 3, 5)

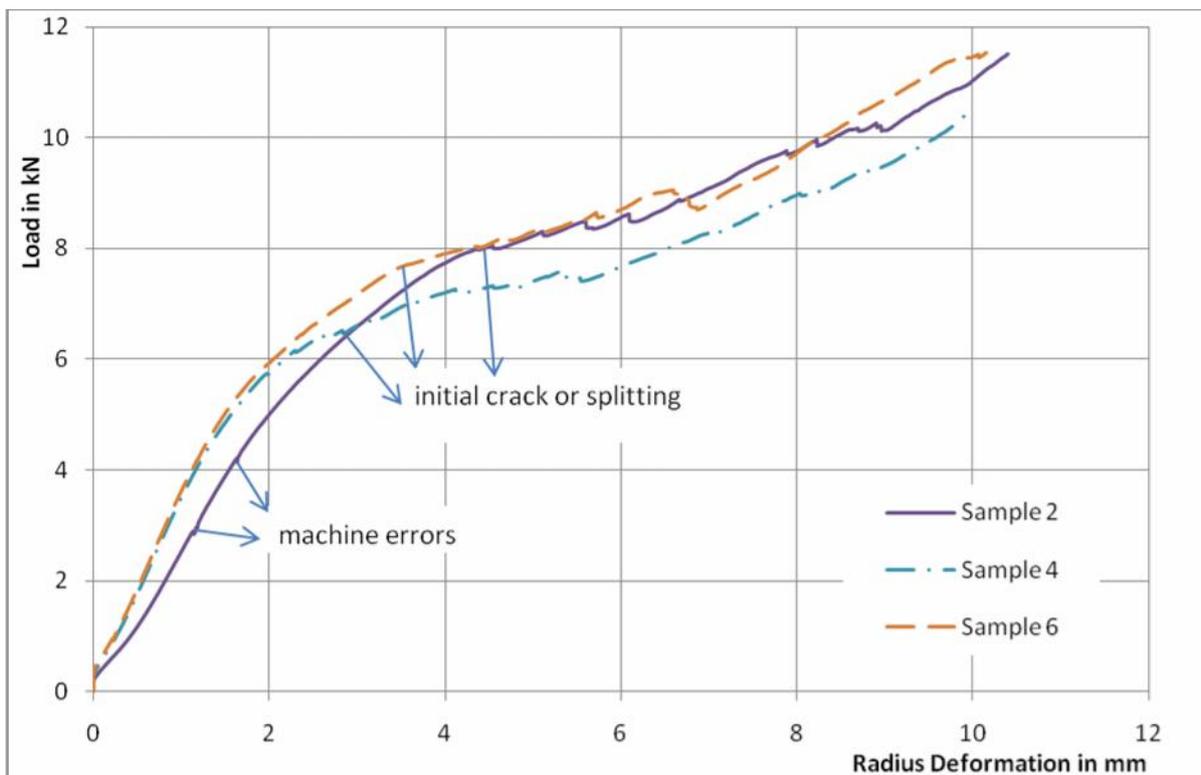


Figure 10. Circumferential compression test result of Test B (Sample 2, 4, 6)

3.1.3 Discussion

In each test, all three tests performed, showed a similar pattern without any big differences. The similar initial failures of crack or splitting in the end of bamboo tubes happened in all six tests were

due to compression to the center of the tube in the middle which generated moment inertia to the opposite direction. After the bamboo was split in several pieces, then these bamboo splits were folded because of the pressure in the middle by the wire.

One factor that causes these behaviors was the presence of only longitudinal fiber in the samples. In the proposed joint, the winding was in between two nodes. In the node the fibers are rather twisted to prevent crack or splitting much better. In this case the strength of the joint under the similar load mechanism must be higher than it showed in these tests, although in the joint there was a reduction of the strength due to the presence of two holes. Because the winding was placed as close as possible to the node, it should even have higher strength.

The tests showed that the number of winding played important role. Samples which compressed with one hemispherical-winding (Test A) performed better than those with three hemispherical-winding (Test B). This phenomenon was beyond the prediction, because it was thought that samples in Test B would perform better because the force would be distributed in a wider surface on the bamboo. Instead of force distribution to the surface of bamboo, multiple winding multiplied the force to the bamboo and made it crack or splitting faster.

This fact gave a very valuable input to next steps of the development of the joint, which then focused to use less winding as possible.

3.2 Tension Test of the Lashing Joint with Eye-Bolt

Tension test was conducted to the samples of simplest joint (Joint H, Figure 5). This method was conducted to be able to determine tension strength of the joint and the phenomenon of the failures for further uses and developments.

3.2.1 Methods and Materials

Three samples were subjects to be pulled in a tension test machine Instron 1185 Series. A pair of eye-nut was attached to each end of joint to be connected with the machine. To measure the deformation of upper part joint a gauge was added because the upper plank of the test machine was the idle part and the bottom table was the moveable part (Figure 11). Deformation of lower part joint was determined by reduction of whole deformation with deformation of upper part joint. The load and deformation of the test was recorded directly in the computer.

There were three samples of Joint H to be tested. This joint provides stability of the rod against torsion when tightening the nut. The failure of this lashing configuration will provide valuable input for further improvement.

These three Samples A, B and C consisted of six typical joints indicated with number 1 to 6. For example, Sample A consisted of joint number 1 in the lower part and number 2 in the upper part of the sample. Each sample consisted of four nodes or three internodes and two joints. The joints were placed between the first and second node from each end of the bamboo, so there was one node between the joint and the end of the bamboo and an undisturbed internode between the two joints.



Figure 11. Equipment setting of the tension test



Figure 12. Sequential failures in Joint 4, Sample B, from left to right: compression on the holes was bigger than on perpendicular sides; bamboo could not hold the ring; the ring slipped inside the hole; wire sliced bamboo; final result

The samples came from two different poles but the same bamboo species, *Guadua angustifolia*, in which Sample B and C came from the same pole. They originated from Colombia and were delivered

to Germany after being preserved. The detail dimensions of the samples are listed in the Table 2 below.

Table 2. Dimensions of the samples

Sample	Joint	Diameter (mm)	Distance Joint – End of Bamboo (mm)	Average Wall Thickness (mm)	Length of the Bamboo (mm)	Average Internodes Length (mm)
A	1	101	190	16	683	172
	2	101	215	14		
B	3	103	162	18	627	161
	4	102	192	15		
C	5	102	224	15	776	193
	6	98	250	12		

For this kind of joint there were only small changes on the bamboo. It was drilled to make two holes with the diameter of 22 mm in an angle of 45° relative to the fiber. The diaphragm of the node in the end of the bamboo had to be broken to be able to put the eye-nut inside the cavity of the bamboo.

In these joints some other materials were used. Steel wire dia. 5 mm, 6x7 RHRL DIN 3055 with ultimate strength of 14.4 kN was used to fix an eye-nut attached to a rod. Two ends of the wire were connected with two wire clips. To protect the holes, two rings were employed. These rings were customized to fit with the round shape of bamboo and provided a minimum bending radius of the wire in the holes avoiding sharp edge of the holes in bamboo.

Two other materials were wooden cap and nuts. One nut was utilized to fix the eye-nut by twisting both in opposite direction so as to push against each other. Another nut was used to pull the rod against wooden cap attached in end of the bamboo as pre-tensioning of the joint, especially to tighten the lashing of steel wire. This pre-tensioning is very important otherwise the deformation of the joint will be greater just to stretch the steel wire in order to be perfectly fit in the joint before the force is fully transferred to the bamboo.

3.2.2 Results

The tests showed two different failures over three tests performed, but the initial failures were same. The common failures started at the hole when the ring failed to hold the pressure afterwards and therefore slipped into the hole. After the wire contacted directly to the bamboo in the holes without any protection from the rings, it sliced the fiber slowly in perpendicular direction, began from the edge of the holes until it sliced the whole section of bamboo almost completely. This happened in Joint 4, Sample B (Figure 12).

The similar failure pattern happened also in Joint 5, Sample C, but with variation in the end of the failure. One ring slipped into the hole and the wire started slicing the fiber. But the bamboo could hold another ring and it caused the ring pressing the fiber in parallel direction, which brought about a shear failure in one side.

Also with similar initial failure in one side of Joint 1, Sample A, in another side the bamboo could hold the ring to stay in the original position. But the wire tore the ring and caused a sharp edge in a V-slice that later cut the wire slowly (Figure 13).



Figure 13. The failure of Joint 1, Sample A: cutting off the wire because of sharp edge of V-slice in the ring (left) in one side; the ring slipped inside the hole and the wire sliced the bamboo in another side (right)

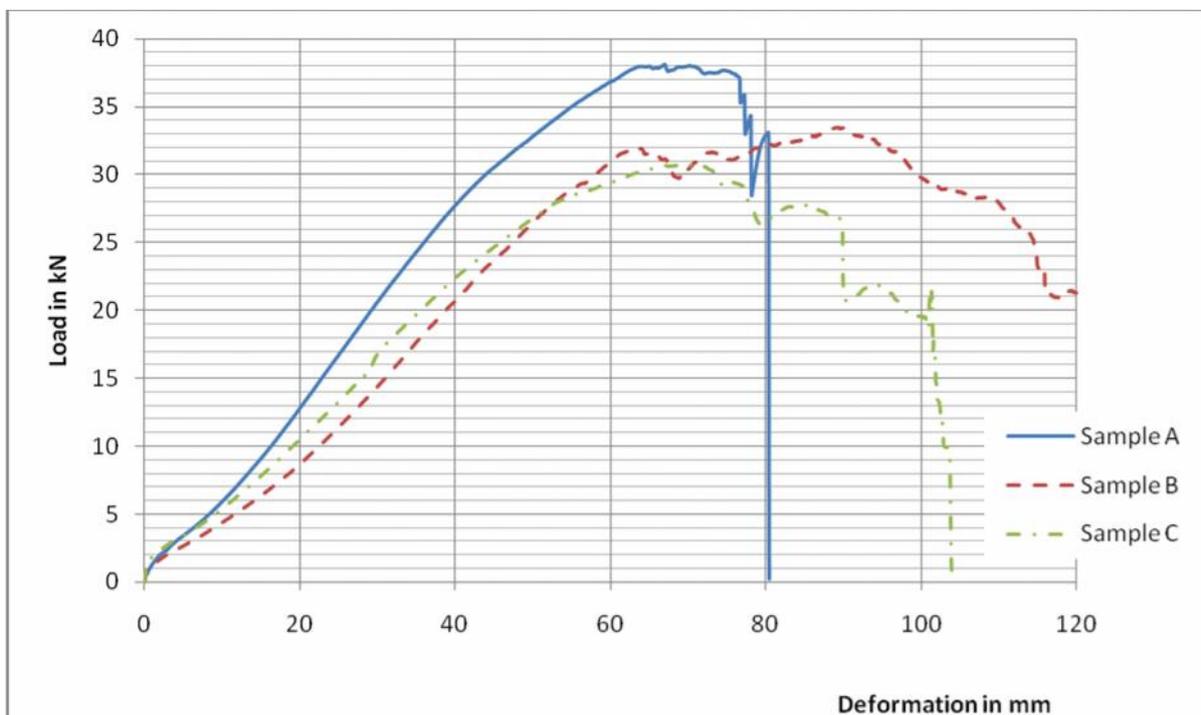


Figure 14. Load-deformation diagram of the three samples

The diagram of correlation between the load and the deformation of the three samples can be seen in Figure 14. From the diagram above it can be seen that Sample A consisted of Joint 1 and 2 reached the highest load level of almost 38 kN. Two other samples reach almost the same load level of approximately 31 kN. It also showed the similar pattern without extreme difference, except the failure in the end stage which was breaking off the wire (Sample A) and cutting off the bamboo (Sample B and C).

3.2.3 Discussions

It has been predicted before that there will be an accumulation of force in the holes because in this place tension force in the rope was changed to circumferential compression perpendicular to fiber. Therefore attention should be paid in this part.

The failure pattern showed that without rings the wire would slice bamboo easily. This was the main reason to utilize customized rings to protect this slicing mechanism as the rings maintained the position of the two wires in the hole from splitting farther apart that caused tearing of the hole. As long as the ring stayed in the position, the wire could not slice the bamboo from outside of the tube to inside, because of the wider contact surface and hard bamboo skin.

As long as the ring could prevent the slicing mechanism it caused a problem of concentration of point load perpendicular to fiber on the bamboo surrounding the hole which had direct contact with the surface of the ring. This point load caused the bamboo tube to deform elliptically (see Figure 12, left) although the circumferential compression force by the wire to the circumference of bamboo tube was considered equal. As the tension increased, this point load also increased until the ring slipped into the hole.

The test proved that the joint showed very good performance. The average ultimate tension strength of these three samples (34.1 kN) almost reached the ultimate strength of the used 5 mm steel wire even one sample have passed it. It was calculated that the wires in the joint would break off when the load reach 34.8 kN (in 45° , double and reduced by the use of rope clip). It means that the joint maximized the strength of its components.

If the tension strength of the whole section was calculated, there was still high tension strength of bamboo which was still unutilized. It means that there was still possibility to add more holes in the bamboo if necessary.

From the test, it was very clear that the ring played very important role. That is why the improvement should be taken to solve the problem on this particular component. The improvement should be in the following aspects:

- The ring should have a higher strength to prevent to the tearing off by the wire
- The ring should have a better curve surface to fit the round shape of bamboo, provide minimum bending curve of the wire and also adapt well to the angle of the hole relative to the direction of the fiber.
- To reduce the possibility of the ring to slip inside the hole, it should have a wider contact surface on the surrounding area of the hole to spread the compression.

4. Conclusion

Lashing joint with eye-bolt performs very well. It maximizes the use of connector, although the tension strength of the whole section of bamboo remains much higher. The test showed the average tension strength of 34.1 kN from three samples or six joints.

Improvement of the joint can be done in many different ways. One of them is the improvement of the rings. Rings at the holes play very significant role, because it prevents the wire slicing bamboo easily. Further development in this element will increase the tension strength.

Using lashing configuration with more holes will distribute the force in wider surface instead of concentrating in a pair of holes. The other solution is by using lashing configuration in which the rope slips inside without being severely bent, so as to reduce the concentration of force in the interaction between the rope and the bamboo wall surrounding the holes.

References

- Janssen, Jules J.A. 1981. *Bamboo in Building Structures*. Eindhoven: Eindhoven University of Technology, Ph.D Thesis
- Dunkelberg, Klaus. 1985. *Bambus als Baustoff*. Stuttgart: Institute for Lightweight Structures
- Hidalgo-Lopez, Oscar. 2003. *Bamboo The Gift of the Gods*. Bogota: s.n., ISBN 958-33-4298-X
- Arce, O. 1993. *Fundamentals of the Design of Bamboo Structures*. Eindhoven: Technical University of Eindhoven. Ph.D Thesis.
- Widyowijatnoko, Andry and Trautz, Martin. 2011. *Tension Bamboo Joint for Spatial Structure*, Proceedings of the IABSE-IASS Symposium: Vol. CD-ROM. IABSE-IASS Symposium. London, UK, 20-23 September 2011

Session 6. Wood treatment

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Applying Neem (*Azadirachta indica* A. JUSS) Seed Oil as a Protectant for Bamboo (*Bambusa vulgaris* SCHRAD.) against Basidiomycetes: Assessing the Effectiveness Based on Tensile Strength Properties

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Abstract

There are increasing worldwide clamours for environmentally benign preservatives for lignocellulosics that will serve this purpose in cheap and cost effective manners. To this end, the effectiveness of mechanically extracted crude Neem (*Azadirachta indica* A. JUSS) seed oil was investigated as a preservative against fungi. The basidiomycete identified as *Pycnoporus sanguineus* (L. EX FR.) MURR. was chosen as a test fungus for the experiment, using a tensile strength assay for bamboo, whose data is limited in the literature. Harvested bamboo stems were converted to split samples for tensile strength tests in conformity with modified ASTM D-143 and treated by soaking two sets differently in the oil at an ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours (A) and 60°C for 4 hours (B) with samples not treated with the oil as controls. The oil-treated (soaked and heat treated) and control samples were all inoculated with cultured *P. sanguineus*, incubated for 12 weeks and then subjected to the tests for mechanical properties. Results obtained showed that Modulus of Rupture (MOR) was lower for controls (117.70 Nmm^{-2}) than for both A (139.79 Nmm^{-2}) and B (126.11 Nmm^{-2}) treatments. Conversely, the Modulus of Elasticity (MOE) was higher for controls ($2,425.94 \text{ Nmm}^{-2}$) than for A ($1,821.74 \text{ Nmm}^{-2}$) or B ($1,886.59 \text{ Nmm}^{-2}$) treatments. Mean values for Energy at Maximum Load, Load at Yield and Extension at Yield respectively roughly followed the trend obtained for MOR. The data obtained were statistically analysed using a basic descriptive tool (mean) and ANOVA while Fishers' Least Significant Difference was applied as a follow-up test to compare means ($P < 0.05$). Conclusion and recommendations were suggested in line with the outcome of the study.

Keywords

Bamboo, Neem, Vegetable oil, Basidiomycetes, Mechanical properties, Tensile strength

Introduction

Bamboos (*Bambusaceae*) have for long been known in different climates for multiple uses. For instance, stems (culms) of many species have been identified and applied for several purposes, such as in certain structural applications, for centuries, most of which are based on the fact that these culms possess good strength/mechanical properties. However, bamboo utilisation for this type of application, particularly when the material is exposed to weather conditions, is constrained by their easy susceptibility to agents of biological degradation like termites, borers and fungi, among others. Susceptibility to these organisms lead to significant reductions in strength and mechanical properties of the bamboo material (INBAR 1998; Erakhrumen 2011b) and cause most of these bamboo structures to be temporary often requiring replacement.

This problem has led to numerous efforts in the past to solve it adequately, in order to realise a truly sustainable use of bamboo fibre, as a valuable renewable natural resource worldwide. Some of these efforts, either based on native intelligence and traditional knowledge or on modern scientific outcomes, were aimed at finding suitable means of protecting lignocellulosic material against biological degradation. In trying to achieve this, particularly along modern scientific lines, many types of synthetic chemicals have been developed and used as preservatives, with various levels of success. Most of these substances have afterwards been discovered to contain certain constituents that are not only dangerous to life forms but also to the environment (Onuorah 2000; Cooper 2001; Erakhrumen 2010) as well as cause concern for their relative cost increase, particularly in the developing countries (Erakhrumen 2011b).

In line with these concerns, there has been a need, especially from developing countries such as Nigeria, for sustainable environmentally benign methods of preserving bamboo and other lignocellulosics which are cheap and cost effective and cause no concern for human health. Studies have shown that these objectives can be achieved by the application of plant-derived extracts such as vegetable oil for this purpose. For example, some among these studies have shown that oil from Neem (*Azadirachta indica* A. JUSS) seed have some anti-microbial properties (Parveen and Alam 1993; Locke 1995; Puri 1999) thereby serving as lead to exploratory studies focussed on the use of the oil as preservative for lignocellulose such as bamboo against biodegradation in West Africa, including Nigeria (Erakhrumen 2010, 2011a, 2011b).

This research was therefore conducted in line with the reported anti-microbial properties of Neem Seed Oil (NSO), to investigate the possibility of protecting the most prevalent bamboo species in south-western Nigeria known as *Bambusa vulgaris* SCHRAD. EX J.C. WENDL. against fungi, experimenting with a basidiomycete, known as *Pycnoporus sanguineus* (L. EX FR.) MURR. Tensile strength parallel to the longitudinal axis (the maximum tensile stress sustained in the direction parallel to the axial direction) and other associated properties were used for the investigation. Tensile strength is a mechanical property of lignocellulose with relatively few data in the literature (Green *et al.* 1999) and it is also less investigated in bamboos' stems/culms (Erakhrumen 2009).

Materials and Methods

Sourcing of Bamboo Culms

The bamboo culms that were converted and experimented upon in this study were sourced from wild clumps at Isale-Togun Forest, Lanlate, Ibarapa, Oyo State, Nigeria (latitude 7° 36' N and longitude 3° 27' E) in October, 2008. This area is located in between the humid and sub-humid tropical climatic zones, where mean annual rainfall ranges between 1,117.10 and 1,693.30 mm. Only mature culms with mean circumferential length of 300 mm at the second node above ground were harvested and cross cut in such a way that only the basal culm portion of 3000 mm length were removed and placed

in jute bags with nylon lined inner surface to avoid contamination from the soil. The harvested culms were transported to, protected and stored in the wood workshop of the Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria, for conversion to the test specimens.

Sourcing of Neem Seeds

The ripe Neem seeds from which oil was mechanically extracted in this study were obtained from *A. indica* trees on the University of Ibadan campus located on the northern edge of the city of Ibadan, Nigeria (latitude 7° 20'N and longitude 3° 50'E). Collection of the seeds was done by placing clean nylon sheets around the stems of *A. indica* trees in such a way that it covered a substantial cross sectional area of the crown in order to directly collect the seeds as they fall. The Neem seeds were secured in the months of June to early August of 2008.

The Neem seeds obtained were thoroughly washed using deionised water to remove dirt and other impurities and then air dried in an open space with regular movement for aeration to ensure proper drying, a method also applied by Soetaredjo *et al.* (2008), to reduce the moisture content (MC) for proper crushing and to facilitate high oil volume recovery during mechanical extraction. The seeds were daily air dried with proper monitoring to prevent spoilage as a result of possible moisture fluctuations.

Conversion of Bamboo Culms to Test Samples

The harvested bamboo stems were carefully sawn into longitudinal strips using circular and vertical breakdown sawing. Each strip was planed on both the inner and outer surface, using a planing machine, in order to obtain mean thickness of 5 ± 0.5 mm for the determination of MC and tensile strength and associated properties. The strips were first conditioned in the laboratory for 14 days and then oven-dried at $103 \pm 2^\circ\text{C}$ to constant weight. They were removed from the oven afterwards and stabilised in the laboratory for 24 hours.

The mean MC of split-bamboo samples after stabilisation was determined using test dimensions of 20 mm (tangentially) x 20 mm (longitudinally) x 5 mm (radially) and calculated in accordance with ASTM D 4442 (2007) while the strips at the same MC were also converted to test specimens with dimension 25 mm (tangentially) x 200 mm (longitudinally) x 5 mm (radially) in conformity with a slightly modified ASTM D 143-94 owing to the nature of this bamboo species. The nodes of the harvested culms were excluded.

Extraction of Neem Seed Oil

The Neem seeds were decorticated after being air dried, separating the kernel from the shells and dirt, and then air-dried again. Dried kernels were carefully pulverised into smaller particles using a seed grinder ensuring no significant loss of seeds' oil. Mechanical extraction of oil was performed by cold pressing the pulverised seeds using an oil expeller at a maximum pressure of 31 MPa (31 Nmm⁻² or 4,500 psi). Mechanical extraction was performed at this pressure until oil stopped flowing.

Treatment of Split-Bamboo Samples with the Extracted Oil

The split-bamboo samples to be treated with NSO and those for control were earlier stabilised in the laboratory to a MC of 11.76%. The stabilised samples to be oil-treated were subjected to two NSO-treatment regimes through (1) soaking a set of samples in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and (2) soaking another set in hot oil at 60°C for 4 hours, removed from the oil afterwards and allowed to cool in a dessicator at an ambient room temperature of $25 \pm 2^\circ\text{C}$.

These methods of NSO-treatments were also earlier adopted in the experiments reported by Erakhrumen (2009, 2010, 2011b); Erakhrumen and Ogunsanwo (2009, 2010). The maximum heat treatment temperature of 60°C was adopted in this research because strength and stiffness values used in practice for most lignocellulosic materials are valid for temperatures below 60°C (Homan and Jorissen 2004).

Sourcing, Identification, and Culturing of Fungal Isolates

In order to obtain a fungal species that attacks *B. vulgaris*, a decaying culm of this bamboo species found on the forest floor where the bamboo samples for this research were harvested was also obtained, kept in aseptically clean container, and taken to the Pathology Laboratory of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria, where samples of the microbes present were identified as predominantly a white rot fungus known as *Pycnoporus sanguineus* (L. EX FR.) MURR.

A nutrient medium of potato dextrose agar (PDA) in deionised water was prepared in line with the method earlier adopted by Erakhrumen, (2010, 2011b). The white rot fungus that was obtained from the decaying culm was first cultured and again sub-cultured, in order to obtain pure culture, in sterilised Petri dishes with the prepared PDA until the nutrient was completely covered by the mycelium of the purely cultured fungus. The prepared PDA in the culture bottles were then inoculated with the pure culture and also allowed to be completely covered by the mycelium of the pure cultured fungus.

Infection of Split-Bamboo Samples with Fungal Isolates

The split-bamboo samples, i.e., both the NSO-treated (soaked and heat treated) and those not treated with the oil (control), to be infected with the pure cultured fungal species were sterilised and placed aseptically into the culture medium in chemically clean amber glass culture bottles with dimension 300 mm (length) x 50 mm (breadth) x 200 mm (height) in which there were cultures of actively growing test fungal species. The split-bamboo samples with dimensions 25 mm (tangentially) x 200 mm (longitudinally) x 5 mm (radially) to be inoculated with the cultured *P. sanguineus* in the laboratory test were placed such that they came in contact with the aerial mycelium of the test fungus and not the culture medium itself.

The culture bottles were then properly covered with Teflon lined lids, also made of glass ware, to prevent external contamination particularly from the surrounding air. Ten test samples for each treatment (soaked and heat treated) and those not treated with oil (control) were so infected. The bottles together with their contents were left in an incubating room with ambient temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5\%$ and monitored for twelve weeks (84 days) based on the method adopted by Erakhrumen, (2010, 2011b) in line with slight modifications to methods described by Leithoff and Peek (2001) and Luna *et al.* (2004).

Evaluation of Tensile Strength and other related Properties for Split-Bamboo

The tests for tensile strength and associated properties were carried out using split-bamboo test samples with specimens dimensions of 25 mm (tangentially) x 200 mm (longitudinally) x 5 mm (radially). The oil-treated and control specimens were subjected to these tests after they have all been exposed to isolates of *P. sanguineus* for 12 weeks. The tests for tensile strength and associated properties along the specimens' longitudinal axis were carried out using a computer controlled Instron® 3363 Universal Testing Machine (UTM) at a constant cross-head speed of 4.00 mm min⁻¹.

The tension load was introduced in the longitudinal plane of the test samples with the assumption that the fibres are orientated parallel to the longitudinal axis of the bamboo stems as most bamboo cells are axially orientated at the internodes (Liese 1985) with their anatomical construction appearing uniform among the about 1,200 species (Liese 1992; Liese 1998; Liese 2003). The UTM used for the tests was located at the Material Testing Laboratory of the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria.

Statistical Analyses

The data obtained for tensile strength and other associated properties evaluated for both the oil-treated and control split-bamboo samples after exposure to fungal attack were subjected to basic descriptive statistical analysis in the form of mean. Analyses of variance (ANOVA), was employed in analysing the data for statistical significant variation ($P < 0.05$) while Fishers' Least Significant Difference (LSD) was applied as a follow-up test to compare means ($P < 0.05$).

Results and Discussion

The values obtained for tensile strength and associated properties, shown in Table 1, revealed that mean value for modulus of rupture (MOR) obtained for control samples was 117.70 Nmm^{-2} while they were 139.79 Nmm^{-2} and 126.11 Nmm^{-2} for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and samples soaked in hot oil at 60°C for 4 hours respectively. The mean values obtained for Modulus of Elasticity (MOE) were $2,425.94 \text{ Nmm}^{-2}$ for control, $1,886.59 \text{ Nmm}^{-2}$ for samples soaked in hot oil at 60°C for 4 hours and $1,821.74 \text{ Nmm}^{-2}$ for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours.

The mean values in Table 1 also showed that MOR was lower for control samples than those for samples treated with NSO at the two temperature regimes. Mean value for MOR was highest for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours followed by that for samples soaked in hot oil at 60°C for 4 hours. Conversely, the pattern was reversed in the mean value for MOE as control samples had mean value that was higher followed by that for samples soaked in hot oil at 60°C for 4 hours while samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours had the lowest mean MOE value.

Mean value for Energy at Maximum Load was slightly lower for control samples (0.12 kJ) but was the same for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours (0.13 kJ) and samples soaked in hot oil at 60°C for 4 hours (0.13 kJ). Load at Yield was also lower for control samples (2, 1211 N) compared with values obtained for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours (2, 3265 N) and samples soaked in hot oil at 60°C for 4 hours (2, 2011 N). Extension at Yield was also lower for control samples (7.63 mm) in comparison to values obtained for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours (8.41 mm) and samples soaked in hot oil at 60°C for 4 hours (9.02 mm).

Result of ANOVA (Table 2) revealed that variations in the evaluated data for all the properties, except that for Energy at Maximum Load, for tensile strength were statistically significant. Fisher's LSD results included in Table 1 revealed that the mean values for MOR were significantly different for all the treatment regimes. The Table 1 also showed that mean values for MOE for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and those soaked in hot oil at 60°C for 4 hours were not statistically significantly different but both were significantly different from mean

value obtained for control samples. Data obtained for Energy at Maximum Load were not statistically significantly different for control samples and those for oil treated samples. However, the data obtained for both Load at Yield and Extension at Yield were statistically significantly different for all the treatments (Table 1).

The values obtained for MOR by tensile testing (Table 1) revealed that the oil-treated samples, i.e., those soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and in hot oil at 60°C for 4 hours had higher values than those for control. It is however, noteworthy that MOR and MOE are often determined from bending tests rather than from axial tests as the case was in this experiment. The mean values obtained in this study imply that in terms of MOR, the control samples perhaps had higher susceptibility to the decay fungus as compared to the oil-treated ones.

These MOR values may imply that the oil-treatment might have had negative influence on certain intrinsic/extrinsic conditions and/or processes that would have aided *P. sanguineus* in biodegrading the oil-treated split-bamboo specimens to the extent of significant reduction in MOR as observed in control split-bamboo samples. This observation is supported by results obtained for some chemical properties of NSO in a study by Erakhrumen (2010, 2011a) which related these properties to this oil's anti-microbial properties recorded in the literature (e.g., Locke 1995; Puri 1999). A similar trend regarding MOR was also obtained in another study by Erakhrumen (2011b).

On the other hand, the values obtained for MOE were higher for control samples in comparison with the oil-treated samples. The reduction in this property in treated split-bamboo samples might be a result of the influence of the oil and perhaps its temperature and not necessarily that the control samples resisted the fungal attack more, in terms of MOE values, than the oil-treated samples or that the cultured fungus was able to reduce this mechanical property in the NSO-treated split-bamboo samples. Reductions in strength/mechanical properties for lignocellulosic materials at high treatment temperature have been variously documented in the literature (e.g. Leithoff and Peek 2001; Wahab *et al.* 2006; Kumar 2007; Manalo and Acda 2009; Erakhrumen 2009, 2011b; Erakhrumen and Ogunsanwo 2010).

In addition, mean values for Energy at Maximum Load which indicated the energy expended in bringing the split-bamboo samples to failure by the UTM during the test showed that the treated samples had slightly higher values than the untreated ones irrespective of the recorded negative influence of high temperature on strength properties of lignocellulose in the literature as earlier highlighted. Mean values for Load at Yield and Extension at Yield also followed similar pattern as Energy at Maximum Load. The likely implication of the mean values for Load at Yield is that after exposure to the decay fungus the oil-treated split-bamboo samples were able to withstand comparatively slightly higher tensile load than those for control samples on the longitudinal plane during the test.

In like manner, Extension at Yield, which is the strain caused by the tensile load within the elastic limit for split-bamboo samples, was higher for oil-treated split-bamboo samples than for the control samples. This may therefore mean that the mean values for MOR are consistent with those obtained for Energy at Maximum Load, Load at Yield and Extension at Yield while those obtained for MOE did not appear so. Thus, the lower mean MOE values obtained for the oil-treated split-bamboo samples may not necessarily be a true reflection of the influence of NSO on the activities of the cultured fungal species as earlier stated.

The results of statistical analyses conducted at 5% probability level (ANOVA in Table 2) showed that there was statistically significant variation in the data obtained for all the evaluated properties except those for Energy at Maximum Load. This was examined by subjecting the pooled data obtained for each evaluated properties for control samples, samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and samples soaked in hot oil at 60°C for 4 hours to ANOVA (Table 2). Subsequently, the results of Fisher's LSD of pair of means for the evaluated properties (in Table 1) showed that there was statistically significant difference in the values of MOR for control, samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and samples soaked in hot oil at 60°C for 4 hours.

The LSD also showed that there was no significant difference in MOE values for samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and samples soaked in hot oil at 60°C for 4 hours but there was a significant difference in mean MOE values between the control and oil treated samples. Fisher's LSD also showed that statistical significant difference did not exist among the data obtained for Energy at Maximum Load evaluated for control, samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and samples soaked in hot oil at 60°C for 4 hours.

However, there was statistical significant difference in the data obtained for Load at Yield and Extension at Yield evaluated for control, samples soaked in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and samples soaked in hot oil at 60°C for 4 hours. The likely implications of the results of statistical analyses were that the NSO-treatment had significant influence on the biodegradability of the NSO-treated split-bamboo samples caused by this fungus including the mechanical properties obtained after the incubation period based on the F-values obtained for ANOVA. The LSD showed that based on MOR values obtained for oil-treated and control samples efficacy of the oil-treatment was not similar while fungal attack on test samples differ significantly.

The trend of LSD values obtained for MOR in this study was similar to that for static bending MOR by Erakhrumen (2011b) as earlier highlighted. However, the MOE values obtained indicated that the efficacy of the oil-treatment was similar for the oil-treated samples as also observed by Erakhrumen (2011b) for static bending MOE for split-bamboo samples subjected to the same NSO-treatments and exposed to the attack of the same fungal species. In addition, the LSD obtained for Energy at Maximum Load showed that oil-treatment did not significantly lead to variation in this property after exposure to fungal attack while oil-treatment had significant influence on mean values for Load at Yield and Extension at Yield after exposure to fungal attack.

Table 1: Mean values obtained for selected tensile strength and other related properties for the oil-treated and control split-bamboo samples subjected to fungal attack including the results of Fisher's Least Significant Difference of pair of means

<i>Treatment</i>	<i>Modulus of Rupture (Nmm⁻²)</i>	<i>Modulus of Elasticity (Nmm⁻²)</i>	<i>Energy at Maximum Load (kJ)</i>	<i>Load at Yield Zero Slope (N)</i>	<i>Extension at Yield Zero Slope (mm)</i>
Control	117.70 ^a	2,425.94 ^a	0.12 ^a	2,1211 ^a	7.63 ^a
Samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours	139.79 ^b	1,821.74 ^b	0.13 ^a	2,3265 ^b	8.41 ^b
Samples soaked in hot oil at 60°C for 4 hours	126.11 ^c	1,886.59 ^b	0.13 ^a	2,2011 ^c	9.02 ^c

Means with the same superscript in the same column are not significantly different ($p < 0.05$)

Table 2: Summary of ANOVA results for data obtained for the selected mechanical properties evaluated for treated and control split-bamboo samples

<i>Source of variation</i>	<i>Selected properties</i>	<i>(F-cal)</i>	<i>(F-tab)</i>
Treatment	Modulus of Rupture	1190.21 [*]	3.35
	Modulus of Elasticity	446.99 [*]	
	Energy at Maximum Load	2.71 ^{ns}	
	Load at Yield	2115.07 [*]	
	Extension at Yield	1106.17 [*]	

*denotes significance, ns denotes not significant ($p < 0.05$)

Conclusion and Recommendations

The results obtained from this investigation indicate that Neem seed oil, reported to possess some anti-microbial properties in the literature, can be applied as a preservative for bamboo stems/culms against white rot fungal infestation and degradation. This type of application, aided with more research endeavours, is expected to contribute to the recent worldwide intensified efforts toward developing cost effective, cheap and environmentally benign organic preservatives for non-durable lignocelluloses, particularly in many developing countries such as Nigeria where there are still challenges facing the present levels of technological development and economic realities.

The use of this oil for preserving bamboo from microbial degradation is also anticipated to encourage both potential and existing cottage and small-scale forest industries including other users that are interested in adding value to bamboo, particularly for income generation in this country where Neem seed is still with little or no economic value. However, there are still needs for studies that will be aimed at optimising the use of this oil for this purpose, most especially as it concerns bringing to tolerable level the variability in the values for some strength/mechanical properties of interest perhaps as a result of this oil-treatment. Similar studies using other methods of preservative application for this oil are also necessary.

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References

- ASTM (AMERICAN SOCIETY FOR TESTING AND MATERIALS), 1994. Standard Methods of Testing Small Clear Specimens of Timber. ASTM D-143 – 94 (2007).
- ASTM (AMERICAN SOCIETY FOR TESTING AND MATERIALS), 2007. Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Base Materials. ASTM D-4442 - 07.
- Cooper, P.A. 2001. Wood Preservation in Canada. The International Research Group on Wood Preservation Document no IRG/WP 01-50166-02.
- Erakhrumen, A.A. 2009. Tensile Strength Properties of Wild Grown *Bambusa vulgaris* Treated with Neem Seed Oil in Southwest Nigeria. *Journal of Bamboo and Rattan*, 8(1&2), 95-102.
- Erakhrumen, A.A. 2010. Potentials of Neem (*Azadirachta indica* A. JUSS) Seed Oil as a Preservative for Bamboo (*Bambusa vulgaris* SCHRAD. EX J.C. WENDL.) against Basidiomycetes. An unpublished Thesis for a Ph.D. degree of the University of Ibadan, Ibadan, Nigeria. xviii + 172pp.
- Erakhrumen, A.A. 2011a. Selected Physical and Chemical Properties of Mechanically Extracted Neem Seed Oil Sourced as a Preservative for Ligno-Cellulose in South-Western Nigeria. *Forestry Studies in China*, 13(4), 263-269.
- Erakhrumen, A.A. 2011b. Evaluating the Efficacy of Neem (*Azadirachta indica* A. Juss) Seed Oil-Treatment for *Bambusa vulgaris* Schrad. against *Pycnoporus sanguineus* (L. ex Fr.) Murr. using Static Bending Strength Properties. *Forest Pathology*, DOI: [10.1111/j.1439-0329.2011.00741.x](https://doi.org/10.1111/j.1439-0329.2011.00741.x)
- Erakhrumen, A.A.; Ogunsanwo, O.Y. 2009. Water Absorption, Anti-Swell Efficiency, and Dimensional Stability Properties of Neem Seed Oil-Treated Wild Grown *Bambusa vulgaris* Schrad ex J.C. Wendl. in Southwest Nigeria. *BioResources*, 4(4), 1417-1429.
- Erakhrumen, A.A.; Ogunsanwo, O.Y. 2010. Influence of Neem Seed Oil-Treatment on Static Bending Strength Properties of Wild Grown Split-Bamboo (*Bambusa vulgaris* Schrad.) in South-West Nigeria. *Silva Lusitana*, 18(2), 167-177.

- Green, D.W.; Winandy, J.E.; Kretschman, D.E. 1999. Mechanical Properties of Wood. Chapter 4 of Wood Handbook—Wood as an Engineering Material. Gen. Tech. Rep. FPL–GTR–113. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 463 pp.
- INBAR, 1998. Decay & Biodeterioration of Culms in Storage. In: Diseases of Bamboo in Asia: An Illustrated Manual by C. Mohanan of Kerala Forest Research Institute, Peechi, Kerala, India, for International Network for Bamboo and Rattan. Available at: <http://www.inbar.int/publication/txt/tr10/>
- Kumar, S.; Shukla, K.S.; Dev, T.; Dobriyal, P.B. 1994. Bamboo Preservation Techniques: A Review. Published jointly by International Network for Bamboo and Rattan (INBAR) and Indian Council of Forestry Research Education (ICFRE). Also available at: http://www.inbar.int/publication/txt/INBAR_Technical_Report_No03.htm
- Leithoff, H.; Peek, R.D. 2001. Heat Treatment of Bamboo. IRG/WP 01-40216. Prepared for the 32nd Annual Meeting of the International Research Group on Wood Preservation held in Nara, Japan, from May 20th to 25th, 2001. 11pp.
- Liese, W. 1985. Anatomy and Properties of Bamboo. In the Proceedings of an International Bamboo Workshop held in China from 6th to 14th October, 1985. pp. 196-208. Also available at: http://www.inbar.int/publication/PDF/INBAR_PR_03_1.pdf
- Liese, W. 1992. The Structure of Bamboo in relation to its Properties and Utilization. In: *Bamboo and its Use* Edited by S. Zhu, W. Li, X. Zhang and Z. Wang. Proceedings of an International Symposium on Industrial Use of Bamboo Supported by International Tropical Timber Organization and Chinese Academy of Forestry held in Beijing, China from 7th to 11th December, 1992. pp. 95-100.
- Liese, W. 1998. The Anatomy of Bamboo Culms. International Network for Bamboo and Rattan (INBAR) Technical Report No. 18. 204 pp.
- Liese, W. 2003. Structures of a Bamboo Culm Affecting its Utilization. In The Proceedings of an International Workshop on Bamboo Industrial Utilization Edited by C. Xuhe, L. Yiping and H. Ying sponsored by International Network for Bamboo and Rattan and hosted by Hubei Provincial Government & Xianning Municipal Government. Available at: http://www.inbar.int/publication/txt/INBAR_PR_13.htm
- Locke, J.C. 1995. Fungi. In: The Neem Tree, Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. Ed. by Schmutterer, H. VCH, Weinheim, Germany. pp. 118-125.
- Luna, M.L.; Murace, M.A.; Keil, G.D.; Otaño, M.E. 2004. Patterns of Decay caused by *Pycnoporus sanguineus* and *Ganoderma lucidum* (Aphyllphorales) in Poplar Wood. IAWA Journal, 25(4), 425-433.
- Manalo, R.D.; Acda, M.N. 2009. Effects of Hot Oil Treatment on Physical and Mechanical Properties of Three Species of Philippine Bamboo. Journal of Tropical Forest Science, 21(1), 19-24.
- Onuorah, E.O. 2000. Short Communication: The Wood Preservative Potential of Heartwood Extracts of *Milicia excelsa* and *Erythrophleum suaveolens*. Bioresources Technology, 75, 171-173.
- Parveen, G.; Alam, M.M. 1993. Bioactivity against Plant Pathogens. In: Neem Research and Development. Ed. by Randhawa, N.S. and Parmar, B.S. Society of Pesticide Science, New Delhi, India. pp. 144-153.
- Puri, H.S. 1999. Neem: The Divine Tree. *Azadirachta indica*. Harwood Academic Publications, Amsterdam. ISBN 90-5702-348-2.
- Soetaredjo, F.E.; Budijanto, G.M.; Prasetyo, R.I.; Indraswati, N. 2008. Effects of Pre-treatment Condition on the Yield and Quality of Neem Oil Obtained by Mechanical Pressing. ARPN Journal of Engineering and Applied Sciences, 3(5), 45-49.
- Wahab, R.; Sudin, M.; Samsi, H.W. 2005. Fungal Colonisation and Decay in Tropical Bamboo Species. Journal of Applied Sciences, 5(5), 897-902.

Influence of Treatment Temperature Regimes and Durations on the Absorption of Crude Neem (*Azadirachta indica* A. JUSS) Seed Oil by Split-Bamboo (*Bambusa vulgaris* SCHRAD.)

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Abstract

Most of the documented studies on the use of vegetable oil as preservatives for lignocellulosics have not adequately reported absorption values, a preservative's property that may influence some others, such as its penetration and distribution in treated material. This study was therefore carried out to contribute to efforts at documenting this property by evaluating absorption of mechanically extracted crude Neem (*Azadirachta indica* A. JUSS) seed oil by split-bamboo (*Bambusa vulgaris* SCHRAD. EX J.C. WENDL.) samples at two different treatment temperature (TT) regimes and durations of treatment (DOT). A multivariate linear regression model was also developed for estimating oil absorption (OA) from TT and DOT. Two sets of split-bamboo samples, with same dimension, from the same source were oven-dried at $103 \pm 2^{\circ}\text{C}$, conditioned to 11.76% mean moisture content, and treated by completely soaking a set in oil at ambient room temperature ($25 \pm 2^{\circ}\text{C}$) for 24 hours and by soaking the other in hot oil at 60°C for 4 hours. Results obtained showed that samples soaked in oil at $25 \pm 2^{\circ}\text{C}$ for 24 hours and those soaked in hot oil at 60°C for 4 hours had mean OA values of 57.02 and 124.30 kgm^{-3} respectively. The regression model developed for predicting OA from TT and DOT had a coefficient of determination of 0.93 with a significant ANOVA result ($p < 0.05$).

Keywords

Bamboo, Neem, Vegetable oil, Absorption, Temperature difference, Treatment duration

Introduction

Certain lignocellulosic materials such as bamboo are known to be susceptible to easy destruction by agents of biological deterioration owing to their poor inherent natural durability. Thus, there has been the need for the application of chemical substances, also known as preservatives, for protecting wood and fibre materials in order to, among other reasons, increase their durability and service life (e.g. Kumar *et al.* 1994; Liese and Kumar 2003; Liese 2004).

These chemical preservatives may either be synthetic, natural, or in some instances, mixture of both. However, in recent decades, public attention to the environmental and health issues concerning the utilisation of most synthetic chemical preservatives, particularly the metal-containing wood and fibre preservatives and the disposal problem of chemically treated wood and other lignocellulosic materials have revived interest in the oldest approach to wood and fibre protection, using natural extracts derived from plants or micro-organisms (Evans 2003).

Studies have shown that some green plants can be sources of innocuous biocides, which are non-toxic to mammals and are more easily biodegradable than synthetic chemicals and therefore, can be explored as a source of organic preservatives (Venmalar and Nagaveni 2005; Singh *et al.* 2006). An example of these is the different types of vegetable oils such as that obtainable from Neem (*Azadirachta indica* A. JUSS) seed (Erakhrumen 2010, 2011b).

Nevertheless, a lack of adequate characterisation of some of the physical and chemical properties of this seed's oil in West Africa, including Nigeria, might have been one of the reasons why this oil's anti-microbial properties, reported in the literature (e.g., Parveen and Alam 1993; Locke 1995; Puri 1999), have not been adequately recognised in this region. Owing to this, studies such as Erakhrumen (2010, 2011a) were carried out to further highlight the potentials of this oil, based on the reported properties in the literature and the outcomes of these studies, as a preservative for lignocellulosic materials.

Furthermore, in the same manner, as innovative as the use of this oil for bamboo preservation might be, there is still a dearth of information on properties relating to the use of this oil in this regard. One of these properties not yet adequately reported is oil absorption (OA) values for bamboos, for instance, when oil-treatment is done at different temperature regimes and duration of treatment. Adequate knowledge of this property, most especially as influenced by treatment processes is necessary since it may have effects on some other properties.

Among these properties which preservative absorption may influence are penetration and distribution of preservatives in treated lignocellulosic materials. Penetration and distribution are some of the several treatment factors upon which the effectiveness of preservatives depends. This study was therefore carried out in order to evaluate absorption values for Neem Seed Oil (NSO) in treated split-bamboo (*Bambusa vulgaris* SCHRAD. EX J.C. WENDL.) at two different treatment temperature (TT) regimes and duration of treatment (DOT). A predictive equation based on linear regression analysis was also developed for NSO absorption using the two variables (i.e. TT and DOT) as predictors.

Materials and Methods

Sourcing of Bamboo Culms

The bamboo culms sourced from wild clumps at Isale-Togun Forest, Lanlate, Ibarapa, Oyo State, Nigeria (latitude 7° 36' N and longitude 3° 27' E) in the month of October, 2008. This area is located in between the humid and sub-humid tropical climatic zones, where mean annual rainfall ranges between 1,117.10 and 1,693.30 mm. Only mature culms with mean circumference of 300 mm at the second node above ground were harvested and cross cut in such a way that only the basal culm portion of 3000 mm length were removed and placed in jute bags with nylon lined inner surface to avoid contamination from the soil. The harvested culms were transported to, protected and stored in the wood workshop of the Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria, for conversion to the test specimens.

Sourcing of Neem Seeds

The ripe Neem seeds from which oil was mechanically extracted in this study were obtained from *A. indica* trees on the University of Ibadan campus located on the northern edge of the city of Ibadan, Nigeria (latitude 7° 20'N and longitude 3° 50'E). Collection of the seeds was done by placing clean nylon sheets around the stems of *A. indica* trees in such a way that it covered a substantial cross sectional area of the crown in order to directly collect the seeds as they fall. The Neem seeds were sourced in the months of June to early August of 2008.

The Neem seeds obtained were thoroughly washed using deionised water to remove dirt and other impurities and then air dried in an open space with regular movement for aeration to ensure proper drying, a method also applied by Soetaredjo *et al.* (2008), to reduce the moisture content (MC) for proper crushing and to facilitate high oil volume recovery during mechanical extraction. The seeds were daily air dried with proper monitoring to prevent spoilage as a result of possible moisture fluctuations.

Conversion of Bamboo Culms to Test Samples

The harvested bamboo stems were carefully sawn into longitudinal strips using circular and vertical breakdown sawing. Each strip was planed on both the inner and outer surface, using a planing machine, in order to obtain mean thickness of 5 ± 0.5 mm for the MC determination and OA tests. The strips were first conditioned in the laboratory for 14 days and then oven-dried at $103 \pm 2^\circ\text{C}$ to constant weight. They were removed from the oven afterwards and stabilised in the laboratory for 24 hours.

The mean MC of split-bamboo samples after stabilisation was determined using test dimensions of 20 mm (tangentially) x 20 mm (longitudinally) x 5 mm (radially) and calculated in accordance with ASTM D 4442 (2007) while the strips for OA test at the same MC were also converted to test specimens with dimensions of 20mm (tangentially) x 60mm (longitudinally) x 5mm (radially).

Extraction of Neem Seed Oil

The Neem seeds obtained from the wild were decorticated after being air dried, separating the kernel from the shells and dirt, and then air-dried again. Dried kernels were carefully pulverised into smaller particles using a seed grinder ensuring no significant loss of seeds' oil. Mechanical extraction of oil was performed by cold pressing the pulverised seeds using an oil expeller at a maximum pressure of 31 MPa (31 Nmm^{-2} or 4,500 psi). Mechanical extraction was performed at this pressure until oil stopped flowing.

Treatment of Split-Bamboo Samples with the Extracted Oil

The split-bamboo samples to be treated with NSO were earlier stabilised in the laboratory to MC of 11.76%. The stabilised samples to be oil-treated were subjected to two NSO-treatment regimes through (1) soaking a set of samples in oil at ambient room temperature of $25 \pm 2^\circ\text{C}$ for 24 hours and (2) soaking another set in hot oil at 60°C for 4 hours, removed from the oil afterwards and allowed to cool in a dessicator at an ambient room temperature of $25 \pm 2^\circ\text{C}$.

These methods of NSO-treatments were also earlier adopted in the experiments reported by Erakhrumen (2009, 2010, 2011b); Erakhrumen and Ogunsanwo (2009, 2010). The maximum heat treatment temperature of 60°C was adopted in this research because strength and stiffness values used in practice for most lignocellulosic materials are valid for temperatures below 60°C (Homan and Jorissen 2004).

Determination of Absorption of Neem Seed Oil by Split-Bamboo

Split-bamboo samples with mean MC of 11.76% and dimension 20mm (tangentially) x 60mm (longitudinally) x 5mm (radially) were treated with the extracted NSO in different containers using the two treatment methods i.e., soaking of bamboo samples in oil at ambient room temperature ($25 \pm 2^\circ\text{C}$) for 24 hours and soaking in hot oil at 60°C for 4 hours and allowed to cool at room temperature in a dessicator. Values for OA was determined for bamboo samples based on mean weight gain over time, a method also adopted by Sobrinho *et al.* (2009). The values were calculated using equation 1.

$$OA = W_o / V_b \quad (\text{Eq. 1})$$

Where: OA = Oil absorption (kgm^{-3}); W_o = weight of oil absorbed (kg); and V_b = volume of bamboo sample (m^3).

Statistical Analyses

The OA values obtained for the ten samples for each treatment type were subjected to basic descriptive statistical analyses such as mean, standard deviation and standard error of mean. Linear regression analysis was employed in developing a predictive equation for OA. The statistical package used for the analyses was Minitab13[®] for Windows[®].

Results and Discussion

The results tabulated in Table 1 showed that OA value was higher for split-bamboo specimens treated by soaking in NSO at 60°C for 4 hours (124.30kgm^{-3}) in comparison with the split-bamboo specimens treated by soaking in NSO at $25 \pm 2^\circ\text{C}$ for 24 hours (57.02kgm^{-3}). However, it is noteworthy that bamboo culms have low treatability. This is partly because they have tissues that are resistant to penetration of liquids (Liese 1998). Radial pathways, like the rays in timber do not exist, thus, uptake of a preservative is restricted mainly to the vessels at the culm ends (Liese 2007).

Bamboos have been demonstrated to be able to absorb different fluids of different densities over time, most likely due to capillary forces within the pore structure (Findlay 1985; Sobrinho *et al.* 2009) giving different absorption values with absorption and penetration noted to be higher in split than in whole or round bamboo with impervious outer layer inhibiting absorption while the permeable inner cuticle enhances it (Liese 2007). The absorption values obtained in this study are relatively high for the two treatment regimes and appeared to be influenced by TT and DOT.

Table 1: Mean values, Standard deviation and Standard Error of mean for Oil Absorption

<i>Treatment</i>	<i>Mean Oil Absorption (kgm⁻³)</i>	<i>Standard Deviation</i>	<i>Standard Error of Mean</i>
Samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours	57.02	3.23	1.03
Samples soaked in hot oil at 60°C for 4 hours	124.30	7.26	1.11

Mean values are for 10 test samples per treatment

Based on the OA values obtained in this study, it appeared that split-bamboo specimens might absorb more or less of NSO if TT and DOT are varied. In ascertaining this, a predictive equation, based on linear regression model for estimating the quantity (in kgm⁻³) of NSO to be absorbed at a combination of any TT and DOT using this type of non-pressure oil-treatment method was developed. The multivariate regression model of the type ($y=b_0+b_1x_1+ b_2x_2$) developed for predicting OA is depicted by equation 2 while the results of linear regression for the equation are tabulated in Table 2.

$$OA = 10.7 - 0.050 DOT + 1.89 TT \quad (\text{Eq. 2})$$

Where:

- OA = Oil absorption (kgm⁻³)
 DOT = Duration of oil treating bamboo (hours)
 TT = Treatment temperature (°C)

Table 2: Results of linear regression for Equation 2

<i>Predictor</i>	<i>Coefficient</i>	<i>SE Coefficient</i>	<i>R² (%)</i>	<i>T</i>
Constant	10.74	24.07		0.45
DOT	-0.0495	0.6286	93.3	-0.08
TT	1.8931	0.3579		5.29

p < 0.05

The coefficient of determination (R^2) obtained for the regression model (Table 2) showed that 93.3% represent the proportion of variation in the OA by split-bamboo samples represented by both DOT and TT as predictors. The value obtained for R^2 was high enough for predictive purposes. Similarly, subjecting the regression equation to ANOVA, the result also showed that the predictive power of the equation was significant with 95% confidence.

Table 2 also showed that the ratio of the corresponding value under coefficient, standard error of coefficients, and T-value reinforced the result reported earlier by also showing that both DOT and TT significantly predicted OA in this study since the calculated values were greater than the pre-selected α -level of 0.05.

Conclusion and Recommendations

The results obtained for oil absorption in this study showed that split-bamboo specimens soaked in Neem seed oil, absorbed higher quantity when soaked in the oil at 60°C for 4 hours in comparison with those soaked in the oil at 25 ± 2°C for 24 hours irrespective of the higher duration of treatment at the latter temperature regime, implying perhaps that treatment temperature influenced oil absorption more than the duration of treatment.

The predictive equation was also shown to be able to estimate oil absorption values from treatment temperature and duration of treatment with 95% reliability. Therefore, in order to prevent ambiguities and discrepancies as it concerns oil absorption values for vegetable oil-treated lignocellulosic materials, particularly bamboo, there will be the need to identify and state the treatment variables that may affect absorption values in different treatment methods to be adopted. Further studies are still necessary in this regard.

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References

- ASTM (AMERICAN SOCIETY FOR TESTING AND MATERIALS), 2007. Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Base Materials. ASTM D-4442 - 07.
- Erakhrumen, A.A. 2010. Potentials of Neem (*Azadirachta indica* A. JUSS) Seed Oil as a Preservative for Bamboo (*Bambusa vulgaris* SCHRAD. EX J.C. WENDL.) against Basidiomycetes. An unpublished Thesis for a Ph.D. degree of the University of Ibadan, Ibadan, Nigeria. xviii + 172pp.
- Erakhrumen, A.A. 2011a. Selected Physical and Chemical Properties of Mechanically Extracted Neem Seed Oil Sourced as a Preservative for Ligno-Cellulose in South-Western Nigeria. *Forestry Studies in China*, 13(4), 263-269.
- Erakhrumen, A.A. 2011b. Evaluating the Efficacy of Neem (*Azadirachta indica* A. Juss) Seed Oil-Treatment for *Bambusa vulgaris* Schrad. against *Pycnoporus sanguineus* (L. ex Fr.) Murr. using Static Bending Strength Properties. *Forest Pathology*, DOI: [10.1111/j.1439-0329.2011.00741.x](https://doi.org/10.1111/j.1439-0329.2011.00741.x)
- Erakhrumen, A.A.; Ogunsanwo, O.Y. 2009. Water Absorption, Anti-Swell Efficiency, and Dimensional Stability Properties of Neem Seed Oil-Treated Wild Grown *Bambusa vulgaris* Schrad ex J.C. Wendl. in Southwest Nigeria. *BioResources*, 4(4), 1417-1429.
- Erakhrumen, A.A.; Ogunsanwo, O.Y. 2010. Influence of Neem Seed Oil-Treatment on Static Bending Strength Properties of Wild Grown Split-Bamboo (*Bambusa vulgaris* Schrad.) in South-West Nigeria. *Silva Lusitana*, 18(2), 167-177.
- Evans, P. 2003. Emerging Technologies in Wood Protection. *Forest Products Journal*, 53(1), 14-22.
- Findlay, W.F.K. 1985. *Preservation of Timber in the Tropics*. Published by Martinus Nijhoff / Dr. W. Junk Publishers, Dordrecht, The Netherlands. ISBN 90-247-3112-7.
- Homan, W.J.; Jorissen, A.J.M. 2004. Wood Modification Developments. *HERON*, 49(4), 361-386.
- Kumar, S.; Shukla, K.S.; Dev, T.; Dobriyal, P.B. 1994. *Bamboo Preservation Techniques: A Review*. Published jointly by International Network for Bamboo and Rattan (INBAR) and Indian Council of Forestry Research Education (ICFRE). Also available at: http://www.inbar.int/publication/txt/INBAR_Technical_Report_No03.htm
- Liese, W. 1998. The Anatomy of Bamboo Culms. International Network for Bamboo and Rattan (INBAR) Technical Report Number 18. 204pp.
- Liese, W. 2004. Preservation of Bamboo Structures. *Ghana Journal of Forestry*, 15&16, 40-48.
- Liese, W. 2007. Protection of Bamboo Structures. Paper presented at XXIII Sympozjum Rogów, Polska Akademia Nauk, Komitet Technologii Drewna held from 5 to 7 September, 2007. 7pp.

- Liese, W.; Kumar, S. 2003. Bamboo Preservation Compendium. International Network for Bamboo and Rattan (INBAR) Technical Report Number 22. 231pp.
- Locke, J.C. 1995. Fungi. In: The Neem Tree, Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. Edited by Schmutterer, H. VCH, Weinheim, Germany. pp. 118-125.
- Parveen, G.; Alam, M.M. 1993. Bioactivity against Plant Pathogens. In: Neem Research and Development. Edited by Randhawa, N.S. and Parmar, B.S. Society of Pesticide Science, New Delhi, India. pp. 144-153.
- Puri, H.S. 1999. Neem: The Divine Tree. *Azadirachta indica*. Harwood Academic Publications, Amsterdam. ISBN 90-5702-348-2.
- Singh, T.; Chittenden, C.; Vesentini, D. 2006. In vitro Antifungal Activity of Chilli against Wood Degrading Fungi. IRG/WP 06-10572. A Paper prepared for the 37th Annual Meeting of The International Research Group on Wood Protection held in Tromsø, Norway from 18 to 22 June 2006. 11pp.
- Sobrinho Jr. A.S.; de Aragão Jr. J.L.; Torres, S.M.; de Barros, S.; Ortiz, S.R.; Barbosa, N.P. 2009. Bamboo pH and Absorption in Different Liquids. Proceedings of the 11th International Conference on Non-conventional Materials and Technologies (NOCMAT 2009) 6-9 September 2009, Bath, UK. Also available at: <http://opus.bath.ac.uk/16170/1/papers/Paper%20155.pdf>
- Soetaredjo, F.E.; Budijanto, G.M.; Prasetyo, R.I.; Indraswati, N. 2008. Effects of Pre-treatment Condition on the Yield and Quality of Neem Oil obtained by Mechanical Pressing. ARPN Journal of Engineering and Applied Sciences, 3(5), 45-49.
- Venmalar, D.; Nagaveni, H.C. 2005. Evaluation of Copperised Cashew Nut Shell Liquid and Neem Oil as Wood Preservatives. IRG/WP 05-30368. Prepared for the 36th Annual Meeting of the International Research Group on Wood Protection held in Bangalore, India, from April 24th to 28th, 2005. 20pp.

The Effect of Site and Culm Height on the Shrinkage of the Wood of *Bambusa vulgaris* in Ghana.

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Abstract

Bamboo wood is gaining acceptance as a suitable alternative to dwindling commercial timber species in many countries. It is evident that the sustainable utilization of bamboo resources is an important part of forest management because bamboo could balance technological advancement and need for construction materials with environmental sustainability in most tropical countries like Ghana. As effort is made to improve the industrial utilization of bamboo resources, information on its behaviour including shrinkage characteristics of native bamboo species is needed. Uneven shrinkage in anisotropic materials like bamboo wood can result in drying defects such as warping, checking, splitting, and loosening of tool handles, gaps in strip flooring, or performance problems that detract from the usefulness of the product. In this study the magnitude of shrinkage in different directions, from green state of round and split bamboo culms to the oven-dried state was assessed in selected regions in Ghana. Results indicated significant difference in outer diameter, culm wall thickness and longitudinal shrinkage of bamboo samples with sites and height but not with season of harvest. The longitudinal shrinkage was negligible in all three sites with values between 0.01- 0.89 %. Shrinkage values vary between 4.70 % and 25.63 % for culm wall thickness and 2.33 % to 14.26 % for the outer diameter. There was no distinct increase or decrease in the shrinkage values from base to top in most of the samples. It was recommended among others, that matured bamboo culms with relatively thicker culm walls within the first five meters of *Bambusa vulgaris* could be considered for use as they prove to be more dimensionally stable. Further research is needed on both round and split bamboo samples for enhanced understanding and industrial application.

Keywords

Bamboo wood, Shrinkage, *Bambusa vulgaris*, utilization

Introduction

Bamboos are distinct and fascinating plants with a wide range of values and uses. They provide numerous services and products for human survival especially in countries where knowledge of their properties and the dissemination of the knowledge have been thoroughly pursued. The trade in bamboo products is increasing around the world and it is estimated that globally, domestic trade and subsistence use worth US\$ 4.5 billion per year and export generating over US\$ 2.7 billion (INBAR 2006). Instead of the use of timber, bamboo wood can be used to produce furniture, flooring and building construction and is widely regarded as an excellent substitute for wood in the form of laminated bamboo board (FAO 2006; MLF 2005). The market for laminated bamboo furniture, for instance, is growing steadily especially in Asia. Despite this potential, bamboo processing and utilization is almost non-existing in Ghana and other African countries compared to the extent of utilization in many countries in South Eastern Asia. Its drying is mostly by air with little or no preservatives. This practice take about two to three weeks to complete and even the desired moisture content of 6- 12 per cent is unachievable by current method. This is partly responsible for the production deficiency in pilot bamboo factory in the country. Removal of moisture from bamboo wood is usually accompanied by shrinkage in different directions in both split and round culms.

For any hygroscopic materials to be successfully used in service, its dimensional stability is a necessity. Shrinkage values in the tangential, radial, longitudinal direction and their ratios especially tangential-radial ratio gives a measure of how stable bamboo will be in service. Higher shrinkage values have been obtained for bamboo in relation to wood of similar densities. Two main reasons that have been given for this observation are the absence of radial elements and the increasing amount of parenchyma cells in the inner culms walls (Liese 1985).

In contrast to wood that shrinks after fibre saturation point, bamboo is reported to start shrinking from the moment it start losing water even in the lumen. In addition, bamboos possess hygroscopic materials in its parenchyma cells and, therefore, take a longer time to dry compared with wood of similar density (Laxmana 1985). Green bamboo also experiences irreversible and excessive shrinkage well above the fibre saturation point with only partial recovery at the intermediate stage.

Bamboo wood changes dimension as it gains or loses moisture. It shrinks when losing moisture from the cell walls and swells when gaining moisture in the cell walls. This shrinkage and swelling can result in drying defects such as warping, checking, splitting, and loosening of tool handles, gaps in strip flooring, or performance problems that detract from the usefulness of the wood product. Therefore, it is important that these phenomena be understood and considered when they can affect a product in which wood is used.

Espiloys (1987) reported an average of 12.30 % and 7.70 % for tangential and radial shrinkage respectively for *Bambusa spp.* Researches on the physical properties of bamboo have revealed variation with significant implication for end use (Gnanaharan 1994). In an earlier work in three sites in Ghana, Tekpetey *et al.* (2007 2008) reported significant variation in some technical properties-moisture content, basic density, and chemical composition- of bamboo from different ecological sites. Little information exist on shrinkage properties of bamboo culms from different sites in Ghana. The main aim of this study was to explore the trend in the shrinkage properties of bamboo wood in different sites in Ghana as a means of ensuring better understanding of the native bamboo and the industrial processing of bamboo resources in Ghana.

Materials and Methods

Two different sampling methods were used for extraction of bamboo samples for the research. In the first method, bamboo culms were harvested from three sites (Akim Oda, Assin Fosu and Manso-Amenfi at about 1.20 m above ground level and were further sectioned as bamboo samples. Marks were made at the lower part of each sample and labelled A - base; B - Middle and C - Top respectively. The samples were put into nylon sacks and transported to the Wood Science Laboratory KNUST, Ghana. The samples were kept in the cold store room at 5 °C to ensure freshness. Internodes from the lower part of each bamboo samples was removed using a vertical band saw. Two to three bamboo rings of 25 mm wide were then sectioned from the internode samples. Test samples of 100mm were obtained from the internodes dimension for shrinkage determination according to ISO 22157-1:2000E.



Fig 1: bamboo samples for shrinkage determination

In the second sampling method, ten matured culms of *Bambusa vulgaris var. vulgaris* were randomly harvested from four areas in southern Ghana: Bamboo culms were taken from Manso Amenfi (Western Region), Kumasi (Ashanti Region), Assin Akropong (Central Region), and Akim Oda (Eastern Region) of Ghana. The samples were harvested in the dry season (December to February) and raining season (April to June, 2009). Sound internodes of culms were marked and the 4th, 8th, 12th and 16th internodes were selected. Samples were refrigerated at 5°C to ensure freshness. The shrinkage samples of 100mm long (Fig 1) were obtained from the lower sections of each selected internode. In both sampling procedure shrinkage samples were air-dried for about three weeks and dimensional change were recorded regularly (every 24hours) until constant readings were recorded.

In both instances, the initial and final height (h), outer diameter (D) and culm thickness (t) were measured. On each sample, 4 diameters, 4 culm wall thicknesses and 2 lengths were measured. After air drying, the samples were finally dried in electric ovens at 105°C until the samples weight remain constant.

The final values were recorded and shrinkage percentage were computed using the formula below:

$$\% \text{ Shrinkage} = \frac{I-F}{I} \quad (\text{Eq. 1})$$

Where I - initial dimensions of samples and F - final dimension of samples

Results and Discussion

In most cases, the shrinkage values in the culm wall thickness of bamboo samples from the three sites were relatively higher than the outer diameter shrinkage as shown in Table 1. The longitudinal shrinkage was small in all three sites with values between 0.01-0.89 %. Shrinkage values vary between 4.70 % and 25.63 % for culm wall thickness in all samples from the three sites and 2.33 % to 14.26 % for the outer diameter. Table 1 further shows decline in shrinkage value especially in culms from Manso-Amenfi. This could be due to the prolonged period of refrigeration of culms of bamboo from the two sites. The differences in shrinkage values at the base, middle and the top among the different culms was significant at 5 % significant level. The base had values range from 2.0 to 7.5 %, the middle 4.0 - 7.5 % and 5.2 to 6.3 % for the top portion.

In an earlier work in Ghana, Ebanyenle and Oteng Amoako (2007) also recorded similar findings though the work was carried out in two ecological zones. Shrinkage values according to Ebanyenle and Oteng- Amoako (2007) were 12.0; 8.7;.0.2 per cent for culm wall thickness, outer diameter and longitudinal respectively in *Bambusa vulgaris* from Wet Evergreen (WE) Forest Type; whilst samples from the moist semi-deciduous (MS forest types) were 6.8 and 6.4 per cent for shrinkage in thickness and diameter respectively. The findings in this work are relatively higher (about two times in culm wall thickness of samples)

Table 1: Shrinkage in Outer Diameter, culms wall thickness and length of *Bambusa vulgaris* from three sites of Ghana.

Site	Outer Diameter (%)			Wall Thickness (%)			Longitudinal (%)
	A	B	C	A	B	C	
Akim Oda	2.33	3.90	5.23	7.60	7.87	7.10	0.01-0.08
	5.33	5.04	5.00	7.20	5.80	2.60	
	5.73	5.33	5.67	6.53	5.62	8.30	
	7.50	7.06	6.30	7.50	7.86	6.80	
	(5.22)	(5.33)	(5.55)	(7.21)	(6.79)	(6.20)	
S.D	2.14	1.31	0.57	0.48	1.25	2.49	
Assin Fosu	6.40	5.55	5.39	7.50	6.02	5.95	0.00-0.26
	10.55	6.76	6.89	12.18	7.08	9.57	
	8.44	7.20	5.60	11.26	7.39	7.92	
	9.86	8.32	5.86	14.00	10.21	6.53	
	(8.81)	(6.95)	(5.94)	(11.24)	(7.68)	(7.49)	
S.D	1.83	1.14	0.66	2.74	1.79	1.61	
Manso Amenfi	14.26	9.93	14.20	25.63	10.80	23.84	0.00-0.89
	9.44	7.84	5.30	10.44	10.35	4.70	
	10.21	6.60	9.33	20.09	14.60	14.00	
	12.70	8.32	10.70	19.71	16.80	17.20	
	(11.65)	(8.17)	(9.88)	(18.97)	(13.14)	(14.94)	
S.D	2.23	1.38	3.68	6.30	3.10	7.96	

S.D: Standard Deviation A-Base, B-Middle, C-Top

Sint et al. (2008) also reported that the longitudinal shrinkage values ranges between 0.119 % in *Dinochloa maclellandi* and 0.262 % in *Dendrocalamus calosstachyus*. Significant effects of species and culm height were observed on the longitudinal shrinkage from green to oven-dry condition at 5 % level of significance. Longitudinal shrinkage when compared to diameter shrinkage and the culm wall thickness shrinkage is negligible. The shrinkage in culm wall, however, was not definitely increasing or decreasing with height but the differences were significant with height at 5 % significant level. It is also known that higher percentage of shrinkage may be related to a higher percentage of moisture content (Sattar et al. 1991). Shrinkage in bamboo is influenced by the stage of fibre maturation and the density of vascular bundles. The indefinite pattern of shrinkage in culm wall thickness may be due to variation in the culm thickness within and among sites of harvesting which decline from the base to the top.

As bamboo wood loses moisture, like wood, there is reduction in size and dimension. Shrinking of the cell wall occurs as bound water molecules escape from the long-chain cellulose and hemicelluloses molecules in the cell wall. The amount of shrinkage that occurs is proportional to the amount of water removed from the cell wall. In other words, the more bound water removed from the culm wall, the more obvious the dimensional change.

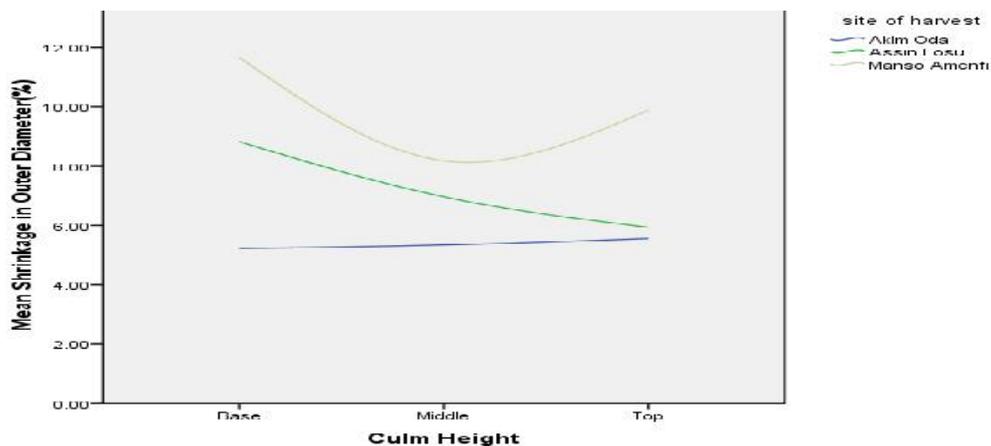


Fig 2: Shrinkage in outer diameter of bamboo culms from three sites in Ghana

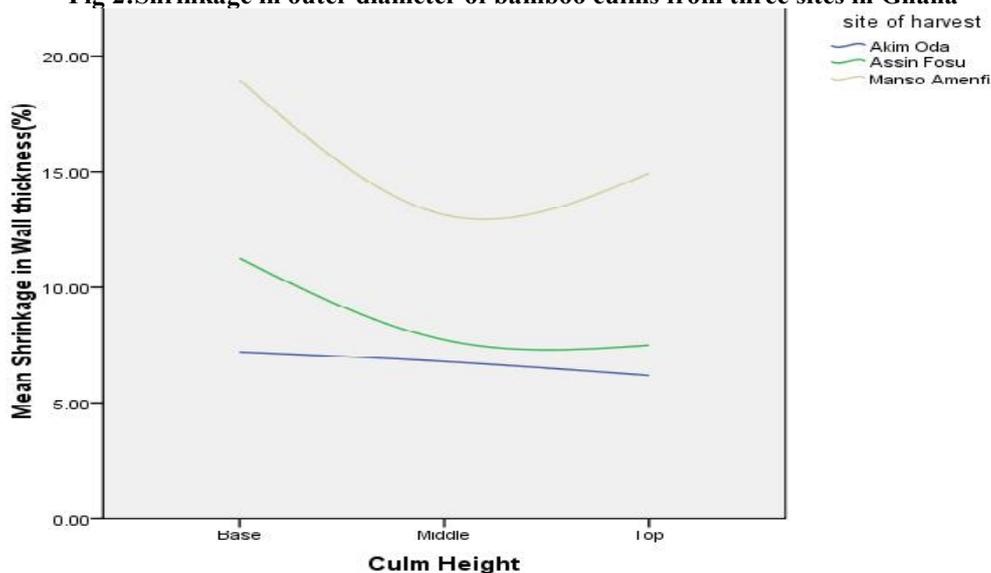


Fig 3: Shrinkage in outer diameter of bamboo culms from three sites in Ghana

Table 2 revealed the mean values of shrinkage in the outer diameter and wall thickness from four major bamboo growing areas in Ghana. There was decline in the mean values of shrinkage in all the four sites from the 4th to 16th internodes. The highest shrinkage value in outer diameter was recorded in culms from Manso-Amenfi which is located in the Moist Evergreen Forest type of Ghana (11.65 per cent) and lowest value in Kumasi located in the Semi Deciduous Forest Types (0.17 per cent).

Unlike the outer diameter shrinkages, bamboo samples from Assin Akropong recorded the highest values of 11.24 percent at the 4th internode whilst the lowest value was from Kumasi on the 12th internode (0.10 per cent). The magnitude of shrinkage in bamboo decreases with the height of the culm and age. Though specific age of culms were not determined in this work, the within culm variation in shrinkage values in this work may be due to age difference that may exist among the culms in the natural stands. Shrinkage in bamboo is influenced by the stage of fibre maturation and the density of vascular bundles and age of bamboo culms. Older culms are dimensionally more stable than younger ones.

Table 2: Mean shrinkage in Outer diameter, culms wall thickness of *Bambusa vulgaris* from four zones of Ghana.

Site/Shrinkage	4 th	8 th	12 th	16 th
Akim Oda (D) (T)	5.22	5.33	5.55	4.43
	7.21	6.79	6.20	3.23
Assin Akropong	8.81	6.95	5.94	4.54
	11.24	7.68	7.49	7.11
Manso-Amenfi	11.65	8.17	9.88	6.56
	8.79	3.14	4.94	3.23
Kumasi	1.09	0.24	0.79	0.17
	2.81	3.18	0.10	0.37

Conclusion

Shrinkage properties of bamboo culms from different sites in Ghana revealed that there is significant variation in the outer diameter and culm wall thickness. The longitudinal shrinkage among samples from the all sites were negligible compared with the outer diameter and cell wall thickness. The amount of shrinkage that occurs is proportional to the amount of water removed from the cell wall. The more bound water removed from the culm wall, the more obvious the dimensional change.

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References

- Ebanyenle, E & Oteng-Amoako A.A,2007. Site differences in Morphological and Physical properties of *Bambusa Vulgaris* Grown in Ghana: 222-225
- Espiloy, Z. 1987. Physico- mechanical properties and anatomical relationships of some Philippine bamboos. In: (A.N.Rao, et al., eds.) Recent research on bamboo. Proceedings of the International Bamboo Workshop, Hangzhou, China, 6-14 October. Chinese Academy of Forestry, Beijing China; International Development Research.Center, Ottawa, Canada: 257-264.
- FAO.2006.Bamboo Furniture Making.Teca Technologies for small holders.www.fao.org (date accessed 5th April,2009)
- INBAR 2006. Bamboo for the Environment, Development and Trade. International Bamboo Workshop, Wuyishan ,Fujian China.
- Laxmana, M.G.1985 Drying of Some Commercial Philippine bamboos. FPRDI Journal, 14: pp 8-19.
- Liese, W. 1985. Anatomy and properties of bamboo. In A.N Rao, G. Dhanarajan and C.B Sastry (Eds.).Recent Research on Bamboo. Proceedings of International Bamboo workshop,China, 1985.Hangzhou,China Chinese Acad. For Beijing, China and IDRC, Canada: 196-208.
- MLF, Ministry of Land and Forestry 2004. Bamboo Timber: Bulletin on Bamboo and Rattan Development Programme in Ghana.
- Mohmod, A.L.,W.T.W., Qarifin and Husein 1991. Variation in physical properties of two malaysian bamboo process of thefourth intern Bamboo workshop held in Chiangmai, Thailand, Nov.27-30, 1991 on Bamboo in asia and Pacific: 232-236
- Sattar, M.A., Kabir, M.F. and Bhattacharji, D.K.. 1991. Effect of age and height position of Muli (*Melocanna baccifera*) and Borak (*Bambusa balcooa*) bamboos on their physical and mechanical properties. Bangladesh J. For. Sci. 19(1&2): pp 29-37.
- Sint, K.M.; Hapla F.; Myint ,C.C. 2008.Investigation on Physical and Mechanical properties of some Myanmar bamboo species. Journal of bamboo and Rattan Vol.7 (3&4) pp 183-192
- Tekpetey, S.L, Frimpong- Mensah, K. and Darkwa N. 2007. Thermogravimetric behaviour and selected physical properties of *Bambusa vulgaris* in Ghana. Journal of Bamboo and Rattan 6(3,4): 199–204.
- Tekpetey,S.L.,DarkwaN.A;Frimpong-Mensah, K.. 2008. *Bambusa vulgaris* in Ghana: chemical composition and phytochemical properties for enhanced utilization. Journal of bamboo and Rattan Vol 7,(3&4) pp243-249

The Thermal Decomposition Property of Moso Bamboo

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Abstract

In this research, thermogravimetry (TG), a combination of thermogravimetry and Fourier transform infrared spectrometer (TG-FTIR) and X-ray diffraction (XRD) were used to investigate pyrolysis characteristics of moso bamboo (*Phyllostachys pubescens*). The Flynn-Wall-Ozawa and Coats-Redfern (modified) methods were used to determine the apparent activation energy (Ea). The TG curve indicated that the pyrolysis process of moso bamboo included three steps and the main pyrolysis occurred in the second steps with temperature range from 450K to 650K and over 68.69% mass was degraded. TG-FTIR analysis showed that the main pyrolysis products included absorbed water (H₂O), methane gas (CH₄), carbon dioxide (CO₂), acids and aldehydes, ammonia gas (NH₃) et al. XRD analysis expressed that the index and width crystallinity of moso bamboo gradually increased from 273K to 538K and cellulose gradually degraded from amorphous region to crystalline region. The Ea values of moso bamboo increased with conversion rate increase from 10 to 70. The Ea values were respectively 153.37-198.55KJ/mol and 152.14-197.87KJ/mol basis on Flynn-Wall-Ozawa and Coats-Redfern (modified) methods. The information is very helpful and significant to design manufacturing process of bio-energy, made from moso bamboo, using gasification or pyrolysis methods.

Key words: Pyrolysis, bamboo, TG, TG-FTIR, XRD

1. Introduction

Bamboo was a kind of biomass material and it owned many advantages such as fast growth and high strength. The bamboo resource was very abundant and had been widely cultivated in the west and south of China. Bamboo had great potential as a biomass material and bio-energy resource of the future. Currently, the area of bamboo was about five million hectares and that of moso bamboo was about 3 million hectares in China (Jiang 2002). Annual yield of moso bamboo was about eighteen million tons and was greater than all other kinds of bamboo, which was widely used to produce furniture, flooring and interior decoration materials.

As a type of natural lignocellulose polymer, bamboo will be subjected to thermal decomposition in manufacturing processes of bamboo composites or in the end use of these products. The study on pyrolysis of moso bamboo is very helpful to better design manufacturing process of bamboo composite or bio-energy, made from bamboo by thermal chemical conversion methods such as gasification and pyrolysis. Thermogravimetry (TG) is often used to analyze thermal degradation of lignocellulosic materials. Travis F et al. (2002) studied on pyrolysis behavior and kinetics of biomass derived materials by TG, Mostashari (2008) investigated combustion pathway of cotton fabrics through TG method, Anker et al. (1998) analyzed the influence of potassium chloride on wheat straw pyrolysis by TG-FTIR, Bradbury et al. (1979) and Koufopoulos et al. (1989) studied kinetic model for pyrolysis of cellulose materials.

Despite previous research is very helpful to understand the thermal decomposition kinetics of cellulose materials, bamboo is a different biomass resource from them. So the pyrolysis characteristics of moso bamboo were studied by TG method in the research. TG-FTIR and XRD analysis was used to investigate bamboo pyrolysis products and residues. The Doyle model-free method was used to determine the apparent activation energy of bamboo. The aim is better design manufacturing process of bamboo composite or bio-energy, made from moso bamboo by thermal chemical conversion methods such as gasification and pyrolysis.

2. Theoretical approach

The fundamental rate equation used in all kinetics studies is generally described as:

$$d\alpha/dt=kf(\alpha) \quad (\text{Eq. 1})$$

where, k is the rate constant and $f(\alpha)$ is the reaction model, a function depending on the actual reaction mechanism. Eq. (1) expresses the rate of conversion, $d\alpha/dt$ at a constant temperature as a function of the reactant conversion loss and rate constant. In this research, the conversion rate α is defined as:

$$\alpha=(w_0-w_t)/(w_0-w_f) \quad (\text{Eq. 2})$$

where, w_t , w_0 and w_f are time t , initial and final weight of the sample, respectively. The rate constant k is generally given by the Arrhenius equation:

$$k=A\exp(-E_a/RT) \quad (\text{Eq. 3})$$

where, E_a is the apparent activation energy (kJ/mol), R is the gas constant (8.314 J/K mol), A is the pre-exponential factor (min^{-1}), T is the absolute temperature (K). The combination of Eqs. (1) and (3) gives the following relationship:

$$d\alpha/dt=A\exp(-E_a/RT)f(\alpha) \quad (\text{Eq. 4})$$

For a dynamic TGA process, including the heating rate, $\beta=dT/dt$, into Eq. (4), Eq. (5) is obtained as:

$$d\alpha/dT=(A/\beta)\exp(-E_a/RT)f(\alpha) \quad (\text{Eq. 5})$$

Eqs. (4) and (5) are the fundamental expressions of analytical methods to calculate kinetic parameters on the basis of TGA data.

The most common ‘model-free’ methods included Friedman method (Friedman 1964), Kissinger method (Kissinger 1956), Flynn-Wall-Ozawa method [Flynn and Wall 1966; Ozawa 1965], Coats-Redfern (modified) method (Brown et al. 2000) and Doyle method (Doyle 1961). In the research, Doyle method was used to analyze the thermal decomposition of bamboo. Its expression was $\ln[-\ln(1-\alpha)]=\ln(AE_a/\beta R)-2.315-0.4567E_a/RT$. In this method, the E_a could be calculated by the slope of line determined by plotting $\ln[-\ln(1-\alpha)]$ against $1/T$ at a series of conversion rate .

3. Experiment

3.1 Material

Moso Bamboo was used in the study. The moisture content of samples was about 8.0%. Bamboo materials were cut off to sample size 40mm (longitudinal) by 20-30mm (radial) by 3-8mm (tangential). Then, they were broken down to particles with a Wiley mill and the size of bamboo particles used in the test was about 250-425 μm . Finally, the particles were dried at 105 °C for 4h.

3.2 Test procedures of thermal decomposition

Thermal decomposition was observed in terms of global mass loss though TA Instrument TGA Q 500 thermogravimetric analyzer (TA Instrument, USA). The bamboo powders were evenly and loosely distributed in an open sample pan and the initial sample weight was about 3-6mg. The temperature change was controlled from room temperature (300 \pm 5K) to 1000K with a heating rate of 10 K/min. A high purity nitrogen stream (flow rate of 60 mL/min and 40 mL/min respectively) were continuously passed into the furnace before thermal decomposition was carried out in order to prevent any unwanted oxidative decomposition. The experimental data could be directly obtained though TGA Q 500 thermogravimetric analyzer, but they were analyzed by using Universal Analysis software from TA Instruments and Origin 8.0 software. E_a values were calculated using a custom program designed within Microsoft Office Excel 2007.

3.3 TG-FTIR test

The experimental setup consisted of a combination of thermogravimetry (TA Instrument, USA) and fourier transform infrared spectrometer (Bruker IFS 66/S, Bruker Optics, Billerica, MA). A helium sweep gas flow of 500ml/min was used to bring the evolved pyrolysis gases from the TGA directly to the gas cell which was heated to 423K. The system collected FTIR spectra every 30s and the sample temperature and mass were logged every 3s. The sample pan was placed close to the end of the furnace, where a steeply decreasing temperature profile existed. This, combined with the high gas flow, minimized the residence time of the evolved gases in the hot zone. In the experiment, sample masses were about 20mg and they were heated from 323 K to 823 K at heating rate 10K/min.

3.4 X-ray diffraction (XRD) analysis of bamboo

XRD test of bamboo, treated in the different temperature for 30min, was carried out with X-ray generator with a Co target ($\lambda=0.1729$ nm) at a scanning speed of 3°/min, and the data were recorded every 0.02° (2 θ) for the angle range of 2 θ =5-45°. The crystallinity index (CrI) and the width of

crystals (t) was respectively calculated according to formula (6) and (7):

$$\text{CrI} = (I_{002} - I_{\text{am}}) / I_{002} \quad (\text{Eq. 6})$$

where, I_{002} is the overall intensity of the peak at 2θ about 22° and I_{am} is the intensity of the baseline at 2θ about 18° .

$$t = k \times \lambda / (B \cos \theta) (\text{\AA}) \quad (\text{Eq. 7})$$

where, k is the Scherrer constant (0.9), λ is the wavelength of the X-ray, B is the half-bandwidth in radians and θ is the Bragg angle.

4 Results and discussion

4.1 Pyrolysis process of bamboo

Figure 1 presented the pyrolysis process of moso bamboo at a heating rate of 10k/min. As shown in Fig. 1, the pyrolysis process of moso bamboo included three steps. The initial temperature of every step was defined as the critical point of weight change in the TG curve. The initial temperature of thermal decomposition for subsequent steps was the same as the final temperature of thermal decomposition for the previous step. In the third step, the final temperature was defined as the critical point where the sample weight had not changed. In the first step, the degradation temperature was from 300K to 450K and weight loss was about 1.59% due to removal of absorbed water from the samples. In the second step, the degradation temperature was from 450K to 650K and the weight loss was about 68.69%. The degradation of cellulose, hemicelluloses and partial lignin happened in this step. The critical temperature of maximum weight loss was 620K for moso bamboo in the pyrolysis process. In the third step, the degradation temperature was from 650K to 850K and the weight loss was about 7.53%. The degradation of lignin residues from the second step or tar and char from the main components resulted in weight loss of the sample in the step. The pyrolysis characteristics of moso bamboo from TG were shown in Table 1.

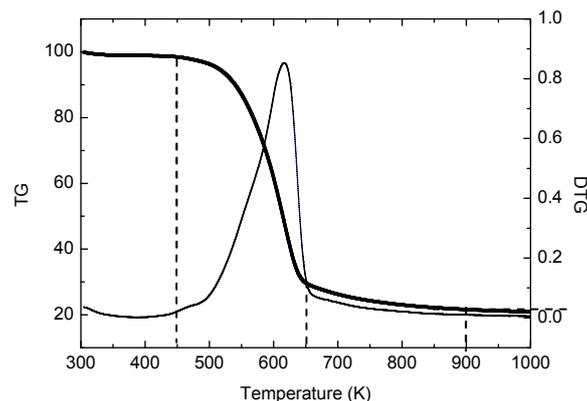


Figure 1 Pyrolysis process of bamboo at a heating rate of 10k/min

Table 1 Pyrolysis characteristics of bamboo from TG

Pyrolysis characteristics	The first step			The second step			The third step		
	Start/K	End/K	Weight loss/%	Start/K	End/K	Weight loss/%	Start/K	End/K	Weight loss/%
Bamboo	300	450	1.59	450	650	68.69	650	850	7.53

4.2 Average apparent activation energy of bamboo

The Flynn-Wall-Ozawa and Coats-Redfern (modified) methods were used to determine the Average apparent activation energy (E_a) of moso bamboo in the research. Both methods were the most 'mold-free' methods in E_a determination of biomass materials. The Flynn-Wall-Ozawa method was the integral method, which led to $-E_a/R$ from the slope of the line determined by plotting $\log(\beta)$ against $1/T$ at any certain conversion rate. The modified Coats-Redfern method was a multi-heating rate application of the Coats-redfern equation. Plotting the left hand side for each heating rate versus $1/T$ at that heating rate gave a family of straight lines of slope $-E_a/R$. The full solution was to be done iteratively by first assuming E_a value and then recalculating the left hand side until convergence occurs. Here, a quick solution, however, was also available by moving $(1-2RT/E_a)$ into the intercept and assuming that it was a constant [7]. The both methods were shown in Table 2.

The typical plots of Flynn-Wall-Ozawa (Figure 2) and Coats-Redfern (modified) (Figure 3) showed a general trend of E_a values for moso bamboo. It was shown in Figure 2 and Figure 3 that the fitted lines were nearly parallel at conversion rate from 10 to 70, which indicated approximate E_a values at different conversions and consequently implied the possibility of single reaction mechanism. Table 3 summarized the E_a values of moso bamboo calculated according to the conversion rate range from 10 to 70 using Flynn-Wall-Ozawa and Coats-Redfern (modified) methods. As shown in Table 3, the E_a values of moso bamboo were around 153.37-198.55KJ/mol at conversion rate from 10 to 70 for Flynn-Wall-Ozawa method. The similar results of Coats-Redfern (modified) method were also confirmed this observation, where the E_a values were around 152.14-197.87KJ/mol. The E_a values of moso bamboo were gradually increased with the conversion rate increase from 10 to 70. The higher E_a values indicated that thermal decomposition of bamboo was more difficult. The information was very helpful and significant to design manufacturing process of bio-energy, made from moso bamboo, using the thermal decomposition methods such as gasification or pyrolysis.

Table 2 Flynn-Wall-Ozawa and Coats-Redfern (modified) model-free method

Method	Expression	Plots
Flynn-Wall-Ozawa	$\log(\beta)=\log(AE_a/RG(\alpha))-2.315-0.4567E_a/RT$	$\log\beta$ against $1/T$
Coats-Redfern (modified)	$\ln[\beta/(T^2(1-2RT/E_a))]=\ln[-AR/(E_a\ln(1-\alpha))]-E_a/RT$	$\ln(\beta/T^2)$ against $1/T$

Table 3 The E_a value of moso bamboo by Flynn-Wall-Ozawa and Coats-Redfern (modified) method

Conversion rate (%)	Average apparent activation energy of moso bamboo (kJ/mol)	
	Flynn-Wall-Ozawa method	Coats-Redfern (modified) method
10	153.37	152.14
20	156.06	154.49
30	161.99	160.41
40	165.89	164.25
50	172.51	171.02
60	181.61	180.39
70	198.55	197.87

Conversion
rate (%)

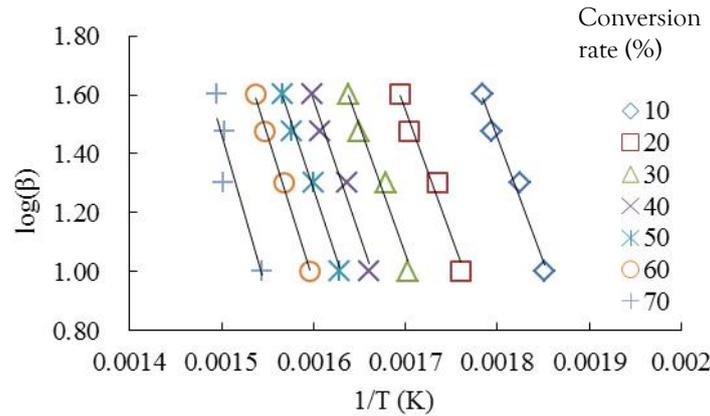


Figure 2 The typical plot of Flynn-Wall-Ozawa for moso bamboo

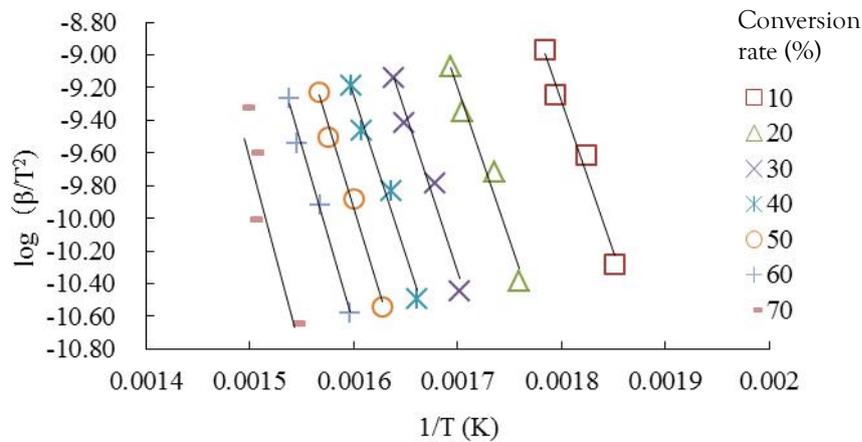


Figure 3 The typical plot of Coats-Redfern (modified) for moso bamboo

4.3 TG-FTIR analysis

In the experiment, approximately 20mg samples were heated to 323 K for 4 min, and then to 823 K at heating rate 10K/min. When temperature reached 823K and was steady for 4 min, the samples were immediately cooled to 323 K. The typical absorbance peaks of pyrolysis products of moso bamboo were shown in Figure 4. According to Figure 4, the typical absorbance peaks and pyrolysis products of moso bamboo were shown in Table 4 and listed as follows: The absorbance at 3435cm^{-1} was O-H stretching vibration and pyrolysis products were mainly absorbed water (H_2O), the absorbance of 2927cm^{-1} was C-H stretching vibration and pyrolysis products were methane gas (CH_4), the pyrolysis products of absorbance (2345 and 650cm^{-1}) were carbon dioxide (CO_2). The absorbance of 1718cm^{-1} was C=O stretching vibration and that of 1617cm^{-1} was C=C stretching vibration which probably came from pyrolysis products of acids and aldehydes, the peak of 1511cm^{-1} and 1388cm^{-1} appeared when nitrogen dioxide (NO_2) was released, the absorbance of 860cm^{-1} expressed ammonia gas (NH_3). Bamboo is a type of biomass material which was composed of several compounds. Some pyrolysis gases can be evolved at the same time, which lead to more difficultly find the absorbance of gas when testing small quantities.

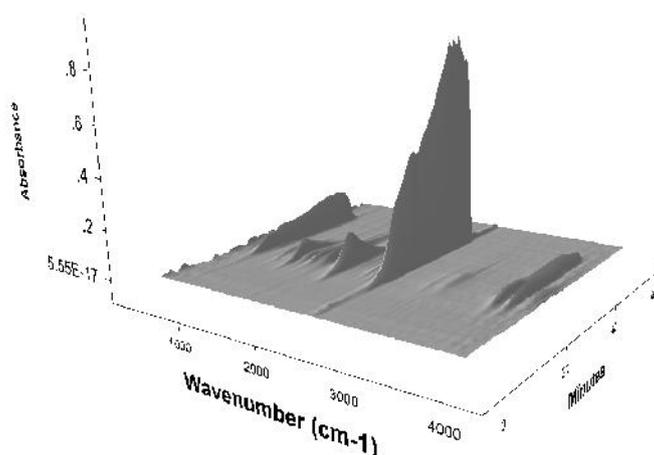


Figure 4 The absorbance spectra stack plot from the pyrolysis of bamboo at heating rate 10K/min

Table 4 The typical absorbance peaks and pyrolysis products of moso bamboo

Typical absorbance peaks (cm^{-1})	The main pyrolysis products
3535 cm^{-1}	Absorbed water (H_2O)
2927 cm^{-1}	Methane gas (CH_4)
2345 and 650 cm^{-1}	Carbon dioxide (CO_2)
2175 and 2117 cm^{-1}	Carbon monoxide (CO)
1795 cm^{-1}	Nitric oxide (NO)
1718 and 1617 cm^{-1}	Acids and aldehydes
1511 and 1388 cm^{-1}	Nitrogen dioxide (NO_2)
860 cm^{-1}	Ammonia gas (NH_3)

4.5 XRD analysis

The bamboo powder, used in the study, also was treated in the heat-treating furnaces with different temperature for 30min. The heat-treating temperatures were selected basis on the pyrolysis temperature of bamboo. For example, temperature point of 433K located in the scope of pyrolysis temperature of absorbance water. Temperature point of 488K was located in the scope of pyrolysis temperature of hemicelluloses. Temperature points of 538K and 588K were located in the scope of pyrolysis temperature of cellulose. The two temperature points (538K and 588K) were selected because cellulose is composited of amorphous region and crystalline region. Temperature point of 723K was located in the scope of pyrolysis temperature of lignin. XRD test of bamboo powder, treated in the different temperature for 30min, was carried out with X-ray generator. The XRD test results were shown in the Figure 5. The crystallinity index (CrI) and the width of crystals (t) was respectively calculated according to formula (6) and (7). The crystallinity index (CrI) and the width of crystals (t) of bamboo treated in different temperatures were shown in Table 5. As shown in Table 5, the index and width crystallinity of bamboo were respectively 28.28% and 3.1488nm, 28.29% and 3.1974nm, 30.38% and 3.2667nm, 32.76% and 3.3441nm, 3.88% and 2.6069nm, 0% and 0nm in the temperature of 273K, 433K, 488K, 538K, 588K and 723K. The experimental results indicated that index and width crystallinity of bamboo gradually increased from 273K to 538K. The main reason was that crystalline region of cellulose was not degraded, but absorbance water, small molecule, hemicelluloses and amorphous region of cellulose had been gradually degraded in the temperature scope. To biomass materials, there are two kinds of the index and width crystallinity of cellulose, respectively absolute and relative index and width crystallinity. The relative index and width

crystallinity were used in the research. So when some matters were degraded except for crystalline region of cellulose, the relative index and width crystallinity of bamboo gradually increased. In the pyrolysis temperature scope of cellulose, temperature point of 538K and 588K were selected in the research. The index and width crystallinity of 588K were lower than that of 538K, which indicated that cellulose pyrolysis started from amorphous region to crystalline region. When temperature was upon to 723K, the crystalline region of cellulose was completely destroyed and the index and width crystallinity of bamboo were 0% and 0nm.

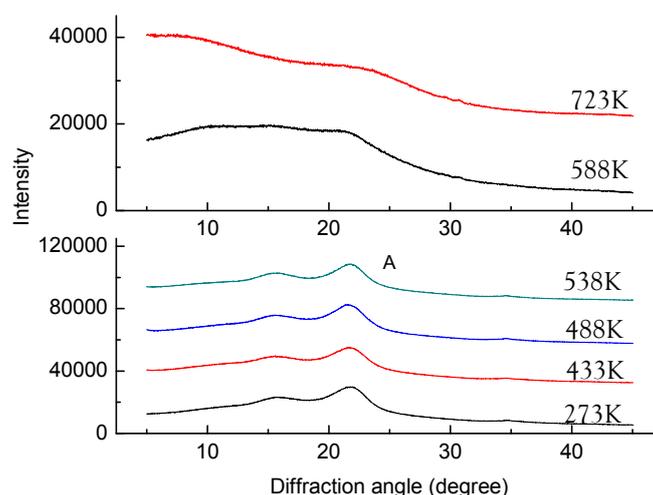


Figure 5 XRD curve of bamboo treated in different temperatures for 30 min

Table 5 The index and width of crystallinity of bamboo treated in different temperatures

Treated temperature	Crystallinity index/%	Crystallinity width /nm
273K (Untreated)	28.28	3.1488
433K	28.29	3.1974
488K	30.38	3.2667
538K	32.76	3.3441
588K	3.88	2.6069
723K	0	0

5 Conclusions

TG was used to investigate the pyrolysis process of moso bamboo. The Flynn-Wall-Ozawa and Coats-Redfern (modified) methods were used to determine E_a value. TG-FTIR and XRD were respectively used to analyze the pyrolysis products and residues of moso bamboo.

The TG curve indicated that the pyrolysis process of moso bamboo included three steps and the main decomposition occurred in the second step with temperature range from 400K to 630K and over 68.69% mass was degraded. The temperature of 620K was the critical point where maximum mass loss occurred in the pyrolysis process of moso bamboo. The main degradation compositions were hemicelluloses, cellulose and partial lignin of moso bamboo in this step.

The E_a values of maso bamboo increased with conversion rate increase from 10 to 70. The E_a values were respectively 153.37-198.55KJ/mol and 152.14-197.87KJ/mol according to Flynn-Wall-Ozawa and Coats-Redfern (modified) methods. The information was very helpful and significant to design manufacturing process of bio-energy, made from moso bamboo, using the gasification or pyrolysis methods.

TG-FTIR analysis showed that the main pyrolysis products included absorbed water (H_2O), methane gas (CH_4), carbon dioxide (CO_2), carbon monoxide (CO), acids and aldehydes, nitrogen dioxide

(NO₂), nitric oxide (NO), ammonia gas (NH₃).

XRD analysis expressed that the index and width crystallinity of moso bamboo gradually increased from 273K to 538K. In the pyrolysis process of cellulose, its pyrolysis gradually occurred from amorphous region to crystalline region. When temperature was close to 723K, the crystalline region of cellulose was completely destroyed.

References

- [1] Jiang Z.H. 2002. World bamboo and rattan. Liaoning Science & Technology Press, China. 43 pp.
- [2] Travis F., Mohammad H., Bruce W., Diane K. 2002. Pyrolysis behavior and kinetics of biomass derived materials. *J of Analytical and Applied Pyrolysis*, (62): 331-349.
- [3] Mostashari S. M., Mostashari S. Z. 2008. Combustion pathway of cotton fabrics treated by ammonium sulfate as a flame-retardant studied by TG. *J of Thermal Analysis and Calorimetry*, 91(2):437-441.
- [4] Anker J., Kim D.J. 1998. TG-FTIR Study of the Influence of Potassium Chloride on Wheat Straw Pyrolysis. *Energy & Fuels*, 12:929-938.
- [5] Bradbury A.W., Sakai Y., Shafizadeh F. 1979 A kinetic model for pyrolysis of cellulose. *J of Applied Polymer Science*, 23(11): 3271-3280.
- [6] Koufopoulos C.A., Maschio G., Lucchesi A. 1989. Kinetic modeling of the pyrolysis of biomass and biomass components. *Canadian J of Chemical Engineering*, 67(1):75-84.
- [7] Yao F., Wu Q.L., Lei Y., Guo W.H., Xu Y.J. 2008. Thermal decomposition kinetics of natural fibers: Activation energy with dynamic thermogravimetric analysis. *Polymer Degradation and Stability*, 93: 90-98.
- [8] Friedman H.L. 1964. Kinetics of thermal degradation of char-forming plastics from thermogravimetry. Application to phenolic plastic. *J Polym Sci Part C-Polym Symp*, 183-195.
- [9] Kissinger H.E. 1956. Variation of peak temperature with heating rate in differential thermal analysis. *J Res Natl Bur Stand*, 57:217-221.
- [10] Flynn J.H., Wall L.A. 1966. General treatment of thermogravimetry of polymers. *J Res Natl Bur Stand Sect A e Phys Chem*, A70:487-523.
- [11] Ozawa T. 1965. A new method of analyzing thermogravimetric data. *Bull Chem Soc Jpn*, 38: 1881-1887.
- [12] Brown M.E., Maciejewski M., Vyazovkin S., Nomen R., Sempere J., Burnham A. 2000. Computational aspects of kinetic analysis. Part A: The ICTAC kinetics project-data, methods and results. *Thermochim Acta*, 355:125-143.
- [13] Doyle C.D. 1961. Kinetic Analysis of Thermogravimetric Data. *J of applied Polymer Science*, 15: 285-292.
- [14] Zhao W.T., Chen H.X., Zhou J.J., Liu N.A. 2009. Characteristics and Kinetics of Forest Peat Pyrolysis. *Acta Phys. Chim. Sin*, 25(9): 1756-1762.

Investigation on optimisation of kiln drying of the bamboo species *Bambusa stenostachya*, *Dendrocalamus asper* and *Thyrostachys siamensis*

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Abstract

Results on kiln drying of the bamboo species *Bambusa stenostachya*, *Dendrocalamus asper* and *Thyrostachys siamensis* are presented. Samples of culm parts at basic and middle sections of the species were dried in a pilot kiln using three different schedules with grades of low, middle and high drying rate. The moisture loss, drying time and drying defects were determined.

Culms of the solid species *Thyrostachys siamensis* are easier to dry than the cavity species *Bambusa stenostachya*, *Dendrocalamus asper*. For fresh culms of *Thyrostachys siamensis* with initial moisture content of over 100 % the drying time to reach a final moisture content of 10% by applying a severe drying schedule was 7 days for the middle part and 9 days for the basic part. *Dendrocalamus asper* is the most difficult species to dry and severely susceptible to checks and splits, so that it needed a mild drying schedule and drying time of 13 days for middle and 16 days for basic part. *Bambusa stenostachya* dries moderately using a relative milder drying schedule with 10 days for the middle and 12 days for the basic part.

Keywords

Bamboo drying, *T. siamensis*, *B. stenostachya*, *D. asper*

Introduction

Bamboo is one of the important vegetative ligno-cellulose resources besides plantation wood. In many tropical countries it is a major raw material for the forest product industry. In recent years, bamboo has become a main material for the industrial manufacturing of round and laminated furniture, parquet and for the worldwide export of culms.

Drying is a key step in processing bamboo products and solving the drying problems will add further value to bamboo resource. Well-dried bamboo culms have the desired appearance, finish and structural properties for the successful export into high value markets. Dried culms are more easily and efficiently processed in steps such as cutting, machining and finishing during production of high quality products. Proper drying also reduces weight, preserves colour, improves the strength of the bamboo, inhibits infestations and minimizes shrinkage in service.

The traditional method of drying bamboo is simple air drying. It has been commonly used for a long time in rural areas and in bamboo factories with small capacities. With proper stacking for air circulation, culms can be dried with no further energy than contained in the ambient air. However, there are some disadvantages. One is the long drying time, which can range from several weeks to several months to obtain the required moisture content for end use. Furthermore, bamboo can be easily infected by fungi, especially moulds during drying. Air drying depends largely on climatic conditions and is undertaken under uncontrollable conditions.

Kiln drying provides means for overcoming these limitations. The significant advantages of kiln drying include higher throughput and better control of the required moisture content. Kiln drying enables bamboo to be dried to any moisture content regardless of weather conditions. For large-scale drying operations, kiln drying is more efficient than air drying and could ensure high level bamboo quality.

In Vietnam the demand for the export of large quantities of quality products has recently increased. Bamboo manufacturers recognized the disadvantages of air drying and have introduced dry kiln techniques. However, considerable problems with drying still exist because the development of bamboo kiln drying has rarely been supported by adequate research efforts.

To contribute to the development of bamboo kiln drying for the benefit of bamboo producers in Vietnam, the project "Investigation on kiln drying of some commercial bamboo species of Vietnam" was initiated. It is supported by the Duy Quy Company of Mechanical Engineering, Ho Chi Minh City and the Bamboo Nature Company, Binh Duong Province, Vietnam. For this project, *Bambusa stenostachya* (Tre Gai), *Dendrocalamus asper* (Manh Tong) and *Thyrostachys siamensis* (Tam Vong) were investigated, which are the most important bamboo species in South Vietnam for production of furniture and export. The goal is to develop suitable kiln dry schedules for culm parts of these species for furniture making.

Materials and methods

The experiments were carried out at the factory of the Bamboo Nature Company, Binh Duong province, South Vietnam during the rainy seasons from May to November in 2008, 2009 and 2010.

Bamboo samples

Mature 3 year old bamboo culms from *Bambusa stenostachya* Hackel, *Dendrocalamus asper* Schult and *Thyrostachys siamensis* Gamble were harvested from a bamboo plantation of the Bamboo Nature Company. Culms were cut about 25 centimetres from ground level and the basic, middle and top parts were marked. The material was transported the same day to the factory for further experiments.

Samples with a length of 140 cm were prepared from the basic and middle culm sections. The epidermis was removed by machine sanding as common for processing. Culm diameter and wall thickness were measured (see Table 1).

Table 1: The dimensions of the samples tested

Species	Length (in mm)		Average diameter (in mm)		Average wall thickness (in mm)	
	Basic	Middle	Basic	Middle	Basic	Middle
<i>T. siamensis</i>	1400	1400	45	38	21	11
<i>B. stenostachya</i>	1400	1400	80	68	20	12
<i>D. asper</i>	1400	1400	88	72	22	13

Lay out of the pilot dry-kiln

Dry-kiln

The experiments were performed in a pilot dry-kiln of 1.7m length, 1.5 m high and 1.2 m width. Its heating system was capable of generating temperatures up to 90°C by electrical heating coils located vertically near the kiln roof. The relative humidity was adjusted by hot water spraying and venting. The air circulation system consisted of two fans with 34 cm diameters. The air velocity was maintained at a constant speed of 3.5 m/s reflecting current industrial standards. The kiln was operated by means of a PLC-controller connected to a PC work station, ensuring control and monitoring of the drying protocol, temperature and relative humidity in the chamber in real time.

Kiln drying

Bamboo samples were dried in the dry-kiln. For the drying of *Thyrostachys siamensis* 154 samples were stacked in 11 rows with 1 cm distance. For *Bambusa stenostachya* and *Dendrocalamus asper*, 64 samples were stacked in 8 rows. Five controls of the sample lot were used to estimate the average moisture content and moisture loss.

During the drying process, the conditions in the kiln were adapted to predefined set point values in the schedule according to the moisture content of the samples at various times during the run. The controls were weighed daily to compute the moisture content.

Drying schedules

The moisture content schedules applied had four grades of drying intensity: mild, medium and severe and very severe. The design of the schedules was based on the studies on bamboo drying by Laxamana (1985),

Yosias (2002), Montoya Argango (2006) and Pham (2006). The drying schedules of tropical wood species published by Boone (1988) were also considered. The applied schedules are presented in Table 2. For each of the bamboo species, three different schedules were tested. Schedule no.1 with mild drying intensity was applied to the cavity species *Bambusa stenostachya* and *Dendrocalamus asper*. Schedule no. 2 with medium drying and schedule no. 3 with severe drying intensity were also applied to these cavity species and also to the solid species *Thyrostachys siamensis*. Schedule no. 4 with very severe drying conditions was tested only on *Thyrostachys siamensis*.

Table 2: The conditions (set-point values) of the four drying schedules

Step	Moisture content (%)	No.1		No.2		No.3		No.4	
		T(°C)	RH (%)						
1	Over 90	45	80	50	80	55	80	65	80
2	90 - 70	45	70	50	70	55	75	65	60
3	70 - 50	50	60	60	60	60	65	70	45
4	50 - 40	50	50	60	50	65	50	70	35
5	40 - 30	50	40	60	30	65	35	70	30
6	30 - 20	55	40	65	30	70	25	75	25
7	20 -10	55	30	65	20	70	20	75	15
Conditioning with 50°C T and 70% RH									

Moisture content

The initial moisture content of the sample control was determined from the moisture sections cut from each end of the control sample. The average moisture content of these two sections and the weight of the sample control at the time of cutting were used to calculate the oven-dry weight of the control sample. The oven-dry weight and the subsequent weights of the sample obtained at intervals during drying, called current weights, were used to calculate the moisture content at those times. The moisture content (MC) of the moisture sections was determined by oven drying and calculated as

$$MC (\%) = 100(W_{or} - W_o) / W_o$$

with W_{or} as original weight of samples and W_o as oven dry weight. The oven-dry weight of control sample (W_{oc}) was computed by using the following formula:

$$W_{oc} = (\text{original weight of control} / 100 + \text{average moisture content of two sections}) \times 100$$

For determination of the average initial moisture content, sections of 5 cm were cut from both ends of the samples. Five controls and five further samples were used. The results are listed in Table 4.

To evaluate the moisture gradient and the final moisture content, sections of 5 cm were taken from both ends and from the middle of 13 samples. The drying rate was determined by the relationship between moisture decreases with drying time.

Drying defects

All culms of the drying experiment were visually inspected for defects like collapse, cracking, and splitting that had occurred during drying. Drying defects were expressed as percentage of all samples in each kiln run.

Table3: Summary of the results for the experiments with three bamboo species

Schedule	Result	<i>T. siamensis</i>		<i>B. stenostachya</i>		<i>D. asper</i>	
		B	M	B	M	B	M
No. 1	IMC (in %)	-	-	100	92	102	89
	FMC (in %)	-	-	10	10	9	8
	Defect (in %)	-	-	3.7	1.9	4.9	3.5
	Time (in hours)	-	-	344	320	362	296
No. 2	IMC	120	110	101	98	104	92
	FMC	8	10	9	9	9	10
	Defect	2.5	1.6	5.1	2.9	17.8	12.5
	Time	294	224	272	249	303	253
No. 3	IMC	118	106	105	96	108	92
	FMC	9	10	9	8	10	9
	Defect	4.8	3.9	15.7	18.9	28.9	19.5
	Time	247	197	249	224	276	230
No. 4	IMC	120	108	-	-	-	-
	FMC	8	10	-	-	-	-
	Defect	5.5	4.2	-	-	-	-
	Time	243	195	-	-	-	-

Table 4: The average initial moisture content with samples n= 20 and the final moisture content with n = 39 of the four experiments

Schedule	Species		<i>T. siamensis</i>		<i>B. stenostachya</i>		<i>D. asper</i>	
	Moisture content		Basic	Middle	Basic	Middle	Basic	Middle
No. 1	Mean (in %)	initial	-	-	103	92	102	89
		final	-	-	10.4	10.1	9.3	8.2
	SD (in %)	initial	-	-	6.8	5	7.5	5.6
		final	-	-	1.4	1.2	1.3	1.1
	VC (in %)	final	-	-	13.8	12.1	14.4	13.6
	min	final	-	-	7.5	6.9	8.3	6.3
max	final	-	-	12.1	12	12.4	12	
No. 2	Mean (in %)	initial	120	110	102	99	105	93
		final	8.5	10.1	9.6	9.5	9.2	10.4
	SD (in %)	initial	8.8	7.2	6.1	5.9	6.9	4.8
		final	1.3	1.1	1.3	1.1	1.3	1
	VC (in %)	final	15.7	11.2	13.9	11.7	13.7	9.8
	min	final	6	6.9	6.9	5.9	6	5.9
max	final	12.4	11.2	11.9	12.2	12.2	12	
No. 3	Mean (in %)	initial	119	106	105	96	108	92
		final	9.7	10.3	9.6	8.3	10.2	9.2
	SD (in %)	initial	8.1	7.2	6.4	4.8	7.1	5.9
		final	1.2	1.3	1.4	1.1	1.4	1.1
	VC (in %)	final	12.8	12.5	14.9	13.1	14.1	11.5
	min	final	6.1	6.8	6	5.9	6.2	5.9
max	final	12.4	11.6	11.9	12.4	11.8	12	
No. 4	Mean (in %)	initial	120	108	-	-	-	-
		final	8.8	10.2	-	-	-	-
	SD (in %)	initial	8.2	7.1	-	-	-	-
		final	1.4	1.2	-	-	-	-
	VC (in %)	final	15.7	11.2	-	-	-	-
	min	final	6	7.3	-	-	-	-
max	final	12.4	11.1	-	-	-	-	

Results and Discussion

All the results for the experiments with three bamboo species are summarized on table 3.

Drying rate and moisture loss

There is a notable difference in drying rate between the solid bamboo species *Thyrostachys siamensis* and the cavity species *Bambusa stenostachya* and *Dendrocalamus asper*. The former showed a higher drying rate, whereas the other two dried more slowly.

This can be partly explained by the differences in specific gravity. In general, the heavier the wood is, the slower the drying rate and the greater the likelihood of defects will be (Simpson, 1992). The study on the physical and mechanical properties of the above mentioned bamboo species by Hoang et al. (2007) showed that the specific gravity of *Thyrostachys siamensis* was 0.41 for the basic and 0.46 for middle part, whereas the species *Bambusa stenostachya* had specific gravity values of 0.69 and 0.74 and *Dendrocalamus asper* of 0.71 and 0.78 respectively.

A difference in drying rate was also measured for the culm section. The middle section showed a higher drying rate than the basic part. This result could be explained by the physical and structural variation of a culm. Though the specific gravity of the middle is slightly higher than the basic part, wall thickness and the diameter of the basic part of the culm are greater than the middle one. Moreover, the middle section contains more vascular bundles than the basic (Liese 1998).

The loss of moisture occurred at a regular rate during all four drying schedules and is presented in Fig. 1, 2 and 3.

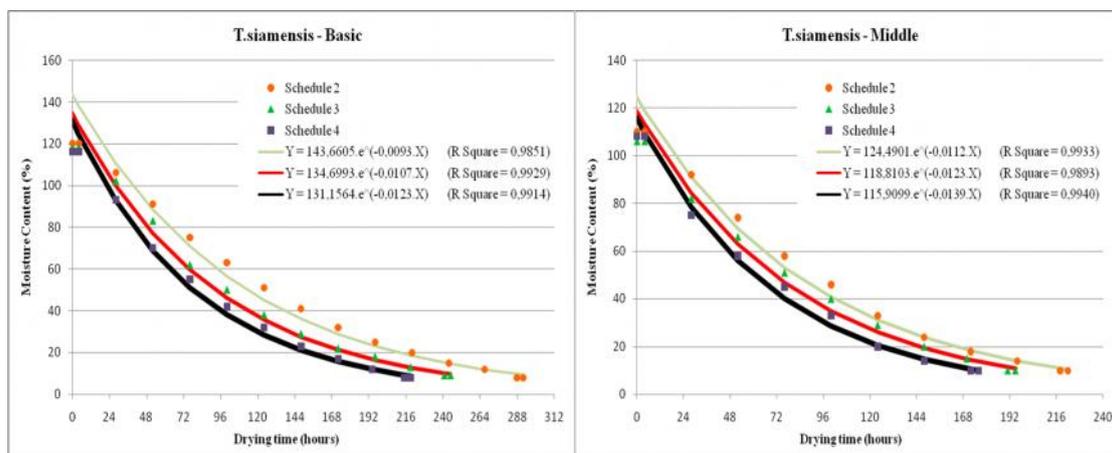


Fig. 1: Relationship between drying time and moisture decrease of *T. siamensis*

Final moisture content

The average final moisture content of the three species is reported in Table 3. In the first drying run of schedule no.1 for *Bambusa stenostachya*, the basic samples showed a great variation of 3 to 16% of moisture content among 13 tested samples. Variations in final moisture content can affect the machining and use of bamboo. To reduce the variation, the conditioning period was increased from 4 to 12 hours during the next drying runs of *Bambusa stenostachya* and *Dendrocalamus asper*. The conditioning of *Thyrostachys siamensis* was kept short with 4 hours.

The average moisture content of the basic and middle parts after drying showed no pronounced differences. The final moisture content of the three species ranged from 6 to 12% for the basic and 7 to 11% for the middle with standard deviations of 1.2 to 1.4 for basic and 1.0 to 1.3 for middle parts.

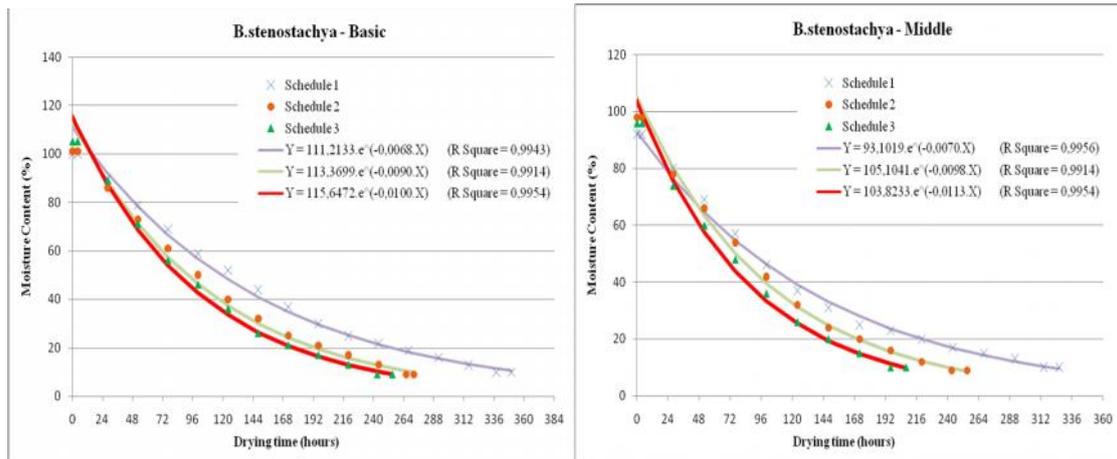


Fig. 2: Relationship between drying time and moisture decrease of *B. stenostachya*

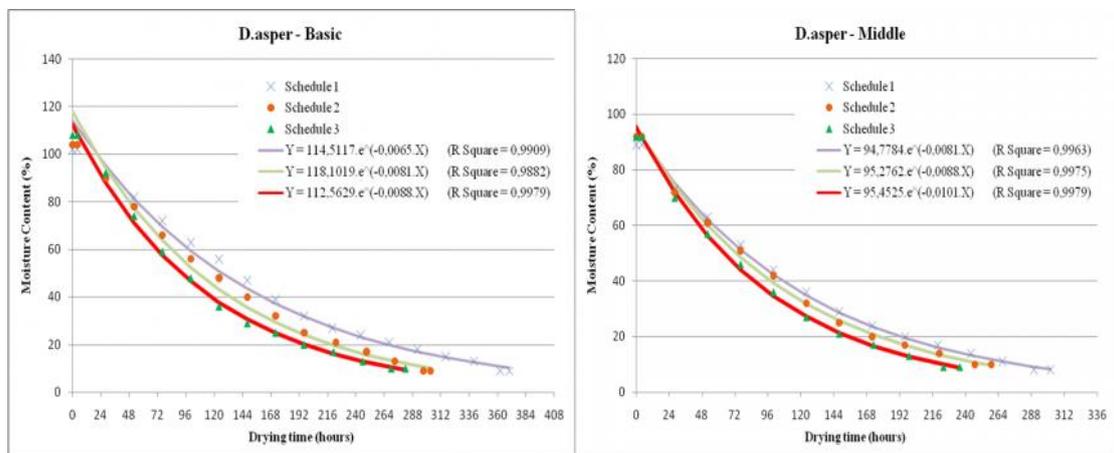


Fig. 3: Relationship between drying time and moisture decrease of *D. asper*

Drying time

The time affected by the drying intensities from mild to severe is presented in Fig. 4, 5 and 6.

When using the milder schedule no. 1 with a final temperature of 50°C and RH of 30% on the species *Bambusa stenostachya*, the drying time for the basic sections was 350 hours for reducing the initial MC from higher than 100% to 9%. The middle parts dried in 326 hours with a reduction of MC from 98% to 9%.

By applying the medium schedule no. 2 with a temperature of 55°C and 20% RH, the time was reduced to 272 hours for the basic and 255 hours for the middle sections. The severe drying schedule no. 3 with a final temperature of 70°C and 25% RH procured drying time of 255 hours for the basic and 208 hours for middle sections. However, severe defects such as splits end and node checks developed in the both parts.

For *Dendrocalamus asper*, the severe drying schedule no. 3 had the shortest drying time of 282 hours for basic and 236 hours for the middle, but serious defects as splits developed. When applying the slightly milder schedule no. 2, the time increased to 303 hours for basic and 253 hours for middle sections, both with notable defects. The milder schedule no. 1 reduced defects, and the drying time was 396 hours for basic and 362 hours for the middle sections.

Schedule no. 3 and the very severe schedule no. 4 with higher temperature of 75°C and lower RH of

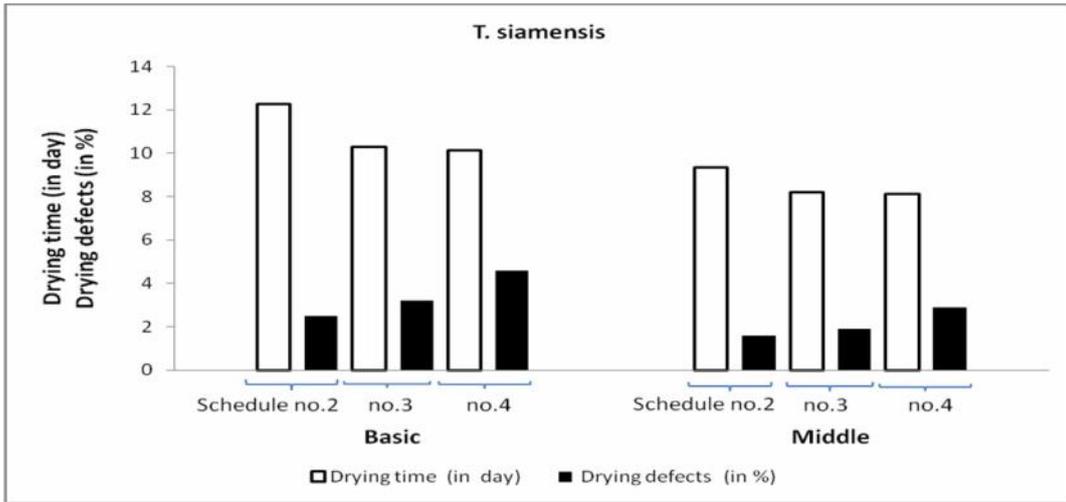


Fig. 4 : Drying time and percentage of defects for *T. siamensis*

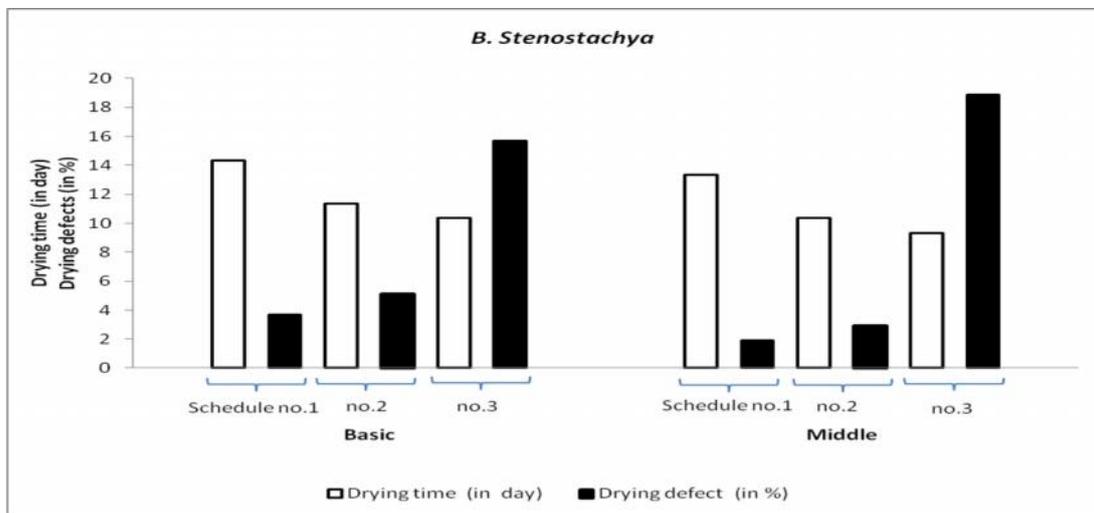


Fig. 5 : Drying time and percentage of defects for *B. Stenostachya*

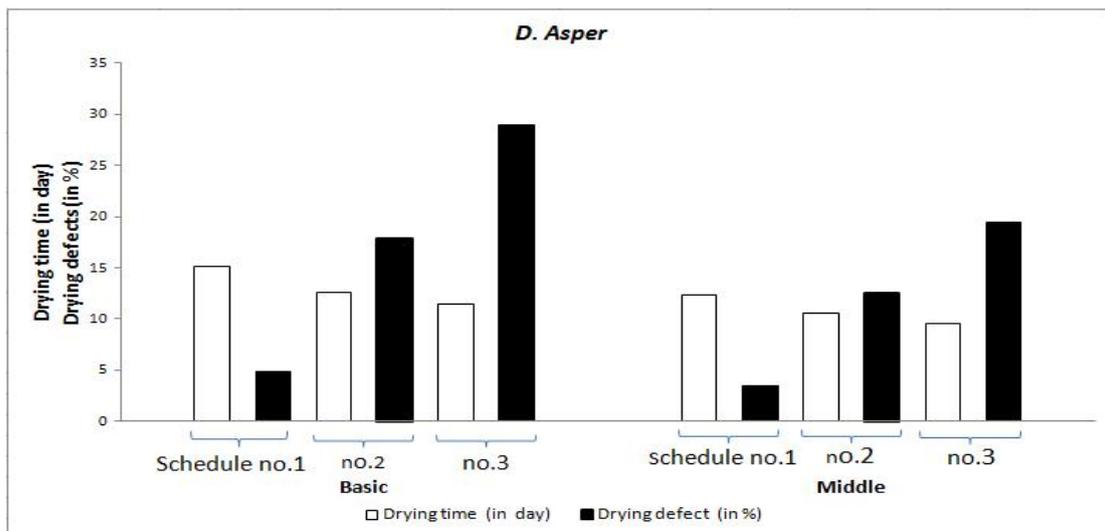


Fig. 6 : Drying time and percentage of defects for *D. Asper*

15% can be applied to reduce drying times for the solid species *Thyrostachys siamensis*. For schedule no. 3, the time was 245 hours for basic and 195 hours for middle sections. The shortest time was achieved with schedule no. 4 with 219 hours for basic and 176 hours for middle sections.

In comparison to the kiln drying results by Laxamana (1985) for the species *Bambusa vulgaris*, *Dendrocalamus merillianus*, *Phyllostachys nigra* and *Schizostachyum diffusum* and the studies on *Guadua angustifolia* by Montoya Arango (2006), the drying time was generally shorter than in these investigated species. Drying time for *Dendrocalamus merillianus* was 128 hours and for *Guadua angustifolia* 118 hours. The shortest time for *Thyrostachys siamensis* was 176 hours, *Bambusa stenostachya* 208 hours and *Dendrocalamus asper* 236 hours.

The difference between the species is partly explained by their physical properties. *Bambusa stenostachya* has a specific gravity of 0.71 and a wall thickness of 20 mm which is less in comparison to *Dendrocalamus asper* (0.78 and 22mm, resp.) and more to *Dendrocalamus merillianus* (0.6 and 10 mm, resp.). *Guadua angustifolia* has a specific gravity of 0.6 and a wall thickness of 23 mm. The solid species *Thyrostachys siamensis* has a low specific gravity 0.46 but its wall thickness is much thicker. In fact, both solid and cavity species have a wide range of structural features and physical properties (specific gravity, moisture diffusion and gas/liquid permeability) that influence the drying behavior.

Drying defects

In kiln drying of bamboo, defects may develop during and after drying. Some common defects are ruptures of culm tissue such as surface checks and splits. Uneven moisture content and discoloration such as mould, blue staining and water staining at the nodes also reduce to drying quality. Most physical defects were end checks, node checks and splits (see pictures 2).

The two cavity species, especially *Dendrocalamus asper* were susceptible to splits, end checks and node checks. The basic part of all species developed more severe defects in comparison to the middle part. The most severe defects in *D. asper* and *B. stenostachya* occurred with the drying schedule no. 3. End splits and node checks lead to 29% defects in basic sections for *D. asper* and to 19% for *Bambusa stenostachya*.

For *B. stenostachya* the slightly milder schedule no. 2 with a final temperature of 55°C and a 20% RH the defect percentage reduced to 6% for the basic and 4 % for the middle parts. Applying the milder schedule no.1 with a low temperature of 50°C and a high RH of 30% for *D. asper* minimized defects at the basic to 6% and at the middle 3.5%.

For the solid species *T. siamensis*, the very severe drying schedule no. 4 with high temperature of 75°C and a very low RH of 15% the defect percentage was 7% for the basic and 5% for the middle parts. End checks at internal layer occurred mainly with the basic samples. The solid species *T. siamensis* is easier to dry and less susceptible to defects than the cavity species *B. stenostachya* and *D. asper*.

In drying bamboo, discolourating fungi such as mould and sap staining can grow on green bamboo in kilns operating at a low temperature and high humidity regime (Tang et al. 2009). In the drying process using the mild schedule no. 1, mould developed on the basic parts of *D. asper* during the initial stage with a temperature of 40°C and a relative humidity of 85%. Mould was prevented by a high temperature treatment with 80°C and a relative humidity of 90% for 2 hours.



Picture 1: Stacking middle parts of *T. siamensis* and *D. asper*



Picture 2: End checks of *T. siamensi* and *D. asper*

Conclusion

The initial experiments have shown that kiln drying of bamboo parts can be conducted successfully using proper schedules of temperature and relative humidity. Drying the solid species *Thyrostachys siamensis* requires a severe drying schedule with high temperature of 65°C and low relative humidity of 60% at the initial stage and 75°C with 17% RH at the final step. The drying time was 10 days for the basic and 8 days for the middle sections. The cavity species *Dendrocalamus asper* is a difficult species to dry and susceptible to drying defects and therefore needs a mild schedule with initial temperature of 40°C and initial RH of 80% and a final temperature of 50°C and RH of 30%; the required drying time was 17 days for the basic and 14 days for the middle sections. *Bambusa stenostachya* dried moderately fast using the relative milder schedule with 65°C temperature and 20% relative humidity and resulted in a drying time of 12 days for the basic and 10 days for the middle. The dry-kiln industry in South Vietnam will apply these effective and feasible schedules for drying longer culms. Additionally, the drying schedules will be further developed for bamboo treated with preservatives based on boron compounds. Since drying is an essential step for

processing bamboo into final products, the investigations should also include other commercial species.

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References

- Arango, M. J. A. 2006. Trocknungsverfahren für die Bambusart *Guadua angustifolia* unter tropischen Bedingungen. Dissertation, University Hamburg. pp. 163-184.
- Boone, R. S.; Kozlik C. J.; Bois, J.P.; Wengert, E. M. 1988. Dry Kiln Schedules for Commercial Woods Temperate and Tropical. General Technical Report FPL-GTR-57. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. pp. 25-26; 37-45.
- Hoang, T. T. H.; Tang, T. K. H. 2007. Structural features and physical properties of some important South Vietnam bamboo species. *Journal of Agricultural- Forestry Sciences and Technology of Nong Lam University of HCM*, (3), 15 - 22.
- Keey R. B.; Langrish, T.A.G.; Walker, J. C. F. 2000. Kiln-Drying of lumber. Springer series in Wood Science. Berlin, Heidelberg, New York. 326 pp.
- Laxamana M. G. 1985. Drying of some Commercial Philippine Bamboos. *FPRDI Journal Volume XIV*, (1 & 2), 8-19.
- Liese W. 1998. The anatomy of bamboo culms. INBAR Working paper No.18. International Network for Bamboo and Rattan, New Delhi, India. pp. 102-112.
- Pham N. N. 2006. Establishing process of kiln drying for *Bambusa procera* and *B.stenostachya*. Project Report to the Department of Science and Technology of Ho Chi Minh-City, Vietnam. 47 pp.
- Simpson W.T 1992. Properties of Wood relative to drying- Dry kiln operators' manual. USDA Agric Handb 188 For Serv For Prod Lab Madison WI. pp. 1-15.
- Tang, T.K.H.; Schmidt, O.; Liese, W. 2009. Environment-friendly Short-term Protection of Bamboo against Moulding. *Journal Timber Development Association of India*, (55), 8-17.
- Yosias G. 2002. Preliminary study on the drying of Bamboo (*Bambusa blumeana*) in a wood waste-fired kiln. Bamboo for Sustainable Development. Proceedings of the Vth International Bamboo Congress and the VIth International Bamboo Workshop San Jose, Costa Rica, 2-6 November 1998. International Network for Bamboo and Rattan, New Delhi, India. pp. 495-510.

Change of the properties of Vietnamese bamboo species by thermal modification

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Abstract

Improving dimensional stability and durability is the general objective of the thermal modification of bamboo. Many physical-mechanical and chemical characteristics of bamboo like the colour, the mass, the density, the moisture sorption and the chemical composition (cellulose, hemicellulose, lignin, ash, extractives and starch etc.) are changed by this modification. The aim of this work was to find relations between the modification parameters and the resulting properties of bamboo by comparison of thermally modified and unmodified twin samples of two Vietnamese main bamboo species.

Keywords

thermal modification, Vietnamese bamboo species, changes of physical properties, changes of chemical composition.

Introduction

Vietnam is a country with tropical climate in the south and subtropical climate in the north. Both of them are very suitable for the growth of bamboo. Vietnam has about 767.122 ha natural pure bamboo forests and 341.273 ha natural bamboo and woody mixed forest (Vu and Le 2005, Tran 2010).

Bamboo is a multipurpose material in Vietnam. It can be used as building material, as raw material for handicraft, for production of chopstick and toothpick and recently for production of pulp, paper and of wood based panels (Vu et al. 2002; Do 2006). Approximately 400 million bamboo culms are used in Vietnam in about 90 factories annually. Four of them produce bamboo based panels with an annual capacity of 15.000 to 130.000 tons (Vu and Le, 2005). The susceptibility of bamboo to fungi and insects is problematic for using (Liese and Kumar 2003; Liese 1998). In order to increase its durability as well as its dimensional stability bamboo should be modified. For this the thermal modification is a promising possibility (Leithoff, H., Peek, R.D. 2001).

Actually the thermal modification used in Vietnam is based only on empirical knowledge. Fundamentally examinations of relationships between thermal treatment and changes of properties are missed up to now. Therefore no reproducible processes are established in Vietnam. The objective of this research is to analyse the changes in chemical composition and the subsequent changes in physical and mechanical properties of bamboo depending on the parameters of the thermal treatment. Only this knowledge permits the processes of thermal modification of bamboo resulting in well defined properties.

Materials and Methods

Two of the main bamboo species growing in Vietnam were chosen for the investigations. These are:

- *Dendrocalamus barbatus* Hsueh et D. Z. Li (Vietnamese: Luong) and
- *Dendrocalamus asper* Backer ex Heyne (Vietnamese: Buong)

The culms were harvested in april and in october 2010 in area of Tan Lac, Province Hoa Binh. They were about 2 till 3 year old and 15 m high. The first 10 m from the bottom were used for the investigations.

These 10 m culms were cut into two halves, the bottom and the top one.

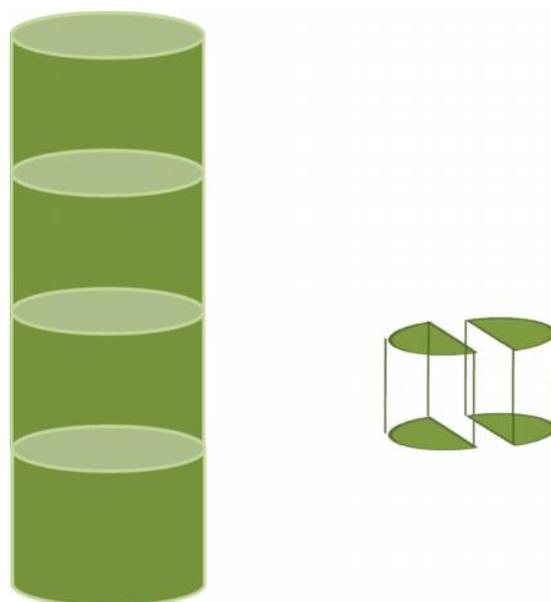


Figure 1: Preparation (cutting) of samples for thermal modification

Each internode was cut in 5 slices. Each slice was split into 2 parts – one part was used for the modification and the other part as unmodified twin sample (Figure 1). The samples were modified for 2 h or 5 h at temperatures between 130 °C and 220 °C.

All samples were dried in three steps (40 °C, 60 °C and 80 °C) before thermal modification. The modification was carried out in a closable treatment chamber (Figure 2). At first the chamber (with samples) was evacuated to 200 mbar and then filled with nitrogen (inert gas). After that the samples were modified under the above mentioned conditions. The treatment conditions are shown in figure 3.

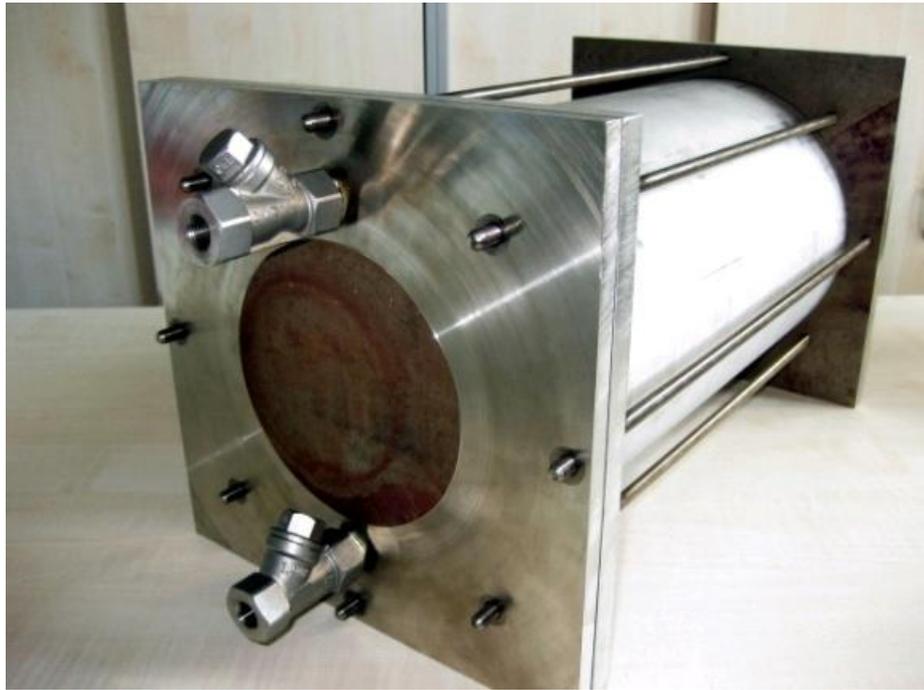


Figure 2: Treatment chamber for thermal modification of bamboo

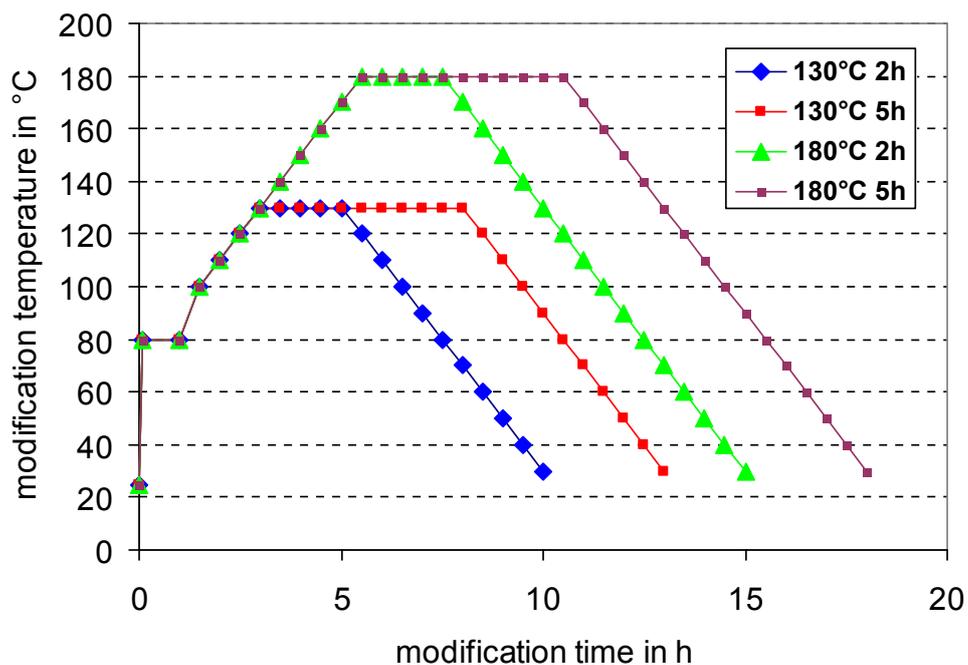


Figure 3: Treatment charts for modification at 130°C and 180 °C

The modified samples and the twin samples were stored at standard conditions (20 °C; 65 % rH) for 2 weeks before cutting into various specimens for different analyses and tests.

The chemical composition of the samples was determined by wet chemical analyses (Table 5).

Table 5: Methods for chemical analyses

Component	Method (Stößer 2011)
Cellulose	Method of Kürschner and Hoffer
Lignin	Klason-Lignin
Holocellulose	Delignification by perchloric acid
Extractives	Extraction with ethanol/toluol

Furthermore the changes in chemical composition of hemicelluloses were investigated by hydrolysis of holocellulose with trifluor acetic acid. The resulting sugar monomers were analyzed by HPLC.

The colour of bamboo was measured with a spectrophotometer (SpectroEye X-rite) under a D65 light source with an observer angle of 10 °. The CIELAB System is characterized by the following three parameters: the lightness L^* , the chromatic coordinates on the green-red axis a^* and the blue-yellow axis b^* . The colour change ΔE was determined according to the following formula:

$$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad (\text{Eq.1})$$

with

$$\Delta L^* = L^*_t - L^*_0 \quad (\text{Eq.2})$$

$$\Delta a^* = a^*_t - a^*_0 \quad (\text{Eq.3})$$

$$\Delta b^* = b^*_t - b^*_0 \quad (\text{Eq.4})$$

(t: after treatment; 0 before treatment)

The mass loss (ML) of the sample was estimated according to the following formula:

$$\text{ML (\%)} = 100 * (m_0 - m_t)/m_0 \quad (\text{Eq.5})$$

with the initial mass of the oven-dried sample m_0 and the mass of the sample after the thermal modification m_t .

The density of the samples was estimated according to the following formula:

$$\rho \text{ (kg/m}^3\text{)} = m/V \quad (\text{Eq.6})$$

where m is the mass and V is the volume of the sample at 20 °C, 65% rH.

The equilibrium moisture content (EMC) was determined according to the following formula:

$$\text{EMC} = 100 * (m_f - m_0)/m_0 \quad (\text{Eq.7})$$

where m_f is the mass of the sample at 20°C and 65 % rH, m_0 is the oven-dried mass.

The compressive strength test was carried out with Tira 28100. For determination of shock resistance a Dynstat Dys-e was used. Scanning electron microscopic investigations were carried out by JOEL JSM-T 330 A. The dimensions of samples for the different analyses and tests are listed in Table 2.

Table 6: Sample dimensions and numbers for different analyses and tests

Method	Sample dimension	Number of measurements per sample
Chemical analyses	powder, particle size < 0,25 mm	2
Colour	twin sample (not cut)	20
Density, EMC, Compressive strength	30 mm (height) * 15mm (width) * wall thickness	10
Shock resistance	15 mm (height) x 10 mm (width) x 1,2 mm (thickness)	2-12

Results and Discussion

Note: All comparisons are based on the results of modified samples and their twin samples.

Chemical composition

Any component content changes during thermal treatment, whereas the degree of change is very different.

Cellulose as the main component has a content of about 50 % in untreated samples. The treated samples show a continuously decrease. This could be caused by a shorting of cellulose chains. Shorter chains are not ascertainable with the used method for cellulose determination. Exemplary the relative mass loss of the autumn sample are shown in Figure 4. Up to 130 °C there is only a small degradation of the chains. The influence of modification time and temperature seems to be similar. But *Dendrocalamus barbatus* undergoes a stronger degradation than *Dendrocalamus asper* at the lower temperatures.

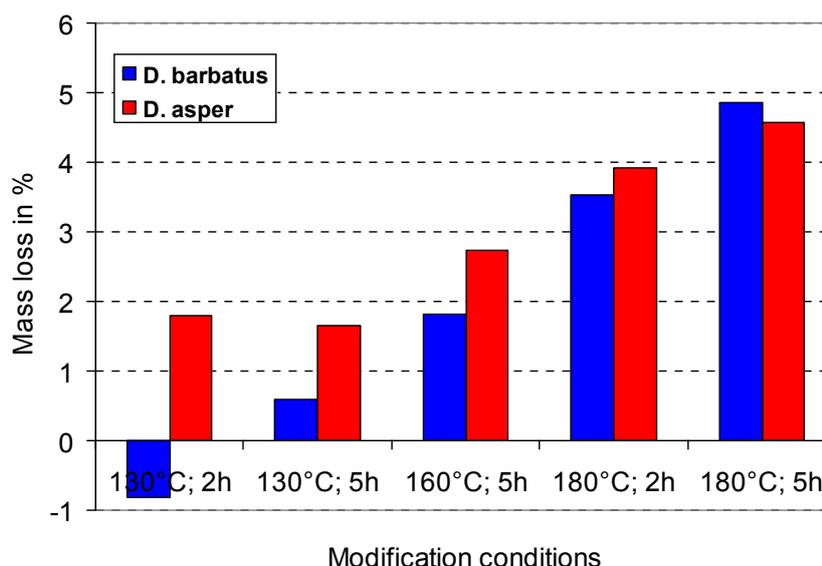


Figure 4: Absolutely mass loss of cellulose during thermal treatment

Hemicellulose content results from the difference between holocellulose and cellulose. We found a strong mass loss of detectable holocelluloses. The calculated relative mass loss of hemicelluloses is

displayed in Figure 5. Already at 130 °C a degradation of hemicelluloses takes place. Bamboo hemicelluloses contain a predominantly content of xylan (Liese 1985), which is the most thermal instable part of hemicelluloses.

A long modification at 180 °C results in a reduction of hemicelluloses of about 70 %, whereas the influence of the modification time grows with increasing temperature.

But this mass loss is not a real mass loss of the sample. Bamboo hemicelluloses contains of about 7 % acetyl groups. These groups were separated from the chains by thermal exposure and form acetic acid. This compound has a catalytic effect on the non-hydrolytic decomposition of the glycosidic bonds of holocellulose. The reactions result in different reactive molecules like furfural or radicals (Fengel 1989), which can react with unsaturated phenolic components of lignin.

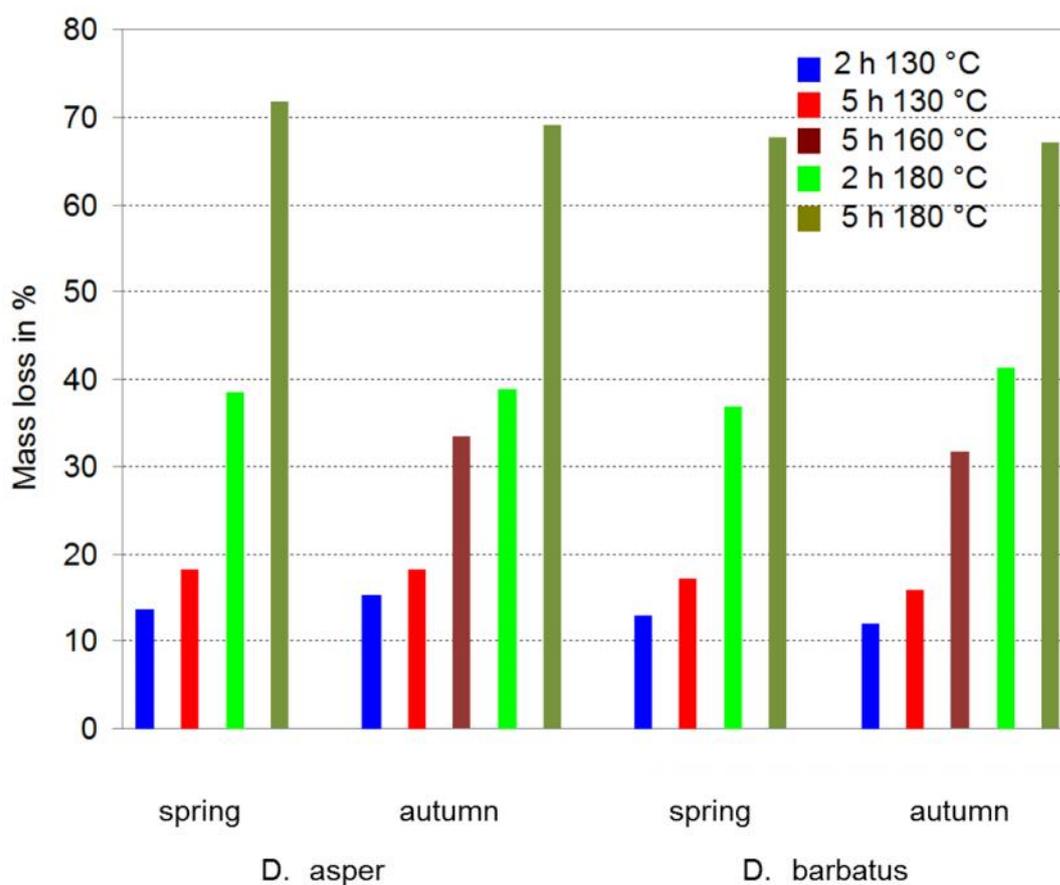


Figure 5: Absolutely mass loss of hemicelluloses calculated as difference from mass of holocellulose and mass of cellulose

The extracted holocellulose of some other samples of autumn was hydrolyzed gently and the resulting sugars were analyzed by HPLC. Beside glucose three monomer sugars were found: xylose, arabinose and galactose.

The ratio in non treated sample of *Dendrocalamus barbatus* is about 18.5 : 1 : 0.6. This ratio shifts to higher xylose content with increasing temperature. The mass loss of the single sugars is visible in figure 6. It shows that mainly arabinose and galactose are reduced up to 180 °C. At 220 °C galactose is completely remote and most of the other two sugars too, what is in a good accordance to the results of Patzelt et al. (2002) for thermal modified wood.

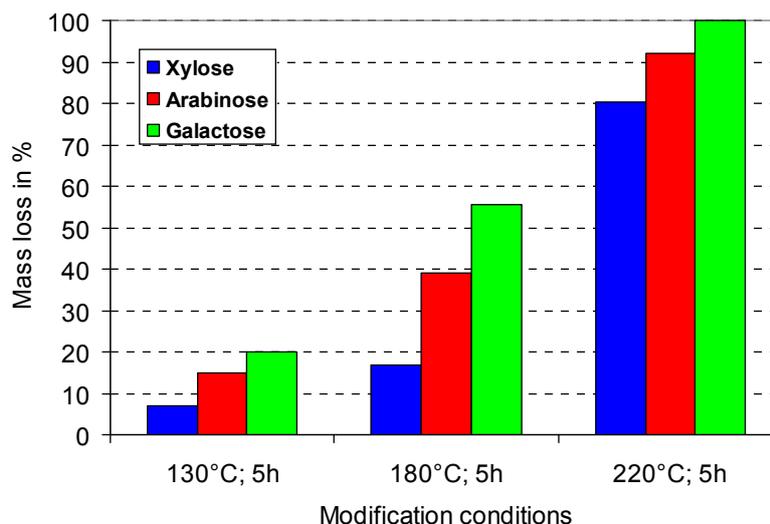


Figure 6: Absolutely mass loss of sugar components of hemicellulose

For lignin as third component an increase of Klason-lignin was found. Analysis of Klason-lignin determines all components which are not soluble in concentrated sulphuric acid. As already described decomposed hemicelluloses components can react with the lignin. Bamboo lignin has a high content of unsaturated phenolic components like it is typical for grass. Furthermore a polymerisation of decomposition products of hemicelluloses is possible. The products of such a polymerisation should be also not soluble in sulphuric acid.

A comparison between the absolutely rise of mass of lignin and the absolutely mass loss of hemicelluloses of a *Dendrocalamus asper* of autumn is illustrated in Figure 7. As shown the changes of these two components are in the same range.

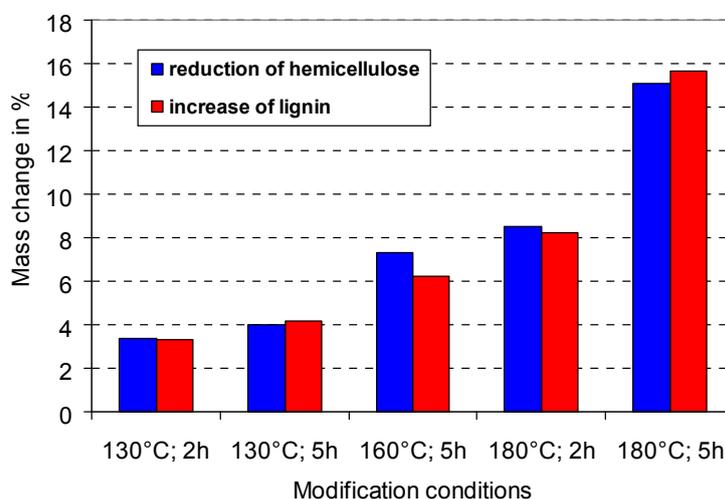


Figure 7: Comparison between reduction of hemicellulose and increase of lignin

Extractives show a mass loss up to 5 % depending on temperature and time. The colours of the extractives change from light green to dark brown. Decomposition of some components like terpenes or fats takes place under formation of volatile compounds. On the other hand a condensation of phenolic compounds is responsible for the dark colour.

Physical properties

The first remarkable change of the bamboo by the thermal modification is the colour. Regardless of the harvest time, the green colour of the two investigated bamboo species shifts into a darker colour region. At higher modification temperatures and/or longer modification time the change of colour becomes stronger. That means the colour difference (ΔE) increases with sharper modification conditions (Figure 8), whereas the influence of modification temperature is higher than the influence of the modification time. This change of colour is caused by the chemical reactions of the decomposition products of hemicelluloses with lignin and extractives. Also condensation of lignin is one factor for colour changing.

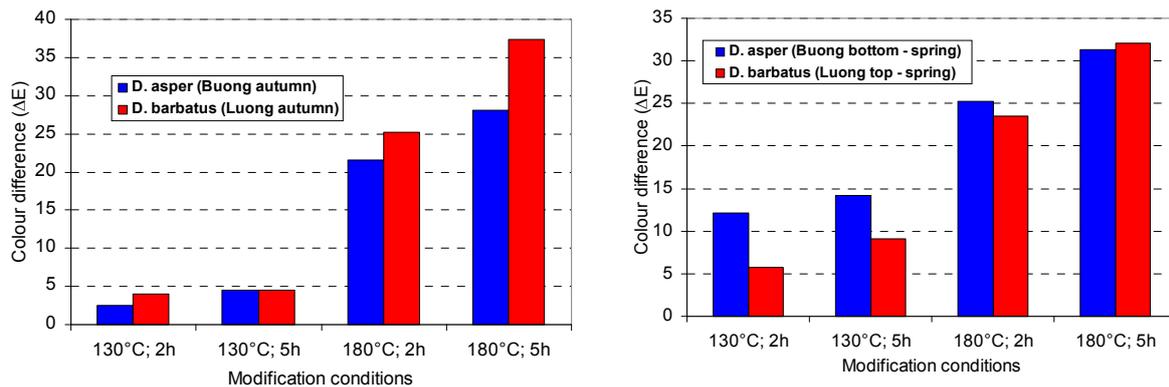


Figure 8: Colour difference of Vietnamese bamboo species as a function of modification conditions

Bamboo is a natural material, so that the colour between the internodes and between locations within one internode is already different. The second remarkable change of the bamboo by the thermal modification is the mass. It decreases due to the evaporation of mainly extractives of bamboo by thermal modification. There is a good accordance to the mass loss of the extractives. So the mass loss increases with increasing treatment temperature and/or time (Figure 9). Similar to the change of colour the modification temperature has a higher influence on the mass loss than the modification time.

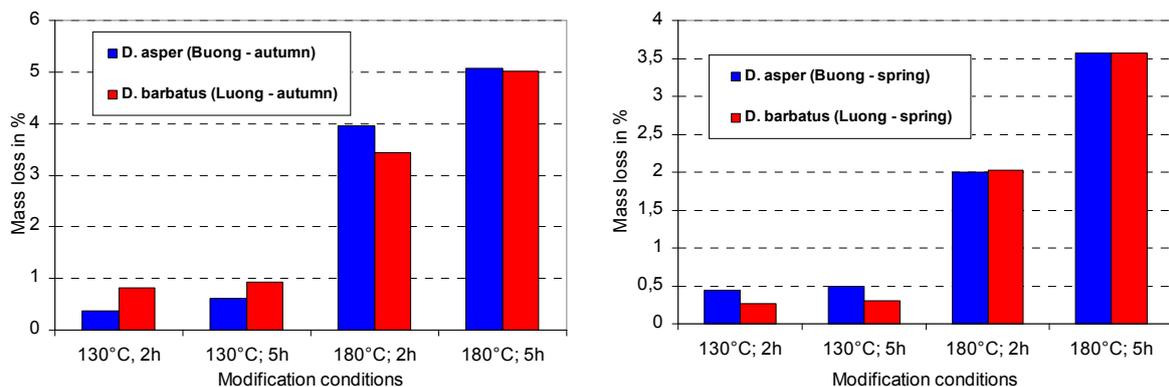


Figure 9: Mass loss of Vietnamese bamboo species as a function of modification conditions

The equilibrium moisture content (EMC) of treated samples decreases by the thermal modification compared to unmodified samples. The difference of EMC between treated samples and their untreated twin samples grows with increasing modification temperature (Figure 10). This is caused by decomposition of hemicellulose by higher modification temperature or longer modification time (see chemical composition). Decomposition of hemicelluloses results in a strong decrease of hydroxyl groups whereby the hydrophilic character decreases.

Furthermore Figure 10 shows the higher influence of modification temperature in comparison to modification time.

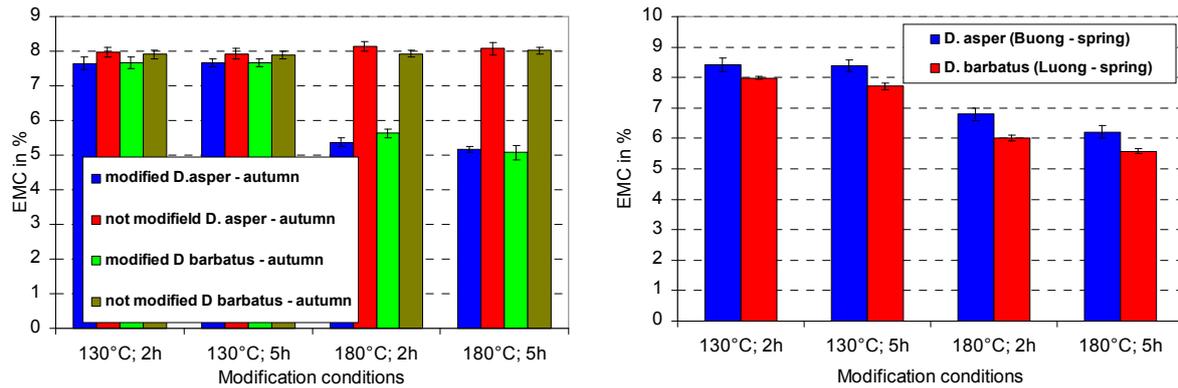


Figure 10: EMC of Vietnamese bamboo species as a function of modification conditions

Compressive strength shows no continuous run but a slightly increasing tendency with increasing modification temperature up to 180 °C with 2 h modification time.

Unexpectedly the density of most treated samples at 20 °C and 65 % rH is slightly higher than their untreated twin samples, although the equilibrium moisture content of these treated samples are lower. But there is no direct correlation between density and compressive strength.

Furthermore the shock resistance of bamboo samples was studied. Shock resistance includes both compressive and tensile stress. Material failure caused by these two stresses result in different morphologies of fracture. Whereas a pulling out of fibres takes place under tensile stress, side fractures can be obtained under compressive stress. So morphology of fracture allows statements about the contribution of tensile and compressive stress.

Samples of the outer and inner part were prepared for these investigations. Experiments results in a complete failure only for the samples of high modification temperatures. For the other samples a side fracture was obtained. Figure 11 shows the values for shock resistance. There are two unmodified samples with similarly the same values. Thermal modification results in a strong decrease of shock resistance, whereas the inner part mostly shows lower values than the outer part. This is caused by the distribution of fibres in the plant.

Morphology of fracture of fibres shows two different characteristics. Parenchyma cells have similar fracture behaviour at all temperatures. These are brittle fracture the lamellae of cell walls. Most of them occur within the intercellular region. Vessels and parenchyma cells shows only one fracture plan. The morphology of fibres changes considerably. Unmodified bamboo shows long fracture with a strong structuring of the individual fibre. Different levels of the single lamellae and some micro fibrils are visible. The fibre bundles mostly have one fracture plan. But the fractures plan of the surrounding parenchyma cells is different (Figure 12).

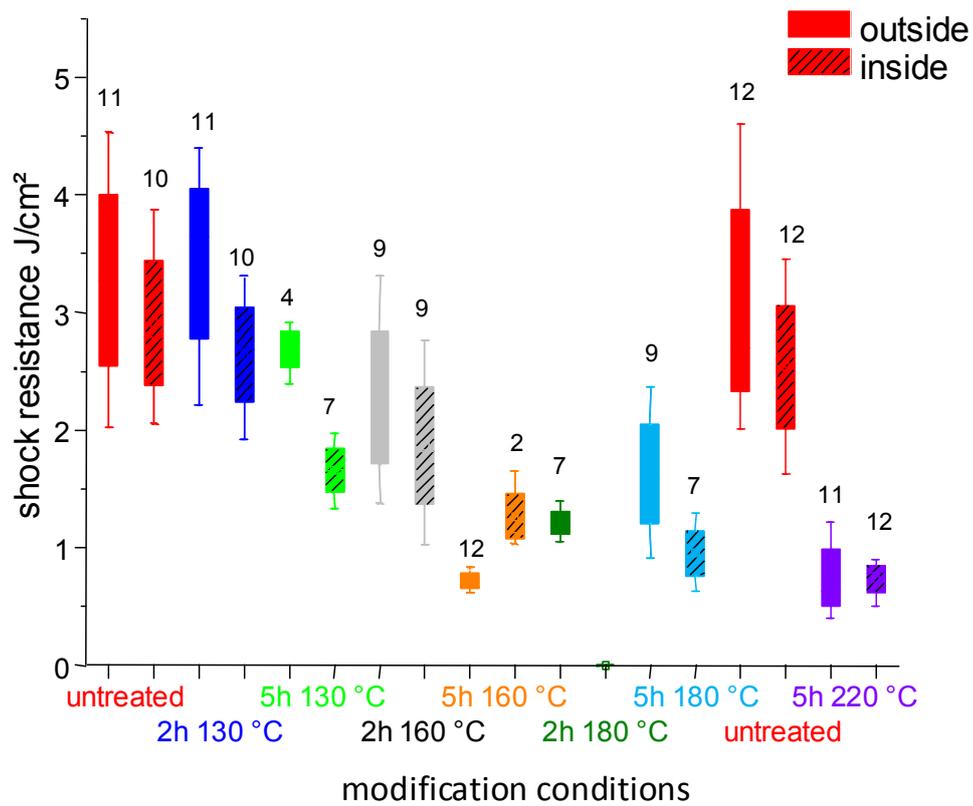


Figure11: Shock resistance of thermal modified bamboo in comparison to unmodified bamboo (Numbers of measured samples above the bars)

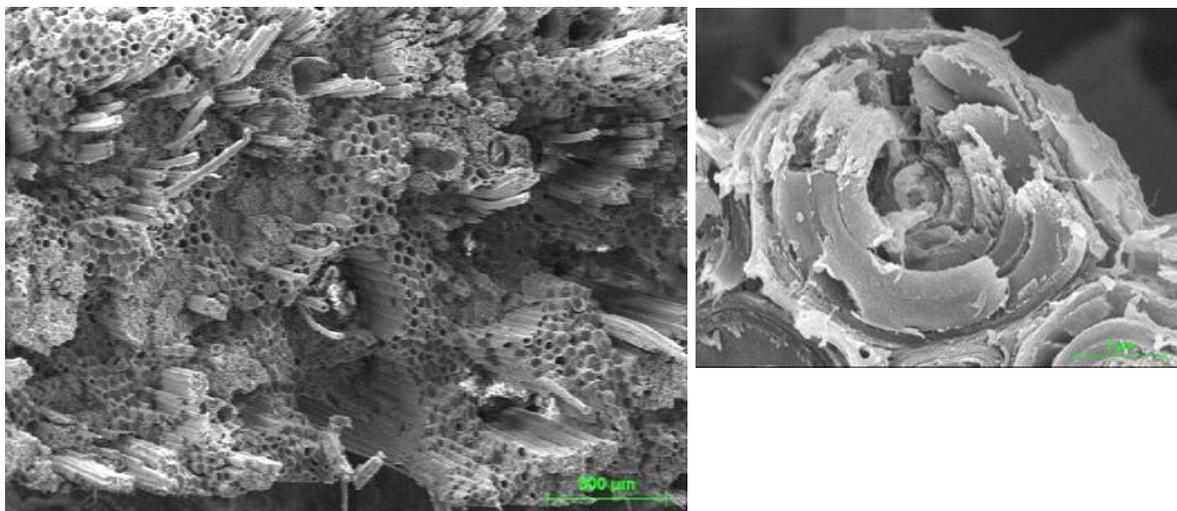


Figure 12: Fracture morphology of an unmodified bamboo sample

With increasing temperature the fibre fractures become shorter. At 220 °C the fractures of fibres and parenchyma cells as well as vessels are similarly in the same plane. The individual fibres show no more structuring (Figure 13).

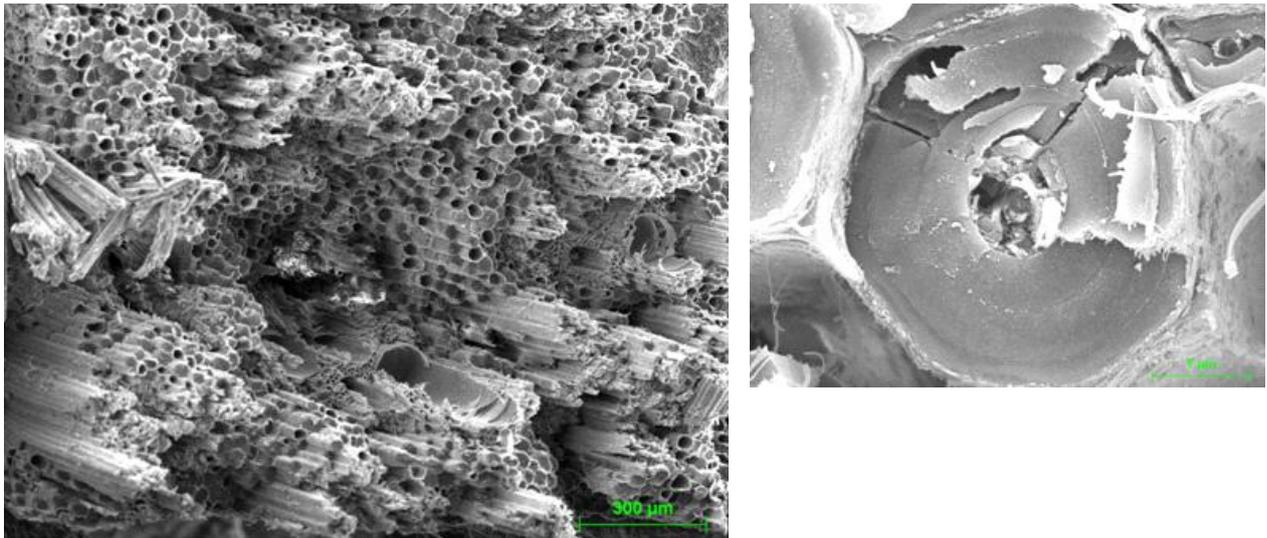


Figure 13: Fracture morphology after modification at 220 °C for 5 h

Between these two states we found an intersection with a separating of fibres.

The drastically decrease of strength shows a good correlation with the decomposition of holocelluloses, which is mainly caused by the decomposition of hemicellulose (Figure 14). Therefore the original function of hemicelluloses is lost. The function of lignin is particularly reduced as well because of the chemical changes in its structure.

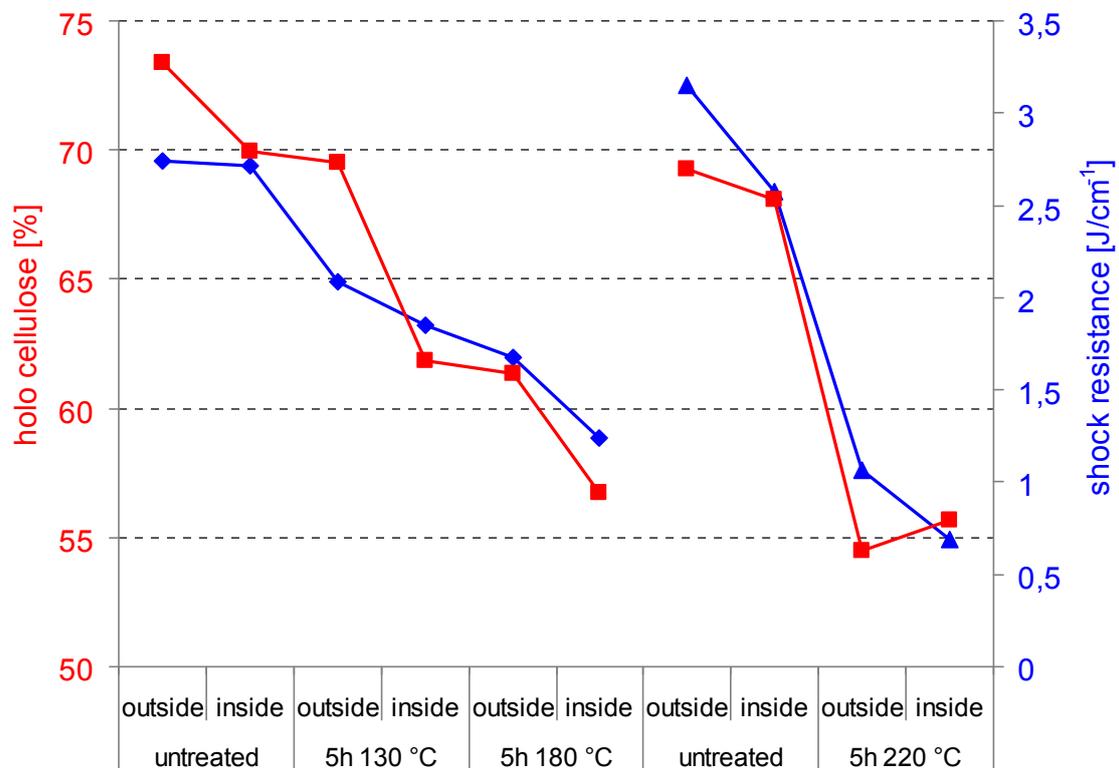


Figure 14: Correlation between shock resistance and mass loss of holocellulose

Conclusions

The thermal modification of bamboo generates many changes in chemical composition. The quantity of these changes depends on the modification temperature and time. Treatments at 130 °C cause only slight changes. Significant changes occur by modification above 180 °C. The influence of modification temperature is significant higher than that of modification time. The most important changes are the strong decomposition of hemicelluloses. Depending on the chemical changes the physical properties are changing too. With increasing temperature the colour becomes darker, the mass loss and density increases slightly and the EMC decreases. But the changes in strength are most important. While the compressive strength along the fibre is slightly improved by thermal modification, the shock resistance shows a strong decrease. Also the fracture morphology shows a soaring brittleness of samples with increasing temperature.

References

- Do V. B. 2006. Mot so loai tre thong dung cua Vietnam can chon de phat trien. Ban tin du an trong moi 5 trieu hectare rung.
- Fengel, D. 1989. Wood: Chemistry ultrastructure reactions. De Gruyter, Berlin, Germany
- Liese, W. 1985. Bamboos – biology, silvics, properties, utilization. TZ-Verlagsgesellschaft, Rossdorf, Germany
- Liese, W., Kumar, S. 2003. Bamboo preservation compendium. INBAR, Beijing, China, Technical Rep. 22, 231 pp.
- Liese, W. 1998. The anatomy of Bamboo Culms, INBAR Beijing, China, Tech. Rep. 18, 204 pp.
- Leithoff, H., Peek, R.D. 2001. Heat treatment of bamboo. IRG/WP/01-40216, 1-11.
- Patzelt, M.; Stingl, R.; Teischinger, A. 2002. Thermische Modifikation von Holz und deren Einfluss auf ausgewählte Holzeigenschaften. Institut für Holzforschung (ihf) und der Verband Holzwirte Österreichs –VHÖ, Hrg. Modifiziertes Holz: Eigenschaften und Märkte, 101-169
- Stößer, K. 2011. Die chemische Zusammensetzung von bambus in Abhängigkeit von Art, Standort und Erntezeitpunkt. Diplomarbeit, HTW Dresden
- Tran, V.H. 2010. Growth and quality of indigenous bamboo species in the mountainous regions of Northern Vietnam. Dissertation, Göttingen.
- Vu V. D. ; Le V. L., 2005. Ket qua nghien cuu tai nguyen tre nua cua Vietnam. Hoi nghi: Khoa hoc cong nghe lam nghiep 20 nam doi moi (1986-2005) phan lam sinh. Hanoi. Thang 4, 2005.
- Vu V. D.; Hoang H. N.; Trinh V.; Nguyen V. T., De Beer J., Ha C. C. et al., 2002: An Overview of the NTFP Sub-Sector in Vietnam. In: Morris, J., An Van Bay (Eds.), Forest Science Institute of Vietnam.

Session 7. Biotechnology

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Biotechnological approaches for propagation, conservation and improvement of important bamboos

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Bamboos belonging to the family Poaceae are woody, perennial, evergreen monocots that inhabit different climatic zones ranging from cold mountains to hot tropics. Bamboos have multiple utilities in wood, pulp, paper, food and textile industries. These fast growing, carbon sequestering, energy efficient plants have emerged as versatile resources for fuel, fodder and environment management. Besides, their use in construction and engineering materials, charcoal and activated carbon, these yet to be exploited plants have great economic and ecological potential for meeting the future needs of mankind. Besides serving the rural community through diversified uses, it is extensively used as a raw material for paper industry. Extensive utilization because of versatility of this natural resource leading to its over exploitation; gregarious flowering in some parts of the country and difficulty in extraction of propagules has resulted in scarcity of this bioresource. This could not be met through labour intensive conventional methods due to several constraints like uncertain flowering cycles, low seed set and viability, limited success by means of culm cuttings and “offsets”.

The problem thus, could be circumvented by devising the ways and means to raise large stock of bamboos to meet the growing needs. Therefore, biotechnological applications like micropropagation of elites, raising new progenies from seeds, conservation and genetic manipulations are important aspects that need to be explored. Presently, the efforts and achievements made by Institute of Himalayan Bioresource Technology (CSIR) Palampur, for the mass production of quality planting material of bamboos have been highlighted. On the basis of commercial importance and species identified by NMBA, TIFAC, DST and DBT (GOI), the Institute has initiated extensive work on selection, micropropagation and field performance of important bamboos like *Dendrocalamus hamiltonii*, *D. asper*, *D. giganteus*, *D. membranaceus*, *Bambusa nutans*, *B. tulda*, *B. bambos*, *B. multiplex*, *Melocanna baccifera*, *Phyllostachys pubescens* and *Guadua angustifolia* together with conservation strategies for slow growth, *in vitro* flowering and genetic transformation of *D. hamiltonii* are being carried out. These species are grown *in vitro* mainly through axillary bud cultures. Somatic embryogenesis has also been successfully achieved in field selected elite mother plants of *D. hamiltonii* and *B. nutans*. Based on availability, seeds are also being used for raising aseptic cultures of *B. bambos* and *Phyllostachys pubescens*.

Despite multiple uses, there are certain problems associated with the use of bamboo in industry. Bamboo only comes in two colours, which can limit its utility for furniture making and flooring as it resists attempts to stain it. Bamboo flooring is more brittle than hardwood. Fading and damage by moisture are other disadvantages of bamboo flooring. Silk and bamboo sheets are more easily damaged by bleaching than other natural fibers. High lignin content of bamboo species interferes with

its use in paper industry and needs to be modified. Adding to the problem is the long and erratic flowering cycle, poor seed set and short seed viability that make perennial seed propagation and genetic improvement by breeding programs nearly impossible. Biotechnological applications, like genetic manipulations and precocious *in vitro* flower induction could thus be utilized for overcoming these problems. *Agrobacterium* as well as biolistic mediated approach is thus explored for the standardization of genetic transformation protocol for a multipurpose species of bamboo i.e. *Dendrocalamus hamiltonii*. The success could further be used for introducing various genes of importance into bamboo species. On the other side, conservation and utilization of plant genetic resources is in fact dependent on the germplasm characterization. In this regards, simple sequence repeat (SSR) markers are valuable tools for many purposes such as phylogenetic, fingerprinting, and molecular breeding studies. Genetic diversity evaluation based on transferred SSR markers derived from rice and sugarcane in 23 species of Indian bamboo indicated a high level of divergence at the species level (73%). However, a relatively low level of diversity was observed within species (25% in 20 accessions of *Dendrocalamus hamiltonii*). Further, cluster analysis revealed that the major grouping was in accordance with the taxonomical classification of bamboo. The study thus, paves better management and utilization of these bioresources. The important details on all these aspects shall be shared during The World Bamboo Congress.

Molecular Characterization of *PeLhcb* Genes in Bamboo and the Structural Analysis of the Complexes Reconstituted by using the Genes expressed *in vitro*

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Abstract

In order to understand the mechanism of the light harvesting chlorophyll a/b complex in bamboos, three genes (*PeLhcb1*, *PeLhcb2* and *PeLhcb3*) encoding light harvesting chlorophyll a/b-binding proteins were isolated from bamboo leaves of *Phyllostachys edulis* by RT-PCR and RACE methods. They shared high identities with *lhcb1*, *lhcb2* and *lhcb3* genes encoding the major antenna proteins of PSII from other plants. The secondary structure analysis showed that all the deduced proteins consisted of signal peptide and mature protein. The expression of these genes was detected in leaf, sheath and stem, with highest in leaf, but undetectable in root. The real time-PCR result showed they were all down-regulated in leaf treated with strong light ($1500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Three genes were overexpressed in *Escherichia coli* and successfully refolded with thylakoid pigments *in vitro*. The mature protein sequences of PeLhcb1 and PeLhcb2 were similar and they were capable of forming homotrimer, while that of PeLhcb3 lacks 10 residues in the N-termini and could not form the homotrimeric structure. The molecular and expression characterization of *PeLhcb* genes, pigment stoichiometries and spectroscopic characteristics of different reconstituted PeLhcb3 reveal that PeLhcb3 differs strongly from PeLhcb1 and PeLhcb2 which indicated they had different function in photosynthesis of bamboo.

Keywords

Bamboo, Light Harvesting Chlorophyll a/b-binding Protein Gene, Expression Analysis, Reconstitution, spectroscopic characteristics

Introduction

Bamboo is a monocotyledonous plant classified into the *Poaceae* family and mainly found in the tropics and the subtropics, which is often noted for its fast growth and being one of the important forest resources. There are two main types of bamboo, one is clump bamboo and the other is runner-type bamboo mainly found in temperate regions. Among the runner-type bamboo, Moso bamboo, *Phyllostachys edulis*, belonging to *Phyllostachys* genus, *Bambuseae* tribe, *Bambusoideae* subfamily and *Poaceae* family is one of the principal commercial species in China which is a large woody bamboo with the highest ecological, economic, and cultural values and accounting for ~70% of total area of bamboo growth and 5 billion US dollars of annual forest production in China (Jiang, 2002).

Moso bamboo is very hardy and known to be one of the fastest growing plants in the world, their fast-growth habit makes them potentially important energy plants for harvesting solar energy and storing it in the format of bio-energy. Growth pattern and photosynthetic activity of different bamboo species were well studied, and the results showed that Moso bamboo contributed in major role to carbon sequestration (14 ± 0.6 kg CO₂ year⁻¹ per culm) compared with the other considered species (on the average 3.0 ± 1.6 kg CO₂ year⁻¹, mean value) (Gratani et al., 2008), which indicates Moso bamboo could be a significant sink for CO₂ carbon sequestration. However, how to fix CO₂ in Moso bamboo by using the solar energy is still unclear.

It is already known that many bio-chemical reactions including the interception of light energy, migration of excitation energy and photophosphorylation are all carried out by the light harvesting complexes on the thylakoid membrane located inside the chloroplasts of many photosynthetic organisms (Yamamoto, 2001). However, little is known about how those reactions are running by the light harvesting complexes in the chloroplasts of bamboos.

A better understanding of the properties of bamboo light harvesting complexes might help to understand the molecular mechanism of photosynthesis in bamboo. To confirm the structural and functional relationship of the light harvesting chlorophyll a/b complex in Moso bamboo, here we reported the cloning of the cDNA encoding PeLHCB1, PeLHCB2, PeLHCB3 and the biochemical characterization of the recombinant PeLHCBs. The result of this study will be not only an important contribution to photosynthetic theory but also can promote the further utilization of bamboos.

Materials and Methods

Plant Materials and Strains

The seeds of *Phyllostachys edulis* were germinated in an artificial climate chamber at 25 °C and transplanted in pots with moistened vermiculite in a culture room (16 h light / 8 h dark at 25°C) when the seedlings were grown with 3 leaves. Fresh leaves of were collected, frozen with liquid nitrogen and stored at -80 °C until needed for use in experiments.

Escherichia coli DH5 α was used as the recipient in routine cloning experiments. *E. coli* BL21(DE3) was used for *lhcb* expression *in vitro*.

Isolation of *Pelhcb* cDNA and Sequence Analysis

Total RNA was isolated from leaves of *Phyllostachys edulis* with Trizol (Invitrogen, Germany) according to an isolation protocol recommended by the manufacturer. First-strand cDNA was synthesized from 500 ng of RNA using the Promega cDNA synthesis system; the cDNA was used as a template to amplify the gene. 3'cDNA and 5'cDNA were synthesized using the SMARTTM RACE kit (Clontech, USA).

Primers (I-F: 5'- ATGGCGACCACCACCATGGCC -3'; I-R: 5'- TCACTTGCCGGGCACGAAGT TG -3'; II-F: 5'- ATGGCCGCGTCGGCGCTGCAC -3'; II-R: 5'- TCACTTCCCTGGGACG AAGTTG -3';

III-F: 5'- ATCGACGATCATGGCCGCCAC-3'; III-R: 5'- TGCACGAAGAATCCGA ACATG -3') were designed on the basis of the sequence of the conservative domain of *lhcb5* from monocots. Gradient PCR amplification was performed with I-F and I-R, II-F and II-R, or III-F and III-R respectively to optimize the annealing temperature. Specific primers were designed according to the sequences obtained from III-1 and III-2 using the procedure described above. The primers used for 5' RACE were III 5-1 (5'- GCCGTTCTTGATCTCCTTCACCTTGAGC-3') and III 5-2 (5'- TGCGGTAG CCCTCGACGAGGCCCATG-3'); those used for 3' RACE were III 3-1 (5'- TCTACCCCGGCGGCCAGTACTTCGACCC-3') and III 3-2 (5'- GCTCAAGGTGAAGGAGAT CAAGAACGGC-3'). Touchdown PCR was performed with III 5-1, III 3-1 and a universal primer mix (UPM, as supplied with the SMARTTMRACE cDNA Amplification Kit), and the PCR amplicons were used as templates for a subsequent nested PCR using the NUP primer supplied in the kit together with either III 5-2 or III 3-2. The PCR fragments were cloned into pGEM-T Easy Vectors (Promega) using a standard protocol and sequenced using an ABI 3730 sequencer (Applied Biosystems, USA). The full length cDNA was obtained by combining the conserved sequence with the 5' and 3' end sequences.

Sequence analysis was carried out with the DNASTAR software package, and the cDNA sequences were subjected to a similarity search against the NCBI database (<http://www.ncbi.nlm.nih.gov>) using the Blastx algorithm with default parameters. A neighbor joining (NJ) tree was constructed using the MEGA4.0 software package and the CLUSTAL algorithm in conjunction with the sequences of Moso bamboo submitted to GenBank (Tamura et al. 2007). On the basis of this assembled sequences, the putative amino acid were analyzed using the software (<http://www.cbs.dtu.dk/services/ChloroP/>) to find the signal peptides and mature protein. Homology modeling was used for the mature proteins.

Tissue Specific Expression by Using Semi-quantitative RT-PCR

For gene expression analysis, total RNA was isolated from leaves, sheaths, stems and roots using Trizol (Invitrogen). First strand cDNAs were synthesized using 500 ng of RNA from the appropriate tissue with AMV Reverse Transcriptase (Promega). PCR was performed with I-F and I-R, II-F and II-R, or III-F and III-R respectively. The a final volume was 20 μ L including 10 μ L of 2 \times GC Buffer I (Mg²⁺ Plus), 3.2 μ L of dNTPs (2.5 mM each of dATP, dTTP, dCTP and dGTP), 2 μ L of PAL-F, and PAL-R (5 μ M, each), 1 μ L template, 1.6 μ L Milli Q- water (MQW) and 0.2 μ L LA Taq DNA polymerase (Takara Biotechnology Co., Ltd).

The PCR program involved an initial denaturation period at 94°C for 5 min, followed by 28 cycles at 94 °C for 1 min, 65 °C for 1 min, and then 72°C for 1 min; after the last cycle, there was a final extension period at 72°C for 10 min. The cDNA fragment arising from *Pe-actin* was amplified as a positive control under the same PCR conditions using Act077-F (5'- ATGGCTGAAGAGGATATCC AGC- 3') and Act077-R (5'- GAAACACTTCATATGGA CGATGG- 3') primers designed on the basis of the *actin* sequence from *P. edulis* (accession no. FJ601918).

Real Time-PCR analysis

Primers were designed from non conserved region of the isolated *lhcb5* using ABI Primer express 3.0, and the amplified fragment was about 50-200 bp. The cDNA template was isolated from the bamboo seedlings treated with strong light (1500 μ mol·m⁻²·s⁻¹) for 2 h, 4 h and 6 h respectively. The amplification was carried out followed the by using Applied Biosystems 7500 Real-Time PCR System and all the data was normalized with respect to *actin* sequence from *P. edulis* (accession no. FJ601918)

Construction of Expression Vector for the Recombinant Proteins

The fragments of *Pelhcbs* encoding the mature proteins were re-amplified respectively by PCR to introduce *Nde* I (forward) and *Xho* I (reverse) sites. Fragments incorporating *Nde* I and *Xho* I sites were cloned into the pET-23a vectors, respectively. The primers used to generate fragments from *Pelhcb1* were ImF (5'- AACATATGCGCAAGACCGGCGCCAAGC -3') and ImR (5'- CTCGAG CTTGCCGGGCACGAAGTTG -3'); The primers used to generate fragments from *Pelhcb2* were IImF (5'- AACATATGCGCCGCACCGTCAAGAGCG -3') and IImR (5'- CTCGAGCTTCC CTGGGACGAAGTTG -3'); The primers used to generate fragments from *Pelhcb3* were III mF (5'- AACATATGAGCAACGACCTGTGGTACGGG-3') and III mR (5'- CTCGAGAGACCCCGGC GCGAACTTG -3'); the *Nde* I and *Xho* I recognition sequences in the primers are underlined. The sequences were confirmed by sequencing (ABI 3730).

After sequencing the fragments encoding the mature proteins were introduced into the multiple cloning sites of pET-23a respectively, the recombinant plasmids were transformed into competent *E. coli* strain BL21(DE3) cells for protein expression. BL21(DE3) cells harboring the recombinant plasmids or an empty vector (pET-23a) were cultured at 37°C, in LB liquid medium containing 100 µg. ml⁻¹ of ampicillin until an OD₆₀₀ of approximately 0.6 was attained. The medium was then supplemented with 0.1 mM IPTG and the *E. coli* cells were cultured at 37 °C for an additional 4 hours to induce synthesis of the recombinant protein.

Recombination of PeLhcb and Pigments

The PeLhcb apoproteins were reconstituted with total thylakoid pigments (Chl *a/b*: 3/1 and Lut/Neo/Vio: 3/1/1) according to the reference (Paulsen *et al.*, 1990) with minor modification. The mixture containing 1 µg·µL⁻¹ apoprotein in a reconstitution buffer (2% (w/v) lithium decyl sulfate (LDS) (Amresco, USA), 100 mM Tris-HCl (pH 9), 12.5% (w/v) sucrose, and 10 mM β-mercapthoethanol) were mixed, under vortex, with thylakoid pigments resolved in ethanol (protein:Chl =1:2.5). After reconstitution, the complexes was loaded to a Ni²⁺-chelating sepharose fast flow column (0.8 cm × 4 cm) (Bio-Rad, Hercules, CA) which was equilibrated with OG buffer (1% (w/v) octyl-β-D-glucopyranoside (OG) (Sigma, USA), 0.1 M Tris (pH 9), 12.5% (w/v) sucrose), and then incubated in darkness at 4 °C for 30 min. The column was washed with 1 mL OG-buffer and 2 mL Triton X-100 buffer (0.05% (w/v) Triton X -100 (Sigma, USA), 0.14 mM 1,2-Dipalmitoyl-sn-Glycero-3-[phospho-rac-1-glycerol](PG) (Avanti, USA), 0.1 M Tris (pH 7.5)). The complexes were eluted with elution buffer (0.05% (w/v) Triton X-100, 0.14 mM PG, 10 mM Tris (pH 7.5), 0.3 M imidazole (Amresco, USA)).

The reconstituted monomeric and trimeric complexes were separated by sucrose density gradients centrifugation. The material eluted from the column was loaded onto a sucrose density gradient in a 12.5 mL centrifuge tube containing 0.1- 1.0 M sucrose density gradient, 2.0 mM dodecyl β-D-maltoside (DM) (Sigma, USA), and 5 mM phosphate buffer (pH7.5) and centrifuged at 230,000 g and 4°C for 18 h. The bands were collected for further experiment.

Pigment Analysis

The band corresponding to the monomers and trimers in the sucrose density gradient after ultracentrifugation were further analyzed for pigment stoichiometries of the recombinant LHCIIB complexes with Waters 600 high performance liquid chromatography (HPLC) (Waters, USA). The pigments were extracted with 2-butanol according to the reference (Martinson *et al.*, 1990). The 2-butanol extraction were applied to an RP-C18 HPLC column (Merck, Germany) and separated with a gradient from 70 to 100% acetone, at a rate of 1 mL·min⁻¹. Pigments were quantified by comparing integrated peak areas to calibrated ones of known pigment amounts.

Spectra Analysis

The absorption spectra were recorded using Shimadzu UV-VIS 2550 spectrophotometer (Shimadzu, Kyoto, Japan) at room temperature. The samples were diluted with the dilution buffer to approximate $5\mu\text{g Chl}\cdot\text{mL}^{-1}$. The wavelength step was 0.5 nm, the scan rate was $100\text{ nm}\cdot\text{min}^{-1}$, and the optical path length was 1 cm.

Circular dichroism (CD) spectra were recorded on a Jasco 815 spectropolarimeter (Jasco, Tokyo, Japan) at $10\text{ }^\circ\text{C}$. The concentration of the samples was adjusted to $\text{OD}=1$ at the Q_y transition of Chl *a*. The spectra were measured from 350 to 750 nm at a scan rate at $100\text{ nm}\cdot\text{min}^{-1}$. The measurements were repeated for four times and averaged.

The fluorescence emission spectra were recorded with a Hitachi F-4500 spectrofluorometer (Hitachi, Japan). The samples were diluted to $0.5\mu\text{g Chl}\cdot\text{ml}^{-1}$ with the dilution buffer. For measuring 77K fluorescence emission, the samples were frozen in a sample tube by immersing the samples in liquid nitrogen. The fluorescence emission spectra were measured from 600 nm to 780 nm, with the excitation wavelength set to 480 nm, slit widths set to 5 nm for excitation and 2.5 nm for emission.

Results

Molecular Cloning and Analysis of *Pelhcb*

Lhcb homolog genes have high similarities among plant species and this facilitate the design of primers for RT-PCR to clone *Pelhcb*. As a result of the RT-PCR with I-F and I-R, II-F and II-R, 798 bp and 792 bp nucleotide fragments including open reading frame were subcloned respectively. Meanwhile, 689 bp with conserved domain was isolated by using III-F and III-R. Subsequently, 625 bp from the 5'-end region and 314 bp from 3'-end region were determined by 5'- and 3'- RACE respectively. Through analyzed the obtained sequences, a 1039 bp full length cDNA was gained containing a 804 bp ORF, a 96 bp 5' untranslated region (UTR) and a 139 bp 3' UTR. Finally, 804 bp of the ORF was determined by end-to-end PCR. The result of blastx showed that those sequences had close homology with *lhcb* genes encoding the major antenna proteins of PSII from other plants such as *Oryza sativa*, *Zea mays* and *Hordeum vulgare*. The genes were designed as *Pelhcb1* (EF405878), *Pelhcb2* (HQ831348) and *Pelhcb3* (EF628208) respectively. Phylogenetic tree was constructed on the basis of light harvesting chlorophyll *a/b*-binding protein genes isolated from Moso bamboo (Fig. 1). There were 35 homologue *lhcb* genes in Moso bamboo which belonged to *lhcb1*, *lhcb2*, *lhcb3*, *lhcb4*, *lhcb5* and *lhcb6* respectively. The proteins encoded by *lhcb1*, *lhcb2* and *lhcb3* had high similarity ($>60\%$) and belonged to macro-antenna proteins, however, the proteins encoded by *lhcb4*, *lhcb5* and *lhcb6* had low similarity ($<50\%$) and belonged to micro-antenna proteins.

Biochemical properties of the three putative proteins (PeLhcb1, PeLhcb2 and PeLhcb3) encoded by *Pelhcb1*, *Pelhcb2* and *Pelhcb3* were analyzed by using DNASTar and. The mature proteins were designed as PeLhcb1m, PeLhcb2m and PeLhcb3m respectively. The result of analysis showed that the precursor of three PeLHCBs had similar molecular weight, but the pI had difference. The amino acid sequence similarities of PeLhcb1 with PeLhcb2 and PeLhcb3 were 82.2% and 64.2% respectively. However, the molecular weight of the mature proteins was much closed. There was difference of isoelectric point among the precursors and mature proteins (Table 1). The alignment for the deduced mature proteins was carried out using ClustalW, the pigments binding sites and the helices were predicated (Caffarri et al., 2004). They all consisted of four α helices, three carotenoid binding sites and chlorophyll *a/b*-binding sites. There was a phosphorylation site in PeLhcb1-2m and PeLhcb2-2m, which was lost from PeLhcb3-2m (Fig.2). The difference of structure indicated that PeLhcb3-2m was unable to participate in the redox controlled regulation of energy distribution.

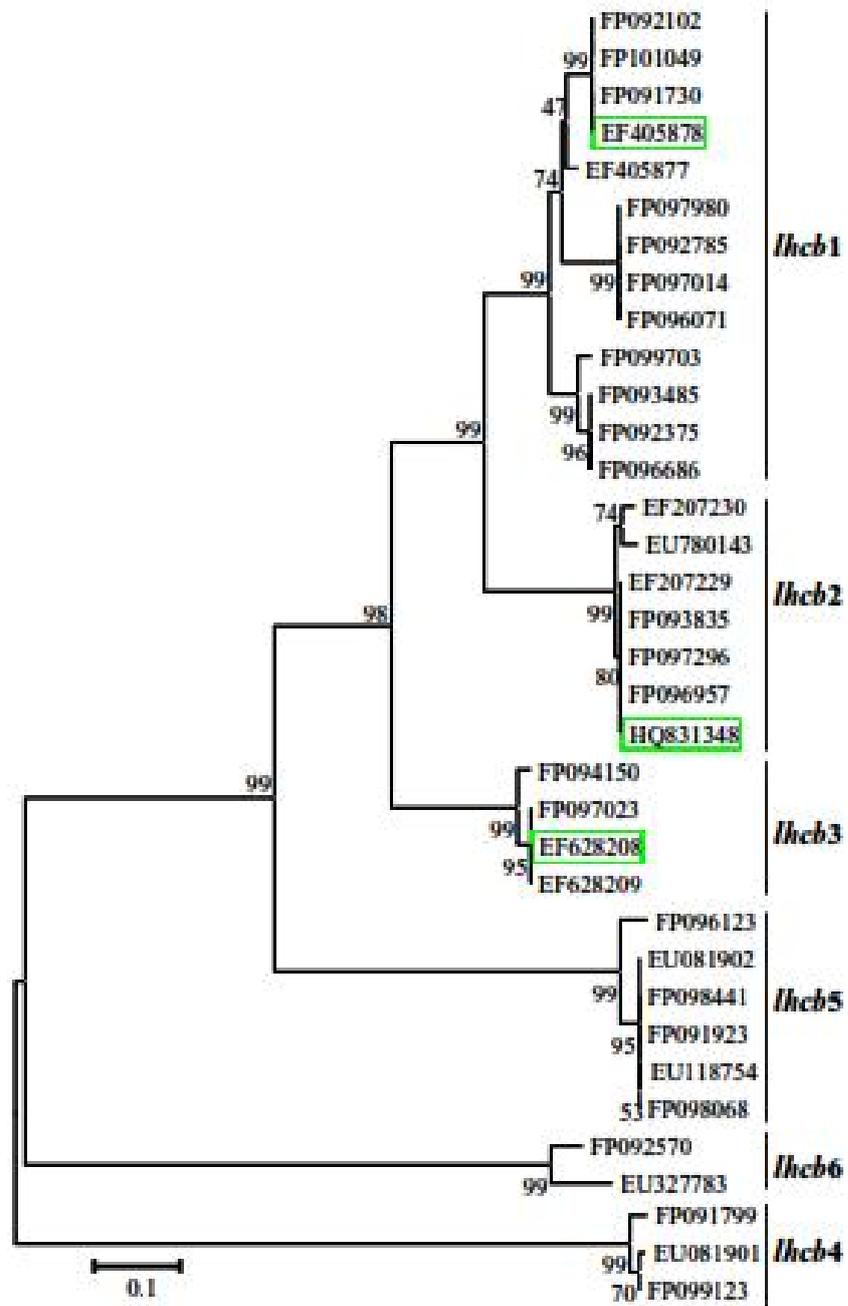


Fig.1 Phylogenetic analysis of *lhcb*s from *Phyllostachys edulis* by using MEGA 4.0.

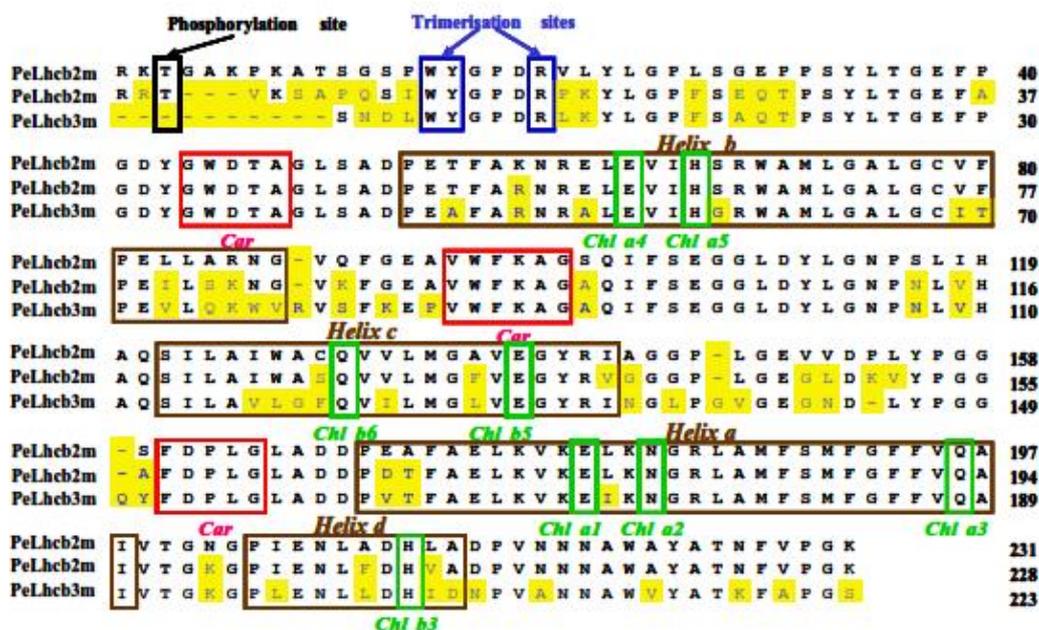


Fig.2 Sequence alignment of PeLhcb1m, PeLhcb2m and PeLhcb3m. Different features are indicated: in yellow the AA different from the PeLhcb1m sequence; black rectangle, phosphorylation site; blue rectangles, trimerization sites; red rectangles, putative carotenoid binding sites; brown rectangles, α -helices; green rectangles, Chl binding sites.

Homology modeling was carried out by using Lhcb1 and Lhcb2 from *Spinacia* for PeLhcb1-2m and PeLhcb2-2m respectively (Fig.3 A, B), and Lhcb3 from *Glycine max* for PeLhcb3-2m (Fig.3 C). The three-dimensional model showed that they all had four helices with much difference which indicated functional diversity.

Table 1 The basic biochemical properties of the putative Pelhcb proteins.

Protein name	Length (aa)	Molecular weight (kDa)	pI
PeLhcb1	265	28.2	5.06
PeLhcb2	263	28.5	5.47
PeLhcb3	267	28.8	5.73
PeLhcb1m	231	24.7	4.69
PeLhcb2m	228	24.8	5.03
PeLhcb3m	223	24.3	4.82

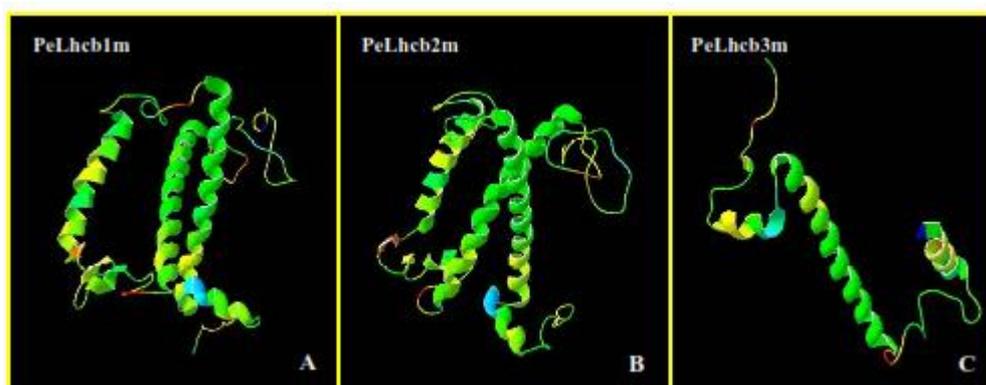


Fig.3 Three-dimensional model analysis of PeLhcb1m, PeLhcb2m and PeLhcb3m.

Expressed Pattern of *Pelhcbs*

Transcription of *Pelhcb* genes in different tissues of *P. edulis* seedling were detected by reverse transcription-polymerase chain reaction (RT-PCR). The expression of *Pelhcb* genes was detected in leaf, sheath and stem, with highest in leaf, but undetectable in root (Fig. 4). The real time-PCR result showed they were all down-regulated in leaf treated with strong light ($1500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), but there was difference for them during treatment. The expression of *Pelhcb1* and *Pelhcb2* decreased rapidly during the former 4 hours, and *Pelhcb2* dropped nearly to zero after 6 hours treatment. However, the expression level of *Pelhcb3* was more than 70% of the control after 4 hours, and then it reduced to less than 5% in short order during the following 2 hours (Fig. 5).

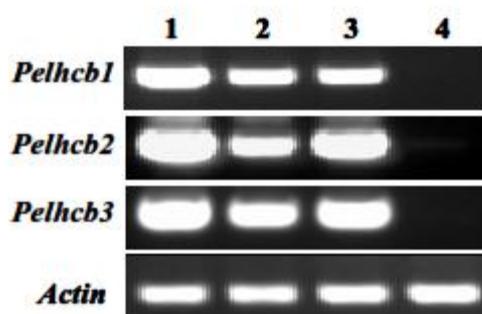


Fig.4 The transcription of *Pelhcb1*, *Pelhcb2* and *Pelhcb3* in different organs with *Actin* as control. 1, Leaf; 2, Sheath; 3, Stem; 4, Root.

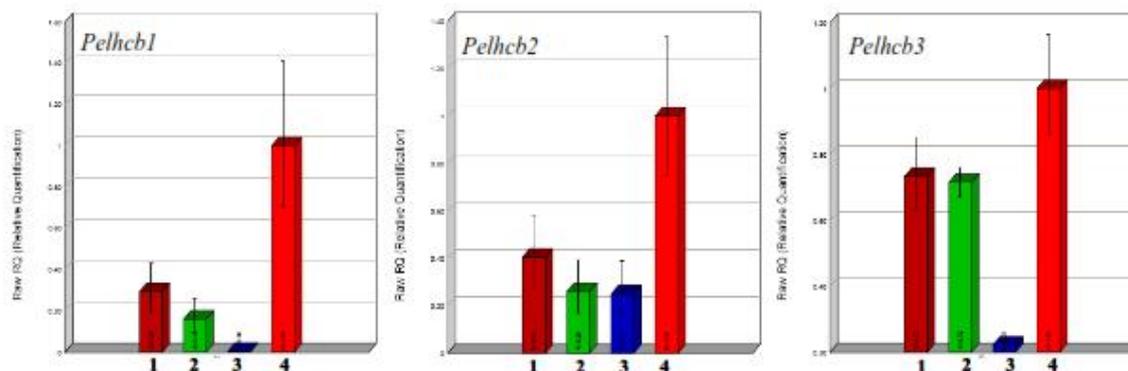


Fig.5 Real time-PCR analysis of *Pelhcbs* in leaves under strong light 1, 2 h; 2, 4 h; 3, 6 h; 4, ck.

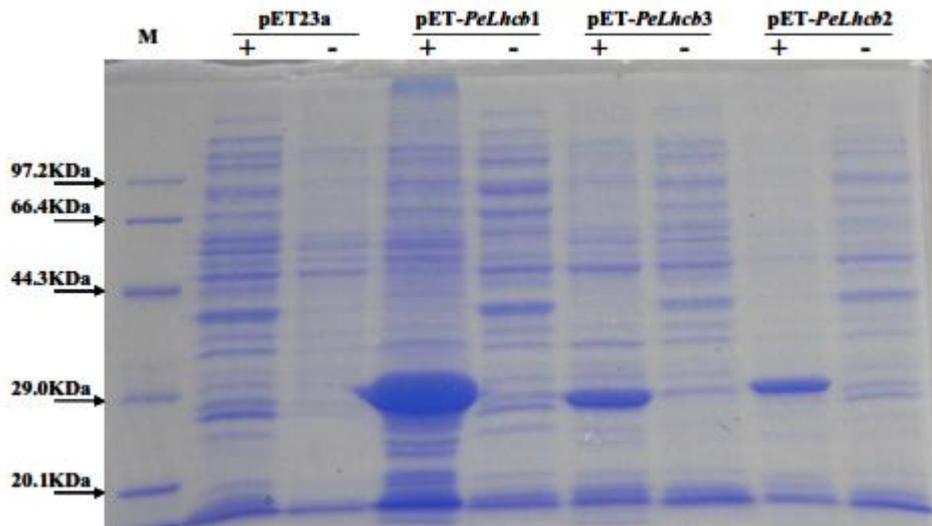


Fig.6 Recombinant proteins of PeLhcb1, PeLhcb2, and PeLhcb3 expressed in *E. coli*

Expression of the Mature Protein and Reconstituted with Pigments *in vitro*

The mature proteins of PeLhcb were expressed successfully in *E. coli* (Fig.6). The recombinant proteins were refolded into pigment-protein complexes *in vitro* using detergent exchange method, which is confirmed by partly denaturing gel electrophoresis, with one obvious band for the monomer of all three samples. Only PeLhcb1m and PeLhcb2m could form trimer, the yield of trimer formation of PeLhcb1m is much better than that of PeLhcb2m (Fig.7), probably because the method to induce trimer formation was optimized for PeLhcb1m.

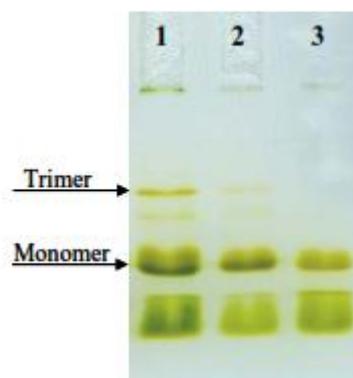


Fig.7 Partly denaturing gel electrophoresis analysis of the reconstituted pigment-protein complexes 1, Pelhcb1m; 2, Pelhcb2m; 3, Pelhcb3m.

Pigment Component of Different Monomers and Trimers

The native LHCII contains Chl a, Chl b, Lut, Neo and Viol. The pigment content of the refolded monomer and trimer was shown in the table 2, calculated on the basis of pigment per 2 Lut followed the reference (Yang *et al.*, 1999). Because violaxanthin lies in the periphery of the complexes, most violaxanthin was lost during experiment for all samples. The Chl a/b ratio of refolded monomer was 1.18, 1.17 and 1.39 for PeLhcb1m, PeLhcb2m and PeLhcb3m, respectively. The reason of high chl a/b ratio of PeLhcb3m possibly was that lhcb3 bound one chl b less than PeLhcb1m and PeLhcb2m. The pigment content of PeLhcb1m and PeLhcb2m trimers was roughly the same, 1.19 and 1.15 respectively

Table 2 Pigment component of different monomers and trimers.

Lhcb	Chl a	Chl b	Lutein	Neoxanthin
Lhcb1M	7.02±0.24	5.90±0.39	2	0.91±0.03
Lhcb2M	6.94±0.28	5.92±0.15	2	0.92±0.01
Lhcb3M	7.72±0.21	5.52±0.14	2	0.74±0.03
Lhcb1T	7.74±0.15	6.48±0.19	2	0.94±0.02
Lhcb2T	7.53±0.13	6.55±0.07	2	0.91±0.01

Absorption Spectra of Monomers and Trimers

The absorption spectra of each isoform were measured at room temperature (Fig.8), normalized to the reddest Chl a absorption in the Qy region. The maxima in the Qy region were around 672 nm and 651 nm, and in the Soret region around 436 nm and 472 nm, for all the isoforms. The fourth deviation of the absorption spectra revealed three components peaking at 666 nm, 672 nm and 680 nm attributed to Chl a absorption in the Qy region. The red-most component (680 nm) was 2-nm red-shifted in PeLhcb3m. In the Soret region, there were three major differences located at 436 nm, 460 nm and 472 nm, respectively. The absorption maximum of PeLhcb3m peaking at 438 nm, and was split into two peaks (435 and 439 nm) for both PeLhcb1m and PeLhcb2m; the absorption maximum at 472 nm for PeLhcb1m and PeLhcb2m was missing in the absorption spectra of PeLhcb3m.

The CD spectrum in the visible range reflects sensitively intramolecular pigment- pigment interactions (Zhang *et al.* 2008). CD spectra of the refolded monomeric and trimeric isoforms were measured at 4 °C. The monomeric spectra of PeLhcb1m and PeLhcb2m were almost identical. The CD signal of PeLhcb3m was very similar to other two isoforms, except that PeLhcb3m had an obvious peak at 470nm (Fig.9 A), which reflect differences in pigment content or pigment interactions. The CD spectrum of trimer was characterized by a strong negative peak around 472 nm, which was absent in the monomer (Fig.9 B).

Fluorescence Spectra of Monomers and Trimers

The energy transfer from chl b to chl a in LHCII can be monitored by the fluorescence emission spectra with an excitation wavelength of 480 nm where Chl a absorption is negligible (Luciński and Jackowski 2006). All samples showed very little chl b emission suggesting that the complexes are intact. The emission peak of PeLhcb1m monomer (Fig.10 A) and trimer (Fig.10 B) were both at

679nm, and those of PeLhcb2m monomer and trimer were blue-shifted about 0.4nm, to 678.6nm. The emission peak of PeLhcb3m monomer differed clearly from the other two, with a peak at 681nm, which red shifted about 2 nm compared to that of PeLhcb1m and PeLhcb2m monomer.

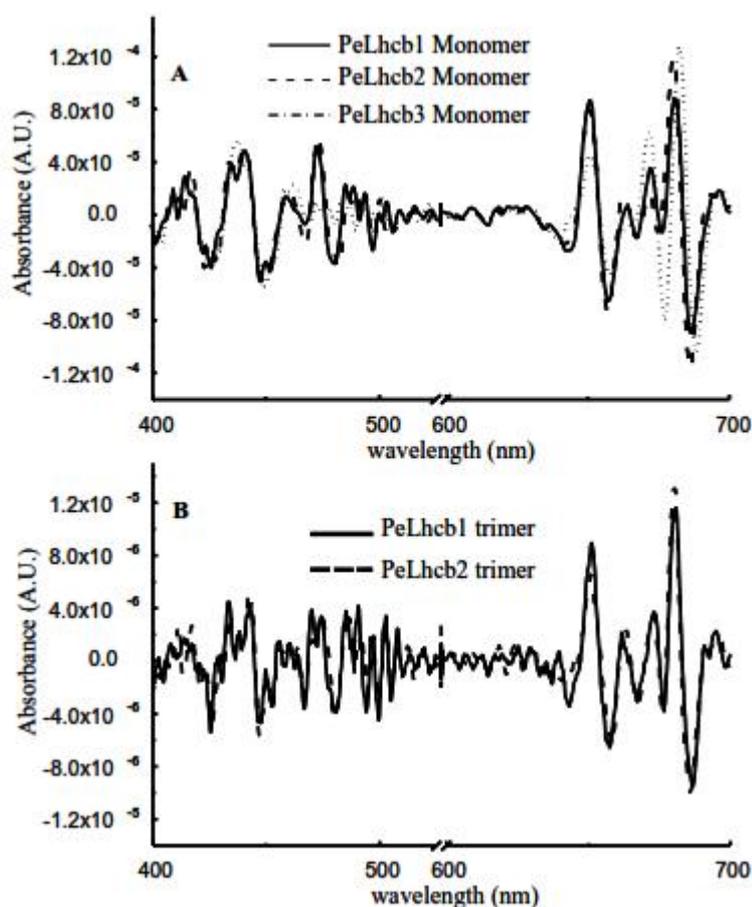


Fig.8 Absorption spectra of monomers (A) and trimers (B) at room temperature.

Discussion

The fast-growing habit of bamboo makes them potentially important energy plants for harnessing solar energy for the production of bioenergy, and it suggests that the LHCIIbs in bamboo have peculiar functions in harvesting and utilizing solar energy. Three genes (*Pelhcb1*, *Pelhcb2* and *Pelhcb3*) encoding light harvesting chlorophyll a/b proteins were isolated and their molecular characterization were analyzed. The tissue specific expressed pattern and the regulation under strong light were detected by semi RT-PCR and real time-PCR. The result indicated the difference of protein component, temporal and spatial expression of the genes would lead to the functional difference for the interception of light energy, migration of excitation energy and photophosphorylation, which was confirmed by the pigment stoichiometries and spectroscopic characteristics analysis of the isoforms reconstituted by using the protein and pigments *in vitro*.

And we refolded all the isoforms *in vitro* and yielded complexes with the same absorption and fluorescence spectra as the native LHCIIb, suggesting that all complexes are functionally in order. The function of LHCIIb depends on the type and number of bound pigments and their conformations and organizations. All the three refolded isoforms of bamboo were highly homologous and all pigment-binding residues were conserved, and compared to native LHCII, they had comparable absorption (Fig.8), CD (Fig.9), and fluorescence (Fig.10) spectra, indicative of a similar pigment organization.

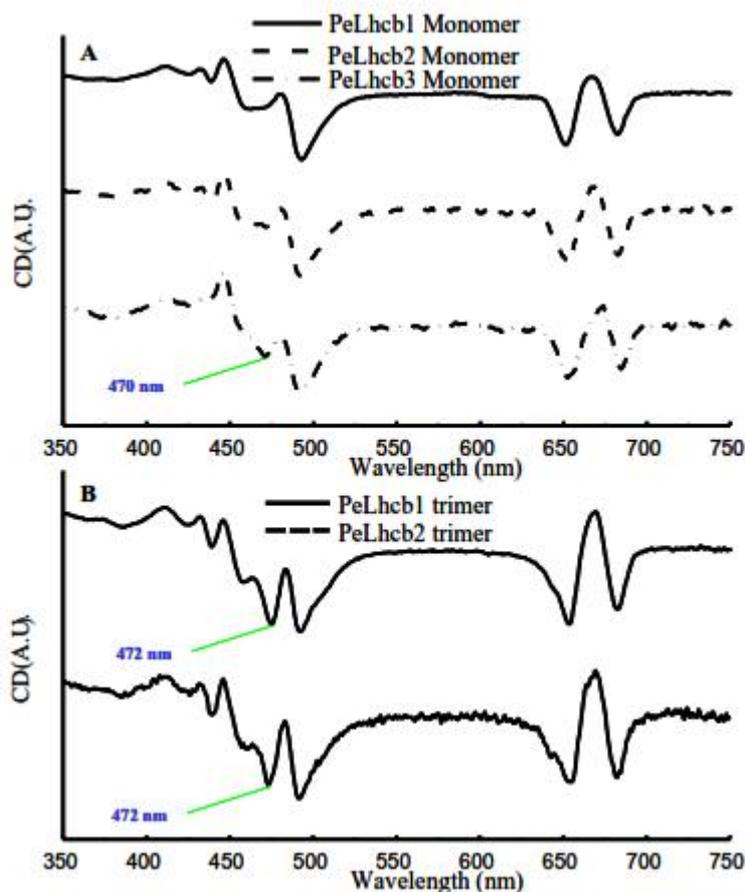


Fig.9 CD spectra of monomers (A) and trimers (B).

Nevertheless, there are significant differences, especially in their fluorescence and absorption properties. The absorption band around 680 nm, which located at the end emitter chlorophyll, differed among the 3 isoforms. Lhcb3 absorbed and emitted light at a wavelength two nanometer longer than the other two isoforms did. In the CD spectrum (Fig.9), Lhcb3 possesses a prominent peak at 470 nm, compared to the other two isoforms. The amplitudes of the peaks at 472 nm in both trimers were almost the same as that at 492 nm. The ratio between the peaks at 472 nm and 492 nm was obviously increased in bamboos as compared to those in the other species, such as pea (Zhang et al. 2008) and *Arabidopsis* (Standfuss et al. 2004), which means that the pigment configuration, mostly of Neo, reflected by the Soret region of CD spectra is quite different in LHCIIB trimers of bamboo as compared to the other species, because the ratio between the amplitudes of the peaks at 473 nm and 493 nm had been proved to be sensitive to the configuration of Neo (Hobe et al. 2006). As is revealed by the crystal structure at near atomic resolution, the Neo locates in the Chl b-rich region, and associates, via an H-bond, with Tyr-112 that locates in the antiparallel strands (Liu et al. 2004). All these residues in bamboo Lhcb isoforms are conservative, which suggests that the difference in Neo conformations was caused by factors other than the binding residues. The different combinations of Lhcb isoforms would cooperate and conduct the regulation between energy absorption and excess energy dissipation, ultimately maintain an appropriate amount of stable energy input for the light reaction in bamboo.

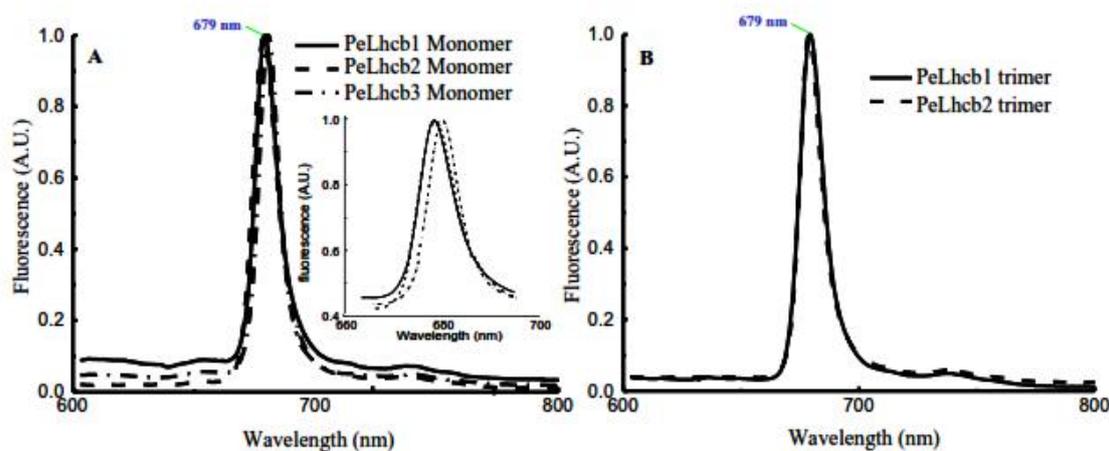


Fig.10 Fluorescence spectra of monomers (A) and trimers (B).

This study will not only help to understand the molecular mechanism of photosynthesis in bamboos, but also contribute to reveal the process of converting solar energy into bioenergy by bamboos, which will promote the further utilization of bamboos.

Acknowledgements

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References

- Caffarri, S., Croce, R., Cattivelli, L., Bassi, R. 2004. A look within LHC II : differential analysis of the Lhcb1-3 complexes building the major trimeric antenna complex of higher-plant photosynthesis. *Biochemistry*, 43: 9467-9476
- Gratani, L., Crescente, M.F., Varone, L., Fabrini, G., Digiulio, E. 2008. Growth pattern and photosynthetic activity of different bamboo species growing in the Botanical Garden of Rome. *Flora*, 203: 77-84
- Hobe, S., Trostmann, I., Raunser, S., Paulsen, H. 2006. Assembly of the major light-harvesting chlorophyll-a/b complex - Thermodynamics and kinetics of neoxanthin binding. *J. Biol. Chem*, 281: 25156-25166,
- Jiang, Z.H. 2002 *Bamboo and rattan in the world*. LiaoNing Science and Technology Published House, Shenyang, China.
- Liu, Z. F., Yan, H. C., Wang, K. B., Kuang, T. Y., Zhang, J. P., Gui, L. L., An, X. M., Chang, W. R. 2004. Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution. *Nature* 428: 287-292.
- Luciński, R., Jackowski, G. 2006. The structure, functions and degradation of pigment-binding proteins of photosystem II. *Acta biochimica polonica*, 53(4): 693-708
- Martinson, T. A., Plumley, F.G. 1995. One-step extraction and concentration of pigments and acyllipids by sec-butanol from in vitro and in vivo samples. *Anal. Biochem*, 228: 123-130.
- Paulsen, H., Ruler, U., Ruiger, W. 1990. Reconstitution of pigment containing complexes from light-harvesting chlorophyll a/b-binding protein overexpressed in *Escherichia coli*. *Planta*, 181: 204-211.

- Standfuss, J., Kühlbrandt, W. 2004. The three isoforms of the light-harvesting complex II – Spectroscopic features, trimer formation, and functional roles. *J. Biol. Chem*, 279: 36884-36891.
- Tamura, K., Dudley, J., Nei, M., Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mole Biol Evol*, 24: 1596-1599
- Yamamoto, Y. 2001. Quality control of photosystem II. *Plant Cell Physiol*, 42: 121-128.
- Yang, C. H., Kosemund, K., Cornet, C., Paulsen, H. 1999. Exchange of pigment-binding amino acids in light-harvesting chlorophyll a/b protein. *Biochemistry*, 38: 16205-16213.
- Zhang, Y., Liu, C., Liu, S., Shen, Y., Kuang, T., Yang, C. 2008. Structural stability and properties of three isoforms of the major light-harvesting chlorophyll a/b complexes of photosystem II. *Biochim Biophys Acta*, 1777: 479-87.

Cytokinin dynamics in cell suspension cultures of *Bambusa balcooa* Roxburgh using UPLC-ESI/MS/MS

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Abstract

Tissue culture of plants is still much more art and skill than science. Rational Plant Tissue Culture involves the quantification of plant growth hormones in plants and media, to gain insight in kinetics and metabolism. We report on the use of UPLC-MS/MS for reliable and highly accurate determination of cytokinins in cell suspension cultures of *Bambusa balcooa*, growing on 2,4-D medium without cytokinins. The cytokinins are almost exclusively isoprenoid cytokinins, and the only aromatic cytokinin detected is BA, at very low concentrations. The predominant forms are glucosides and ribosides and the main cytokinins in cell suspension cultures are *trans*-zeatin-*O*-glucoside and *trans*-zeatinriboside-*O*-glucoside. Between days 8 and 14-16, when the growth of the cultures slows down the levels of *trans*-Z-*O*-G and *trans*-ZR-*O*-G decrease drastically, and from day 15 onwards up to 20 pmol of Z-N9-G is observed.

Keywords

Cytokinins, UPLC, Mass Spectrometry, cell suspension cultures, *Bambusa balcooa*

Abbreviations

2,4-D	2,4 dichlorophenoxyacetic acid
Trans-ZR	<i>trans</i> -zeatin-N9-riboside
Cis-ZR	<i>cis</i> -zeatin-N9-riboside
DHZR	dihydrozeatin-N9-riboside
DHZ	dihydrozeatin
DHZ-N9-G	dihydrozeatin N9-glucoside
Z-N7-G	zeatinN7-glucoside
Z-N9-G	Zeatin-N9-glucoside
iPG	N ⁶ -isopentenyladenineglucoside
iPA	N ⁶ -isopentenyladenosine
iP	N ⁶ -isopentenyladenine
MS-iP	Methylthio-N ⁶ -isopentenyladenine
MS- iPA	Methylthio-N ⁶ -isopentenyladenosine
MS-Z	Methylthio- <i>trans</i> -zeatin
MS-ZR	Methylthio- <i>trans</i> -zeatinriboside
BA	benzyladenine

BAR	Benzyladenineriboside
BA(9)G	Benzyladenine- <i>N</i> 9-glucoside
¹³ C-o-T	<i>ortho</i> -topolin
SPE	Solid Phase Extraction

Introduction

Bamboos and their distribution

Bamboo is one of the most useful plants to mankind. As a giant grass it has evolved to thrive under a variety of natural conditions, and consequently one finds bamboos all over the world from the northernmost part of Chili, up to the northern parts of China and Japan, and from sea level up to 3000-4000 meter (Himalaya, Andes). In most cases bamboo grow as part of forest ecosystems, but under certain conditions pure bamboo forests are well known.

It occurs worldwide with main centers of diversity in Asia and the Americas. In the New World over 400 different species have been described in South and Central America, from the North of Chili up to Mexico. In North America only one genus is endemic, namely *Arundinaria*, with three different species (Triplett et al., 2006). In Africa the genetic diversity is relatively low (with large areas of *Arundinaria alpina* in Central Africa and of *Oxytenanthera abyssinica* in Eastern African countries like Ethiopia, Sudan), but from Madagascar over 40 different species have been described.

Bamboos come in many variants, from the tall woody bamboos in Asia, to climbing and clambering species, like *Dinochloa* (Wong, 1987) or various American bamboo species (Judiewicz et al., 1999). Bamboos play a key role in forest dynamics because of their ability to take advantage of small or large-scale disturbances (Widmer, 1997).

Major movements of germplasm have occurred by human intervention, and as a consequence over 400 different types of bamboo, which are mainly ornamentals, occur in Europe and North America. The reintroduction of bamboo in Europe remedies its extermination during the last Ice Age. As a consequence, many Asian bamboos are found now throughout Africa and America.

Bambusa balcooa cell suspension cultures as model system

Bambusa balcooa Roxburgh is one of these examples. This species originates from Northeast India, and is originally found in Nepal, Bangladesh, Myanmar and Thailand. In South-Africa, it has become naturalized, where it is known as ‘common bamboo’. It thrives under typical monsoonal climates with ample rain in the growing season (2500-3000 mm) and long dry seasons. It is one of the most useful species of bamboo supplying material for building and scaffolding, paper and pulp, boards and mats, handicrafts, and its young shoots are even consumed as vegetable.

As for most commercially interesting bamboos, hardly anything is known about their genetic basis (Gielis, 1999). Only recently, there has been some focus on exploring wild accessions of *Bambusa balcooa* in order to identify superior traits in terms of fiber quality among natural accessions (Bhattacharya et al., 2010; Rai et al., 2011).

Because of its economic potential *Bambusa balcooa* is also one of the species for which successful mass propagation methods through plant tissue culture have been developed, either via axillary branching (Gielis 1999; Das & Pal, 2005; Mudoi, K.D., Borthakur, M, 2009; Negi & Saxena, 2010), or via somatic embryogenesis (Gillis et al., 2007). Cell suspension cultures have been derived from these embryogenic cultures.

Cell suspension cultures, in combination with feeding experiments, provide excellent model systems

for studying biochemical pathways, developmental pathways (for example cell wall and microfibrils), gene expression and genetic transformation (Ojita et al., 2011), or serve as a source of protoplasts. However, optimization of cell suspension cultures is essential if we wish to use these as model systems.

One of the key factors in this optimization strategy is the role of plant growth regulators PGR. Cytokinins and auxins are the key ingredients of culture media to control (1) the growth of cells and calli, (2) the regeneration and maturation of somatic embryos and (3) organogenesis, more specific branching and rooting, in micropropagation. However, little if anything is known about their uptake, metabolism and efficiency.

Understanding the metabolism of phytohormones may even be more critical for genetic transformation of bamboo. Despite many published or unpublished attempts, genetic transformation has hitherto been unsuccessful, other than mainly transient expression. Already in the early eighties it was shown that *Agrobacterium* cells do not adhere to bamboo cells (Douglas et al., 1985) and major deterrents to successful genetic transformation were only studied very recently (Sood et al., 2011).

In this paper we present results on the optimization of cell suspension cultures as a model system and on the qualitative and quantitative analysis of the dynamics of endogenous cytokinins over time.

Materials & Methods

Bambusa balcooa cell suspension cultures and ploidy levels

The mother plant of *Bambusa balcooa* Roxb. originates from the Oprins Plant collection and has been kept at the Botanical Garden of Ghent. The same genotype has been used to induce somatic embryogenesis (Gillis et al., 2007) and for the analysis of phytoactive components (Van Hoyweghen et al., 2010). The cell cultures have been derived from somatic embryos originally induced from pseudospikelets of motherplants (Gillis et al., 2007).

The cell cultures were cultivated on MS medium supplemented with 2,4-D 1,5 mgL⁻¹ or 0,5 mgL⁻¹ and 30 gL⁻¹ sucrose. They grew in darkness at 25°C +/- 2°C on a liquid shaker (Innova 44 Incubator Shaker Series, New Brunswick Scientific).

For the callus cultures 2g/L Gelrite (Duchefa) was added. For the experiments stocks of the cell culture were kept and each new experiment was initiated from these stock. Ploidy levels of cell suspension cultures were determined using methods described by Gielis et al. (1997).

Sample preparation for analysis

For the analysis 20 mL of cell culture solution was used, and the medium was separated from the cells by vacuum filtration (Whatman, filter paper Grade 12: 11 µm, cat.n° 1001-055.) After filtration the cells were ground in liquid nitrogen using a mortar and pestle.

Cytokinins (isoprenoid and aromatic), methylthio-cytokinins and cytokinin-*O*-glucosides were extracted from the samples using Bielecki mixture (Methanol/chloroform/formic acid/ water in 12/5/1/2 ratio) (Bielecki, 1964) which was added at 4mg/40 mg plant material at -20°C for 16 hours. After extraction, cell debris was removed by centrifugation (5min, 4°C, Eppendorf Centrifuge, 3220g). The resulting pellets were dissolved in 4 ml 80% Methanol HPLC grade and extracted again at -20°C for 45 minutes. After centrifugation the supernatant fractions were pooled and concentrated to waterphase with a rotavapor (Rotary Evaporator Büchi R110, Büchi water bath B-480 at 37°C). The water phase was diluted with 15 mL distilled water and adjusted to pH 7.

Solid Phase Extraction using DEAE-C18 and OASIS MCX columns

For the SPE with C18: Bond ElutR Solid Phase Extraction, Varian 6mL, 30/PK; 500 mg (Agilent,

Santa Clara, CA, USA) was used. For the purification Solid Phase Extraction SPE was used with an anion exchange columns (DEAE Sephadex) and an inverted phase RP-C18 (LC-18 octadecyl bonded, Agilent) in series. The pH was adjusted to 7 and loaded onto the DEAE Sephadex-RP-C18 column. After rinsing with 20 mL doubly distilled water the RP-C18 column was uncoupled and eluted (Supelco Bulletin 910: Guide to Solid Phase Extraction) two times with 1,5 mL 80% methanol and 1 mL 100% methanol (20° HPLC grade methanol). Then the samples were dried at 27°C for 16 hours.

For the SPE with Oasis MCX columns (OASIS: Waters sample extraction products, OASIS MCX 6cc (150 mg) extraction cartridges (Waters, Milford, MA, USA) were used. During SPE all experiments were kept on ice during the procedure to prevent degradation of cytokinins.

When columns were almost dry, these were rinsed with 20 mL double distilled water. Then the column was rinsed two times with 2mL 100% methanol HPLC grade, followed by a rinse with 5mL of distilled water. The following step was the elution of the column with 1mL 5% NH₄OH in water for the elution of cytokinin phosphates and cytokinin-*O*-glucosides. A second elution step, with two times 1,5 mL 5% NH₄OH in 100% methanol HPLC grade was performed for the elution of isoprenoid and aromatic cytokinins and methylthio-cytokinins. To prevent adsorption of cytokinins silanylated glassware was used to capture the fractions. The fractions were dried using a Turbovap LV evaporator.

For both experiments the dried samples were dissolved in 100 µL HPLC-grade water and in 50 µL 100% HPLC grade methanol. Cell debris was removed by centrifugation (Microcentrifuge 5415D) for 3 minutes at 5,9g. The samples were dried under a continuous stream of nitrogen and then redissolved in 50 µL 10% methanol HPLC grade prior to the measurements. To compare both methods (DEAE-C18 versus OASIS) the experiments were performed according to standard protocols in triple. To be able to account for extraction efficiency and ionization efficiency internal standards were added (Olchemim, Olomouc, Czech Republic) (d-DHZR, d-DHZ, d-DHZ-N9-G, d-Z-N7-G, d-iPG, d-iPA, d-iP, d-MS-iP, d-MS- iPA, d-MS-Z, d-MS-ZR, d-BA, d-BAR, d-BA(9)G, 13C-o-T, 20 pmol each).

UPLC-MS/MS Analysis

To measure the samples UPLC-MS/MS was used (Ultra High Pressure Liquid Chromatography, Acquity-TQD, Waters, Manchester UK). For data analysis Masslynx C V4.1 and Quanlynx (Waters, Manchester) were used. Separation is performed on a BEH-C18 column (Acquity UPLC BEH C18 1.7 µL, Waters Manchester UK) at 40 °C. 6 µliter of sample was injected at 4°C. The UPLC gradients are given in Table 1.

Table 1

Time (min)	Flow Rate	% A (ammoniumacetaat)	% B (100% methanol)
Initial	0,3	100	0
7,5	0,3	58,3	41,7
9	0,3	33,4	66,6
9,1	0,3	0	100
10	0,3	0	100
10,1	0,3	100	0
12	0,3	100	0

Mass spectrometry was executed on a TQC triple quadrupole mass spectrometer (Aquity-TQD, Waters, Manchester) with a positive electrospray ES⁺ (cone 20-30V, source temperature 120°C, dissolution temperature 450°C, cone gas flow 50l/hour, collision gas flow 0.20 L/min and collision energy 18-25 eV. The data were translated into the corresponding diagnostic transitions for Multiple Reactant Monitoring MRM.

For the statistical analysis a significance level of 5% was used. Because of the low number of replicates non-parametric tests were used.

Results

Quantitative growth of cell suspension cultures

The fresh weight of the cell cultures is given in Figure 1. The growth and development follow a classic sigmoid curve. Starting from a fresh weight of 40 gL^{-1} the weight increases fourfold within ten days, and further increases to fivefold after 12 to 14 days.

Whereas in normal vegetative leaves the ploidy level was always $2n$, in cell suspension cultures various ploidy levels are observed corresponding to $2n$, $4n$ and $8n$ (Figure 1). No breakdown of DNA is observed. The composition of the population did not change significantly over time (results not shown).

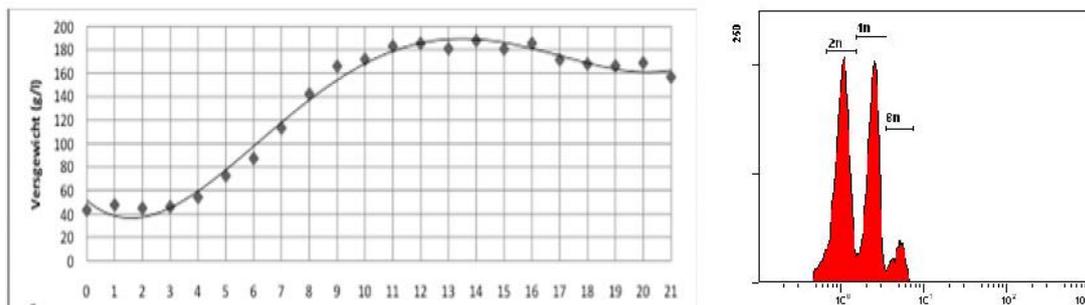


Figure 1: Left: Fresh weight of cell suspension cultures of *Bambusa balcooa* over a period of three weeks. Right: Flow cytometric analysis of the cell suspension culture.

Comparison of SPE methods

The comparison of the extraction efficiency of cytokinins between OASIS and DEAE columns for various cytokinins in cell cultures of *Bambusa balcooa* is displayed in Figure 2. The comparison was performed on both cells and the medium in which they grew. These measurements are the average of 5 replicates \pm SD.

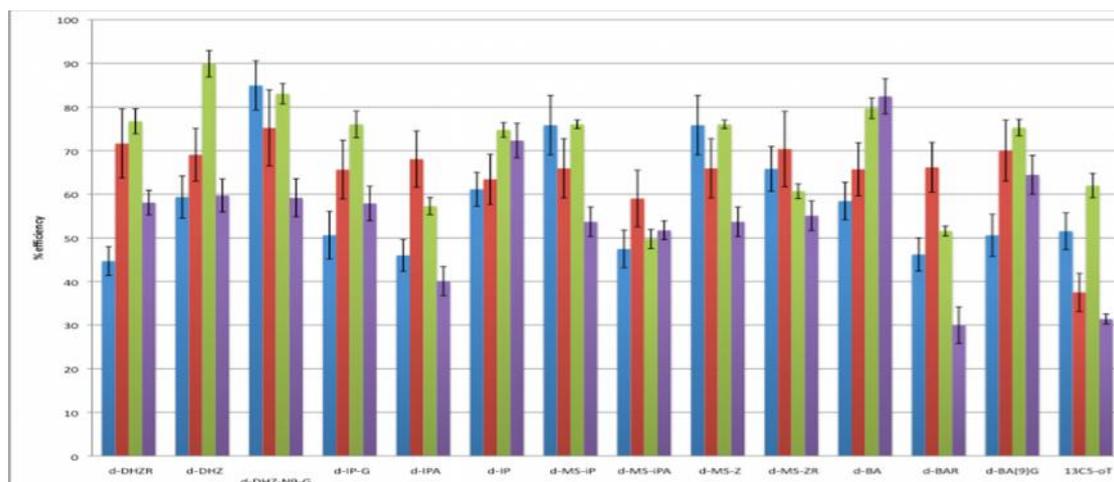


Figure 2: Extraction efficiency using OASIS and DEAE columns in cells and the medium. Blue = cells OASIS; Red = cells DEAE; Green = medium OASIS; Violet = medium DEAE

Cytokinin dynamics in cell suspension cultures

The predominant forms of cytokinins in cell cultures are *trans*-zeatin-*O*-glucoside and *trans*-

zeatinriboside-*O*-glucoside (Figure 3). At day zero the *trans*-Z-*O*-G is very high, stabilizing around 20 pmol/g. Between days 8 and 14-16 the levels of *trans*-Z-*O*-G and *trans*-ZR-*O*-G decrease drastically. From day 9 to 12 a slight increase of *trans*-ZR is observed, but from day 15 onwards up to 20 pmol of Z-N9-G is observed. The predominant forms are glucosides and ribosides. Free bases are hardly present. The cytokinins are exclusively isoprenoid cytokinins. Aromatic cytokinins as BA are hardly above the detection limit.

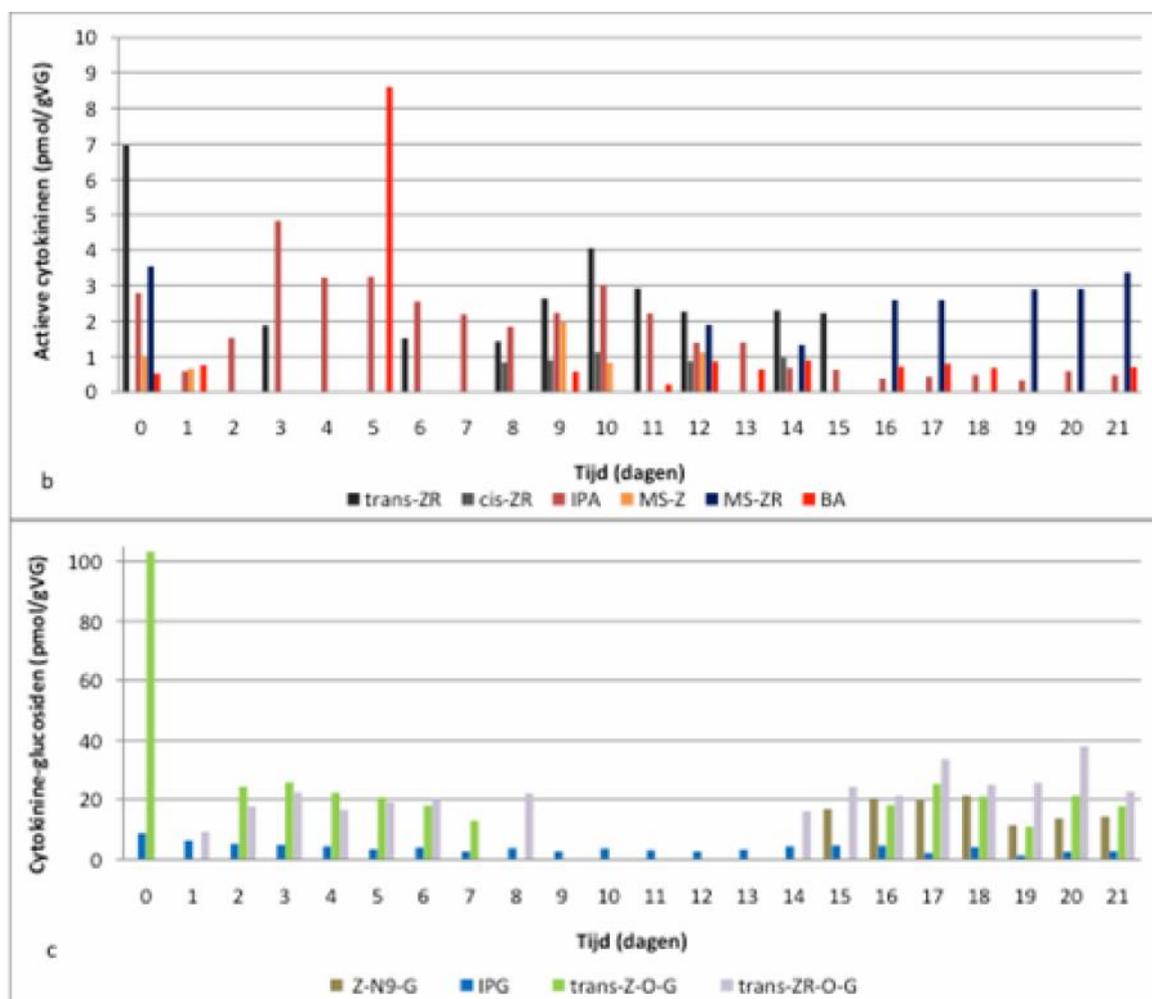


Figure 3: Kinetic profile of the endogenous cytokinins present in a *Bambusa balcooa* cell suspension culture during a 21 days culture period.

Discussion

A plant hormone or phytohormone is an organic substance other than a nutrient active in very minute amounts, which is formed in certain parts of the plant and which may be translocated to other sites, where it evokes specific biochemical, physiological and/or morphological responses. The classic groups of phytohormones comprise cytokinins, auxins, gibberellins, abscisic acid and ethylene (Davies, 2004). More recently, other phytohormones have been discovered including jasmonic acid, brassinosteroids, polyamines and strigolactones. All type of phytohormones are present and active in very small concentrations in plant tissue ranging between 0.01 – 1000 pmol/g fresh weight.

The qualitative and quantitative determination of cytokinins and cytokinin metabolism (and phytohormones in general) has been the focus of intense research efforts in the past two decades. HPLC is still one of the most widely used methods to separate cytokinins (Tarkowski, 2009), based on gradients in polarity (Chen, 1987). The identification and quantitative analysis of cytokinins is

performed with Mass Spectrometry. The preferred method of analysis is the combination of LC with Electrospray Ionisation (Novak et al., 2003). Tandem MS, ESI-MS/MS, is preferred for its increased sensitivity and lower background noise (Prinsen et al., 2005).

In recent years Ultrahigh Performance Liquid Chromatography has come into focus or hormone profiling in plants (Kojima et al., 2009). Compared to HPLC a UPLC column can withstand pressure up to 1000 bar, which ensures a very fast and improved separation of the components under study (Dolezal et al., 2007; von Schwartzberg et al., 2007). Due to the superior separation, in combination with robustness, UPLC-MS/MS is considered as the most accurate and reliable method up to now. In this project we used UPLC coupled with mass spectrometry.

This method can be used to quantify all plant growth hormones, not only cytokinins, and can be developed into high-throughput systems. We stress that the analysis is extremely sensitive and results, even within repetitions may greatly differ and differences are also found between two methods of SPE. Actually various parameters, apart from the different SPE methods, were extensively tested to optimize the method as a whole (results not shown), prior to the analysis of cell suspension cultures. Also prior to the analysis the cell cultures were grown for at least 3 subcultures under exactly the same conditions. The ploidy levels of the cells remained quite constant throughout the subculture with 2n, 4n and 8n peaks.

When the quantitative analysis was performed daily for the cell suspension cultures, a decrease of *trans-Z-O-G* and *trans-ZR-O-G* from around 20pmol/gFW to zero was observed when biomass increase in the cell suspension cultures slowed down. In the final week the original concentrations of these compounds were reached again, with a concomitant increase of *Z-N9-G* to about 20 pmol/gFW in the last week. IPG concentrations remained constant.

While this report is limited to cell suspension cultures were also used to quantify the cytokinin content in micropropagation, in leaves from mature bamboo and other species (monocots, poplar and willows) and to analyze cytokinin biosynthesis and metabolism in feeding experiments. These analytical methods allow for high-throughput measurements of cytokinins, auxins, gibberellins, abscisic acid and much more.

In mature bamboos the main cytokinins are isoprenoid cytokinins and mostly glucosides or ribosides. In *Fargesia rufa* (unpublished results) the glucosides IPG and *Z-O-G* were present at levels above 10 pmol/g FW (up to 60 pmol/gFW for *Z-O-G*). The only aromatic cytokinins observed are the free base BA and its glucoside BA(7)G. In mature *Bambusa balcooa* (unpublished results) the main cytokinins found were *trans-ZR*, *Z-N9-G* (17 pmol/gFW), *Z-N7-G*, *trans-Z-O-G* (266 pmol/gFW) and *trans-ZR-O-G* (26 pmol/gFW) and the methylthio-forms MS-Z and MS-ZR (15pmol/gFW) and IPG. These measurements were based on 3 samples with 5 repetitions each.

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References

- Bhattacharya, S.; Ghosh, J.S.; Sahoo, D.K.; Dey, N.; Pal, A. 2010. Screening of superior fiber-quality-traits among wild accessions of *Bambusa balcooa*: efficient and non-invasive evaluation of fiber developmental stages. *Ann.For.Sci.* 67: 611-620
- Chen, C.; Linkens, H.F.; Jackson, J.F., 1987. High Performancy Liquid Chromatography in Plant Sciences. Springer, Berlin Germany p. 23.
- Das, M.; Pal, A., 2005. In vitro regeneration of *Bambusa balcooa* Roxb.: Factors affecting changes of morphogenetic competence in the axillary buds. *Plant Cell Tiss Organ Cult.* 81: 109-112
- Davies, P.J. (Ed.) 2004 *Plant Hormones: Biosynthesis, signal transduction, Action!* Kluwer Academic Publishers.
- Dolezal, K.; Popa, I.; Hauserová, E.; Spichal, L.; Chakrabarty, K.; Novák, O.; Krystof, V.; Voller, J.; Holub, J.; Strnad, M.; 2007. Preparation, biological activity and endogenous occurrence of N⁶-benzyladenine. *Bioorganic & Medicinal Chemistry* 15, 3737-3747.
- Douglas, C.; Halperin, W.; Gordon, M.; Nester, E., 1985. Specific attachment of *Agrobacterium tumefaciens* to bamboo cells in suspension cultures. *J. Bacteriol.* 161(2):764-766.
- Gielis, J., 1999. Strategic role of biotechnology in germplasm improvement of bamboo. In: Raychaudhuri S.P., Maramorosch K. (Eds.) *Biotechnology and Plant Protection in Forestry Science.* Science Publishers, Inc., Enfield, NH, USA, 13-38.
- Gielis, J.; Valente, P.; Bridts, C.; Verbelen, J-P., 1997. Estimation of DNA content of bamboos using flow cytometry and confocal laser scanning microscopy (CLSM). In: Chapman, G. (Ed.) *The Bamboos.* Linnean Society Symposium Series 19. Academic Press, London, 215-223.
- Gillis, K.; Gielis, J.; Peeters, H.; Dhooche, E.; Oprins, J., 2007. Somatic embryogenesis from mature *Bambusa balcooa* Roxburgh as basis for mass production of elite forestry bamboos. *Plant Cell Tiss Organ Cult* DOI 10.7007/s 11240-007-9236-1.
- Judiewicz, E.; Clark, L.G.; Londono, X.; Stern, M.J., 1999. *American Bamboos.* Smithsonian books, Washington.
- Kojima, M.; Kamada-Nobusada, T.; et al. 2009 Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography-tandem mass spectrometry: an application for hormone profiling in *Oryza sativa*. *Plant & Cell Physiology* 50(7): 1201-1214. et al.
- Mudoi, K.D.; Borthakur, M., 2009. In vitro micropropagation of *Bambusa balcooa* Roxb. through nodal explants from field-grown culms and scope for upscaling. *Current science*, Vol. 96, NO. 7, 10
- Negi, D.; Saxena, S., 2010. Ascertaining clonal fidelity of tissue culture raised plants of *Bambusa balcooa* Roxb. using inter simple sequence repeat markers. *New Forests* 40:1-8.
- Novak, O.; Tarkowski, P.; Tarkowská, D.; Dolezal, K.; Lenobel, R.; Strnad, M., 2003. *Anal. Chim. Acta* 480.
- Ojita, S.; Kikuchi, N.; Nomura, T.; Kato, Y., 2011. A practical protocol for particle bombardment-mediated transformation of *Phyllostachys* bamboo suspension cells. *Plant Biotechnology* 28:43-50.
- Prinsen, E.; Redig, P.; Van Onckelen, H.A.; Van Dongen, W.; Esmans, E.L., 2005. Quantitative analysis of cytokinins by electrospray tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 9(10): 948-953.
- Rai, V.; Ghosh, J.S.; Pal, A.; Dey, N., 2011. Identification of genes involved in bamboo fiber development. *Genedoi:10.1016/j. gene.2011.01.004*
- Sood, P.; Bhattacharya, A.; Sood, A., 2011. Problems and possibilities of monocot transformation. *Biologia Plantarum*, Volume 55, Number 1, pp. 1-15(15)

- Tarkowski, P.; Ge, L.; Wan Hong Yong, J.; Ngim Tan, S., 2009. Analytical methods for cytokinins. Trends in Analytical Chemistry, Vol. 28, No. 3.
- Triplett, J.K.; Weakley A.S.; Clark, L.G., 2006. Hill cane (*Arundinaria appalachiana*), a new species of bamboo (Poaceae: Bambusoideae) from the southern Appalachian Mountains", *Sida* 22 (1): 79 – 95
- Van Hoyweghen, L.; Karalic, I.; Van Calenbergh, S.; Deforce, D.;Heyerick, A. 2010. Antioxidant Flavone Glycosides from the Leaves of *Fargesia robusta*. Journal of Natural Products 73(9): 1573-1577.
- Von Schwartzberg, K.; Núñez, M.F.; Blaschke, H.; Dobrev, P.I.; Novák, O.; Motyka, V.; Strnad, M., 2007. Cytokinins in the Bryophyte *Physcomitrella patens*: Analyses of Activity, Distribution, and Cytokinin Oxidase/Dehydrogenase Overexpression Reveal the Role of Extracellular Cytokinins. Plant Physiology 145, 786.
- Widmer, Y., 1997 Life history of some *Chusquea* sp. In old-growth oak forests in Costa Rica. In: Chapman, G. (Ed.) The Bamboos. Linnean Society Symposium Series 19. Academic Press, London, 17-31.
- Wong, K.M., 1987. The growth architecture and ecology of some tropical bamboos. J. American Bamboo Society Vol 8 N° 1&2: 43-58.

Somatic embryogenesis of *Drepanostachyum falcatum* an important hill bamboo- a rapid means of micropropagation

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Abstract

Drepanostachyum falcatum is an evergreen bamboo with more than 100 culms in a clump. It grows at altitudes upto 2100m in high slopes, high terraces in moist, sheltered shady conditions and thus popularly called as hill bamboo in India. It is put to diverse uses by the locals, flowers gregariously with seedling cycle of 28-30 yrs. Due to its vast usage it is cut indiscriminately pushing to it as endangered species. Micropropagation protocol was attempted for its rapid multiplication so as to meet the increased demand. Somatic embryogenesis was induced and plantlets were developed. *In vitro* multiplied shoots (raised from axillary bud culture) were source material for explant. Callus was developed from nodular region and from basal portion of the *in vitro* leaves. 2, 4-D was found to be best at 20 μ M for induction of callus in 85% of nodal segments and in 70% from leaf bases. The callus so developed when transferred on 10 μ M 2, 4-D + BAP (0.66 μ M – 1.10 μ M) medium produced embryogenic callus. Different types of developmental stages of somatic embryos were observed globular, white cup shaped (scutellar), and yellow tubular (coleoptillar) embryo in the embryogenic callus cultures. Globular stage was prominent on multiplication medium (10 μ M 2, 4-D + 0.88 μ M BAP), whereas scutellar and coleoptillar stages were prominent on maturation medium (MS + 3.5 μ M ABA / MS +4-6% sucrose). Coleoptillar stage somatic embryos developed shoots and roots when these were transferred to MS medium supplemented with BAP (5 μ M - 15 μ M). Maximum germination frequency of 65% was observed on MS + 5 μ M BAP under 16/8 hrs (light/dark) photoperiod and 25 \pm 2 $^{\circ}$ C temperature. Remaining embryos formed only microshoots which were *in vitro* multiplied and rooted to produce plant. The germinated embryos were placed in polybags containing soil, sand and FYM in 1:1:1 proportion and hardened and acclimatized in mist chamber and agronet shade house before field plantation.

Keywords

Micropropagation, Somatic embryogenesis, *Drepanostachyum falcatum*, Bamboo, Tissue culture

Introduction

Bamboo, the world's fastest growing and environment friendly giant grass, has now gained international recognition and priority, leading to its recognition as an important non-timber woody resource. World-wide interest in bamboo as a source of biomass in sustainable agriculture and agroforestry system has increased rapidly in recent years. An economically important bamboo *Drepanostachyum falcatum* also known as hill bamboo in India, was taken with the aim of developing their propagation technology through tissue culture technique for rapid and mass scale propagation. It is found in hills of North- Western India & Nepal. This species was also introduced in Europe, South and North America, Laos and Entebbe Botanic Garden in Uganda. It belongs to the irregularly flowering group with the flowering and seedling cycle of 28-30 years. Also this bamboo is a slow grower that can go shabby in winter, losing leaves, but revive in spring with masses of new foliage again, before it shoots. This popular bamboo is a very cold hardy plant marketed as "blue bamboo". The importance of this species is highlighted by the diverse uses it is put to by the locals. It forms the basic raw material for their huts, tools, utensils and other items (Mc Clure, 1956). Leaves are used for thatching, roofing, animal fodder in winter and young shoots are edible. Due to its immense use there is much demand of this bamboo which is now far exceeding then its natural regeneration potential. As natural strands are cut before it can flower and flowering cycle is also long (20-30 years) which makes unavailability of seeds for its natural regeneration. The species is going to be endangered (personal observations for past eight years) if no efforts are made for its plantations. The conventional methods of propagation of *Drepanostachyum falcatum*, sexual as well as vegetative are beset with many problems that restrict their multiplication on a large scale so as to meet the market demand. Efficient *in vitro* propagation could be a reliable and useful method for establishment of this bamboo as they offer an attractive alternative to conventional methods for mass propagation of bamboo species (Kondas, 1982; Rao and Rao, 1988; Arya and Arya, 1997). By successful micropropagation, bamboos could be multiplied according to commercial need.

Somatic embryogenesis is a preferred method of plant propagation *in vitro*. The high volume multiplication of embryogenic propagules is the most commercially attractive application of *in vitro* somatic embryogenesis. As commercially conceived, the system involves harvesting maturing embryos from a continuously proliferating embryogenic culture of elite genotype, and converting the harvested cloned embryos to seedling transplants or synthetic seeds for delivery to the grower. Majority of reports in bamboos on somatic embryogenesis are from explants obtained from reproductive tissue like zygotic embryos, seeds, inflorescence tissues, immature embryos, anthers, etc and only 2-3 reports describe somatic embryogenesis from juvenile vegetative parts like nodes, leaves, root, rhizome and internodes. As flowering in bamboo is unpredictable due to its long intermast period thus, somatic embryogenesis dependent on reproductive tissue (seed) explants will be dependent on time and cannot be utilized at the time of need. Convenience of utilizing vegetative tissues thus can be relied on towards availability of explants throughout the year for induction and establishment of the somatic embryogenesis protocol. Somatic embryogenesis also resets the zero stage in bamboos thus plants produced can be relied on to live their full life span. Keeping all this in view, protocol for somatic embryogenesis in *Drepanostachyum falcatum* using vegetative explants (nodal and leaf) was developed.

Materials and methods

Induction of callus

Source of explant

In vitro multiplied shoots were developed from axillary bud used as explant material for initiation of somatic embryogenesis. For this, nodal segments measuring 2-4 cm in length and 0.4-0.8 cm in diameter were selected from mature clump of bamboo *Drepanostachyum falcatum*. After removal of leaf sheath and surface cleaning of explants with 70% alcohol, these were sterilized with 0.1 % HgCl₂ for 10-12 min and washed thoroughly with sterilized distilled water. The explants were then cultured on MS (Murashige and Skoog, 1962) medium supplemented with 5.0 mg/l BAP. Axillary shoots proliferated were excised and multiplied on MS medium supplemented with 3.0 mg/l BAP. These *in vitro* raised shoots were used for initiation of embryogenic callus. Nodal segments and leaves were dissected from these *in vitro* shoots and were inoculated on MS and B₅ (Gamborg *et al.*, 1968) medium supplemented with 2,4-D. Explants were inoculated in horizontal position to maintain continuous contact with medium. Callus was induced within 4 weeks under dark culture conditions.

Effect of phytohormones

In order to assess the effect of auxin on induction of callus, both the explants nodal segments and *in vitro* leaves were cultured on medium supplemented with different concentration of 2, 4-D (10-50µM).

Effect of basal media and callus induction frequency

Induction frequency of callus was studied on different basal media. MS medium and B₅ medium were used for initiation of callus. Media were solidified with 0.8% agar (Hi-media laboratories, India) and supplemented with 2% (w/v) sucrose. The pH was adjusted to 5.8 prior to autoclaving. Callus induction frequency was recorded after 4 weeks of incubation under dark conditions. To assess the culture conditions required for induction of embryogenic callus, explants were incubated both in complete dark and light conditions (16 hrs) at a constant temperature of 25±2⁰C. After 4 weeks of incubation callus induction frequency was recorded and amount of callus produced was noted down as fresh weight of callus. Data were collected from five replicates with four propagules in each replicate. Variance was calculated as standard error by applying CRD design of experiments using statistical packages viz. SPSS ver. 10.0 and Excel ver. 2.0 and valid inferences were drawn from it. Induction frequency was calculated as:

$$\text{induction frequency} = \frac{\text{number of explants in which callus was induced}}{\text{total number of explants cultured}}$$

After 12 weeks of incubation on induction medium calli were transferred to multiplication medium. Cultures were scored at 30 days at the end of experiment in terms of induction frequency and fresh weight of callus.

Multiplication of embryogenic callus

12 weeks old compact embryogenic calli with proembryos were transferred and cultured on maintenance medium. In the multiplication experiments, an initial amount of 50mg embryogenic callus was cultured on various media and the final fresh weight was recorded after 4 weeks. Different sets of experiments were performed to study the multiplication of embryogenic callus. Non-embryogenic calli were scored out. Callus showing proembryos were counted. To study the effect of

auxin alone and in combination with cytokinin on proliferation of embryogenic callus, 50 mg callus was cultured on medium supplemented with various combinations of phytohormones. Callus was maintained initially on same medium for their induction. For improvement in proliferation rate of embryogenic callus, it was transferred to other hormonal combinations. 2, 4-D was used (0-50 μM) alone and in combination with BAP. IAA was also used with 2, 4-D and BAP in different concentrations. Cultures were scored for embryogenic frequency, multiplication rate and for embryogenic efficiency of callus after 4 weeks of incubation in dark.

Effect of basal medium strengths on multiplication of callus

In order to assess the effect of media strengths on callus proliferation, MS and B₅ supplemented with 10 μM 2, 4-D + 0.88 μM BAP were tested in their salt strengths of 1/4x, 1/2x, 1x and 2x. Four pieces of callus per flask (50 mg each) were cultured in each replicate. Callus was incubated in dark conditions. Observations were recorded after 4 weeks of culturing by weighing fresh weight of callus and counting number of embryos per piece of callus.

Effect of sucrose concentration

Effect of sucrose concentration was studied on multiplication rate of callus and embryogenic efficiency. To assess the effect of sucrose concentration, MS medium supplemented 10 μM 2, 4-D + 0.88 μM BAP with varied concentration of sucrose (1-6%) was used. Number of embryos produced per callus was recorded. Embryos were observed under stereozoom microscope (Leica) in all cases.

Effect of subculture duration

Effect of subculture duration was studied to assess its effect on multiplication rate and embryogenic efficiency of callus. Callus was subcultured for varied durations of 2, 4, 6 and 8 weeks on MS medium supplemented with 10 μM 2, 4-D + 0.88 μM BAP and was incubated in dark conditions. Fresh weight of callus was recorded after each subculture duration. Compact nodular callus that developed with globular embryos was multiplied under different sets of experiments. 50 mg of callus was cultured on the medium and after 4 weeks final fresh weight of callus was recorded. Under stereozoom microscope different developmental stages of all embryos were identified and counted. The mean number of total embryos per embryogenic callus were recorded. Data were collected from five replicates with four propagules (callus pieces per flask) in each replicate. Variance was calculated as standard error by applying CRD design of experiments using statistical packages viz. SPSS ver. 10.0 and Excel ver. 2.0. Each experiment was repeated thrice. Cultures were scored at 30 days (4 weeks). Four parameters were used to assess embryogenic response as reported by Lazzeri *et al.* (1987).

Mean embryo number = Mean number of somatic embryos per embryogenic callus (FCW).

Embryogenic efficiency = Embryogenesis frequency x Mean embryo number

Maturation of embryogenic callus

Embryogenic calli that developed on multiplication medium showed compact nodular callus with globular embryos. Formation of second stage scutellar embryos in the callus was sporadic on multiplication medium. Exposure to ABA and sucrose is essential for further development of embryos. ABA was supplemented in MS medium from 0.5-20 μM to assess its effect on maturation of globular stage embryo to scutellar and coleoptillar stage embryo. After 2 subcultures, cultures were

scored for its developmental stages. Sucrose was also adjuvated in MS medium at concentration from 2-10% to assess its effect on maturation of somatic embryos. Matured embryos of scutellar and coleoptillar stages were scored under stereozoom microscope and efficiency of embryogenesis was calculated.

Embryogenic efficiency of total embryos = Embryogenesis frequency x Mean embryo number (all stages).

Embryogenic efficiency of matured embryos = Embryogenesis frequency x Mean embryo number (Scutellar and Coleoptillar stages only).

Germination

For germination coleoptillar embryos were cultured on MS medium. For this hormones like BAP, GA₃ (0-15 μM) were tried in MS medium. Germinated embryos were scored when mature coleoptillar stage embryos germinated on germination medium producing shoot and root.

$$\text{germination frequency} = \frac{\text{number of embryos that germinated}}{\text{total number of embryos cultured}}$$

Majority of somatic embryos did not germinate into shoot and root when transferred on germination medium. They only produced shoots. These shoots were *in vitro* multiplied in large number and later *in vitro* rooting was induced in them.

In vitro multiplication of shoots

Embryos that formed only shoots and failed to produce roots were multiplied. Shoots of size up to 2-3 cm were multiplied on MS medium supplemented with BAP and multiplication rate was calculated. After first shoot multiplication the shoot cultures were excised into a group of 3-4 shoot clusters called as a propagule (subculture units) and later they were regularly subcultured and maintained on fresh medium supplemented with cytokinin (BAP) at an interval of 3-4 weeks. The number of propagule cultured and number of propagule derived at the end of subculture is regarded as the rate of multiplication. Shoot multiplication rate was calculated after 4 weeks of culturing. Observations were recorded as average shoots number, shoot length and shoot quality.

4 propagules (3 shoots) were cultured in each replicate. Data were collected from minimum of 5 replicates per treatment. Each experiment was repeated twice. Variance was calculated as standard error by applying CRD design of experiments using statistical packages viz. SPSS ver. 10.0 and Excel ver. 2.0 and valid inferences were drawn from it. Multiplication rate was calculated as:

$$\text{multiplication rate} = \frac{\text{average number of shoots obtained}}{\text{average number of shoots cultured}}$$

In vitro rooting

Experiments on *in vitro* rooting of shoots were attempted with 3-4 cm long shoots produced during shoot multiplication experiments. Propagules of four shoots were cultured for initiation of roots and observations were recorded after 4 weeks interval. For *in vitro* rooting, MS medium was supplemented with auxins i.e. IBA, NAA in the range of 1.0-11.0 mg/l. After 4-6 weeks, data were collected on the percentage of rooted propagule, the number of roots per propagule and the duration of root initiation along with root length. Analysis of variance (ANOVA) was calculated as standard error by applying CRD design of experiments using statistical packages viz. SPSS ver. 10.0 and Excel ver. 2.0 and valid inferences were drawn from it.

Hardening and acclimatization

Plantlets obtained from direct germination of embryo

Individual plantlets of 4-5 cm in size along with clusters of 2-4 plantlets were transplanted for hardening and acclimatization. These plantlets were transferred for hardening to polybags containing soil: sand: FYM. The plants were reared inside mist house conditions of $30\pm 2^{\circ}\text{C}$ temperature and 80-90% RH for 7-15 days. These were regularly fed twice a week with half strength MS salt solution without organics. After 15 days of hardening these plants were shifted to agro net shade house to protect plants from strong sunlight. These plants were acclimatized under shade house for 1-2 month.

Plantlets obtained through rooting of *in vitro* shoots produced by the somatic embryos

In vitro raised plantlets need to be hardened and acclimatized before their field transplantation as tissue culture raised plants are heterotrophic in their mode of nutrition and cannot withstand environmental conditions without proper hardening and acclimatization. Therefore, to assess the effect of hardening and acclimatization on tissue culture raised plantlets following methodology was adopted. Rooted shoots from four-week-old cultures were transferred to soil under shade house either directly (in rainy season) or after hardening. For hardening the plantlets were taken out from the flasks, washed to remove adhered agar and then transferred to autoclaved 250 ml screw cap glass bottle containing 1/3 volume of vermiculite. These plantlets were nurtured with half strength MS medium (without organics) twice a week for two weeks and were kept in tissue culture room. After two weeks these bottles were shifted to mist chamber having relative humidity of 80-90% with a temperature of $30\pm 2^{\circ}\text{C}$. The caps of bottles were removed and plantlets were allowed to remain in the bottle for 3-4 days before they were transferred into polybags containing a mixture of sand, farmyard manure and soil in a ratio of 1:1:1. In mist chamber, the plants were kept for three weeks and were irrigated with half strength MS medium. After mist chamber hardening, these polybags were shifted to high-density double deck agronet open shade house for acclimatization. After one month in shade house the plants were transferred to bigger polybags/pots containing same soil composition. During this acclimatization the plants were irrigated with tap water. Generally, plants are kept in shade house for two months.

Field transfer

Hardened and acclimatized plants were directly planted in the field during monsoon and after monsoon season (July to December). Simple silvicultural practices were followed during plantation with 6m x 6m spacing. The field-transferred plants were regularly irrigated with tap water after every 15 days for first 3 months and were also supplied with manure

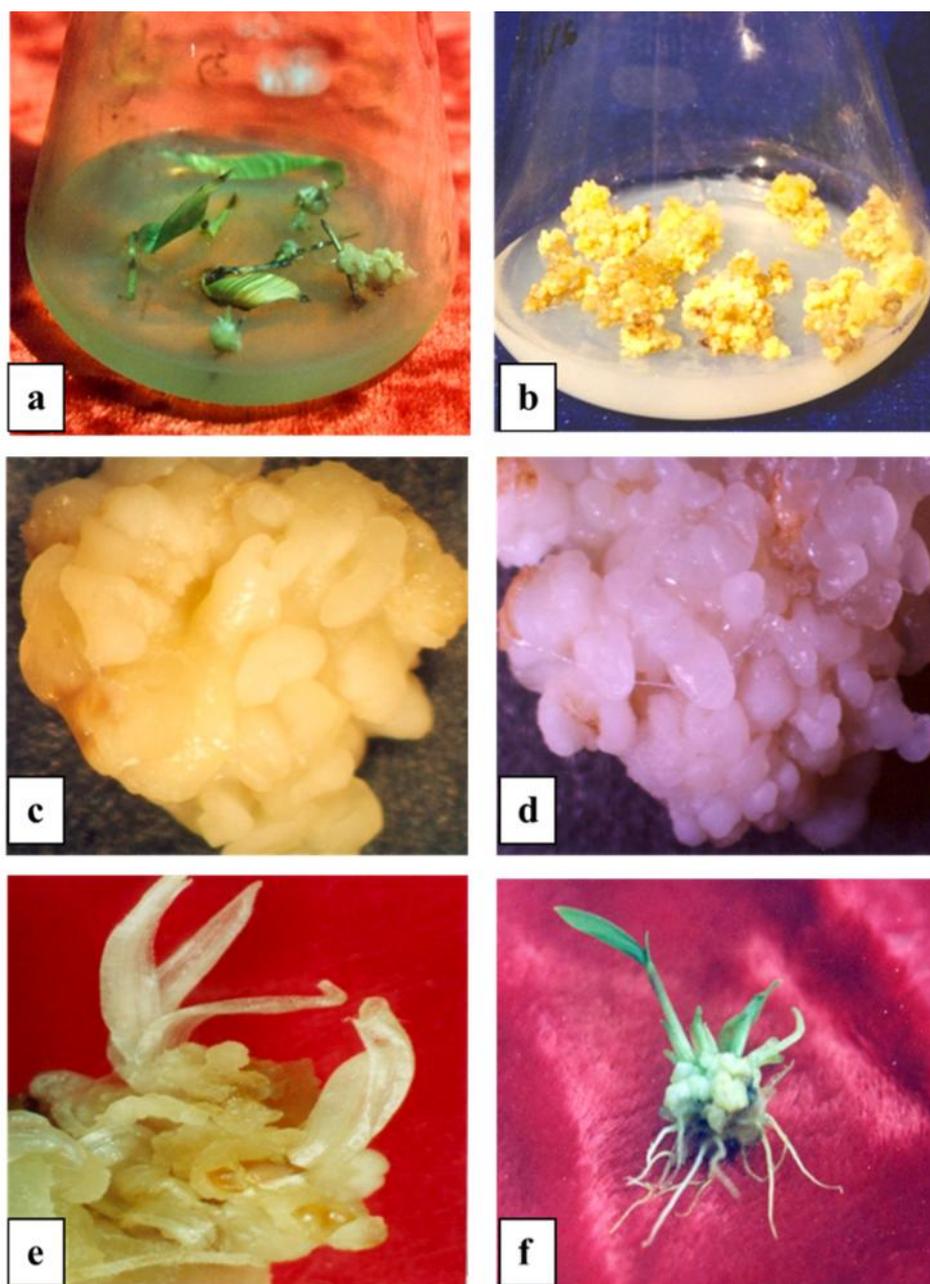


Figure 1. Somatic embryogenesis in *Drepanostachym falcatum*. a) Callus induced from nodal segments and leaves bases on MS+20 μ M 2,4-D. b) Embryogenic callus formation after 6-8 weeks on MS+20 μ M 2,4-D. c) Development of globular embryoids in embryogenic callus after 8-10 weeks of culture. d) Proliferating embryogenic callus showing organization of globular, scutellar embryoids after 12 weeks of culture. e) Coleoptillar stage somatic embryo. f) Coleoptillar embryos in embryogenic callus forming shoots and roots on germination medium in light conditions.

Results

Induction of callus

Effect of phytohormones

Nodal segments and leaves excised from *in vitro* multiplied shoots produced callus in 2 weeks of culture, but a reasonable amount of callus developed only after 4-6 weeks of incubation on MS medium supplemented with 10-50 μM 2, 4-D (Table 1). Callus originated from nodular region of nodal segments and from basal portion of leaves. (Fig.1a). Of the various concentrations of 2, 4-D tried, 20 μM was found to be the best for induction that had resulted in 85% callus development in nodal segments and 70% callus was induced from the leaf bases. The callus size varied from 2-4 mm in diameter weighing from 9-30 mg in fresh weight within 4-5 weeks. At increased 2, 4-D concentration (50 μM) induction frequency decreases and browning of callus was noticed. On hormone free medium, no callus was induced. Morphogenic differentiation of callus started after 8 weeks of culture in dark conditions (Fig. 1b). Proembryos were developed after 10-12 weeks of culture. After 10-12 weeks of culture of callus on 20 μM 2, 4-D supplemented MS medium three types of calli were observed as 1) Yellow friable callus that perpetually remained non-embryogenic 2) White compact nodular embryogenic callus 3) Translucent mucilaginous callus which is partly embryogenic. During each subculture the friable non-embryogenic callus was removed which grows faster than the embryogenic callus.

Effect of media and light conditions

MS medium was found to be suitable for callus induction as compared to B₅ medium where callus formation occurred quickly when supplemented with 20 μM 2, 4-D. Fresh weight of callus developed was nearly double (27 mg) on MS medium as compared to B₅ medium (14 mg). For callus induction and proliferation, darkness is required as in light conditions very less callus was formed. Callus weight increases to 2-3 times in dark conditions as compared to light conditions. Also, in light the callus turns green without multiplication and further development.

Multiplication of embryogenic callus

Effect of phytohormones

Variation in developmental potential of somatic embryos was observed among the callus multiplied on MS medium supplemented with 0-40 μM 2, 4-D. Prolonged subculturing on MS medium supplemented with 2, 4-D alone (0 μM -40 μM) did not improve the production of somatic embryos. Addition of BAP was found to enhance the rate of callus multiplication and formation of somatic embryos. It was observed that the cultures, which were established on 20 μM 2, 4-D became highly embryogenic when transferred on MS medium supplemented with reduced level of 2, 4-D i.e. 10 μM 2, 4-D along with BAP (0.66 μM -1.10 μM). On this medium large number of well-developed globular embryos with 18.6 (embryos per culture) efficiency and 2.8 folds callus proliferation rate was observed (Table 2). Therefore, after 12 weeks of culture on induction medium callus with proembryo initials were transferred to multiplication medium (MS + 10 μM 2, 4-D + 0.88 μM BAP) which resulted in differentiation to globular stage of embryos. These well-developed globular somatic embryos were formed after 2-3 subcultures on multiplication medium in dark. Thus, best multiplication of callus and somatic embryos was obtained on MS + 10 μM 2, 4-D + 0.88 μM BAP medium. Therefore, all cultures were maintained on this multiplication medium and regular subculturing was carried out every 4 weeks to multiply the embryos and to maintain the embryogenic potential of callus. On this medium callus retains its potential for more than 24 months with same

efficiency. Addition of IAA to this multiplication medium did not improve the multiplication frequency of embryos .

Microscopic studies revealed the presence of proembryos after 10-12 weeks on induction medium that developed to globular stage somatic embryos after 2-3 subcultures on multiplication medium in dark conditions (Fig. 1c). Different developmental stages of somatic embryos were present in established embryogenic callus as observed under stereozoom microscope (Fig.1d). Three types of embryos were observed i.e. white round glossy smooth bodies (globular), white cup shaped (scutellar) and yellow tubular (coleoptillar) somatic embryos. Globular stage was prominent on multiplication medium whereas scutellar and coleoptillar stages were prominent on maturation medium. Also fused somatic embryos were seen along with development of secondary embryos in fast growing embryogenic callus.

Effect of basal media

Concentration of salts present in medium also plays critical role in determining the callus morphology and callus proliferation. MS medium was found to be the best multiplication medium (Table 3). MS medium in its full strength supported embryogenesis as it showed efficiency value of 18.4 embryos per culture. B₅ medium was found to be inferior in all strengths used as compared to MS medium. Multiplication rate on MS medium was 2-3 folds more than on B₅ medium. On increasing the salt concentrations of MS medium non-embryogenic mass increased along with decreased efficiency value of somatic embryogenesis.

Multiplication and subculture cycle

Different concentration of sucrose 1-6% in MS medium was tried for multiplication of embryogenic callus. Optimal sucrose concentration for callus proliferation and somatic embryogenesis ranged from 1-2%. Sucrose at 2% showed best response in terms of embryogenic frequency (75%), multiplication rate (3 folds) and optimal efficiency value of 19.8 embryos per culture (Table 4). At this concentration large number of globular stage embryos were developed. At 1% sucrose in MS medium a slight reduced mean number of embryos and efficiency was obtained. At higher concentration of sucrose (4-6%) mean number of embryos and embryogenic efficiency decreased. Subculture interval of 4 weeks was most efficient in maintaining embryogenic callus cultures with embryogenic efficiency of 20 embryos per culture (Table 5). Subculture interval of more than 4 weeks resulted in the formation of non-embryogenic callus with a general decrease in growth rate. Delayed embryogenic calli subcultured on fresh medium showed development of secondary embryogenesis in 10-11 weeks. Secondary embryos were formed directly on the primary scutellar stage somatic embryos resulting into chains of globular somatic embryos. Embryogenic callus proliferated faster in dark conditions than the light conditions as reflected from increased multiplication folds (2-3 folds) in dark conditions. Multiplication in light conditions resulted in development of chlorophyll with retarded growth in callus formation.

At the end of each multiplication cycle, embryos and callus with embryos at different stages of development were selected and subcultured on medium for maturation. For optimal utilization of all globular embryos to attain maturity to scutellar embryos and to coleoptillar staged embryos, a maturation step was needed, which later formed shoots and roots and subsequently plantlet development. Maturation of embryos is carried out either on ABA or high sucrose medium.

Table 1: Effect of hormone (2, 4-D) on induction of callus from leaf base and nodal segments on MS medium. Data recorded after 4 weeks.

Hormonal concentration 2,4-D (μ M)	Response (%) (Leaf base)	Response (%) (Nodal segments)	Fresh Callus wt. (gm) (Nodal segments)	Fresh Callus wt. (gm) (Leaf base)
Control	-	-	-	-
10	60.00 \pm 0.52	65.00 \pm 0.23	0.0258 \pm 0.0013	0.0156 \pm 0.0014
20	70.00 \pm 0.58	85.00 \pm 0.29	0.0302 \pm 0.0009	0.0196 \pm 0.0007
30	65.00 \pm 0.29	65.00 \pm 0.29	0.0270 \pm 0.0008	0.0186 \pm 0.0010
40	49.90 \pm 0.19	55.00 \pm 0.17	0.0156 \pm 0.0009	0.0178 \pm 0.0009
50	40.00 \pm 0.23	45.00 \pm 0.17	0.0090 \pm 0.0007	0.0120 \pm 0.0008
Significance	***	***	***	***
CD at 5%	1.24	0.74	0.003	0.004

***-Significance at 0.1%

\pm Values represent the Standard Error

Table 2: Effect of phytohormonal combination (2, 4-D + BAP) on multiplication of embryogenic callus on MS medium. 50 mg of fresh wt. of callus was inoculated initially. Data recorded after 4 weeks.

Medium with phytohormones 2,4-D (μ M)	BAP (μ M)	Embryogenic response (%)	Fresh callus wt. (gm)	Callus multiplication rate (folds)	Mean no. of embryos	Embryogenic efficiency
10	0.66	77.00 \pm 0.58	0.037 \pm 0.003	2.616 \pm 0.036	10.8 \pm 0.583	8.316 \pm 0.449
10	0.88	90.00 \pm 0.58	0.142 \pm 0.002	2.832 \pm 0.033	18.6 \pm 0.678	16.740 \pm 0.610
10	1.10	75.03 \pm 0.32	0.098 \pm 0.002	1.964 \pm 0.033	12.0 \pm 0.548	9.000 \pm 0.411
20	0.66	73.00 \pm 0.29	0.121 \pm 0.001	2.412 \pm 0.028	11.0 \pm 0.707	8.030 \pm 0.516
20	0.88	71.00 \pm 0.29	0.103 \pm 0.002	2.056 \pm 0.047	14.8 \pm 1.625	10.508 \pm 1.154
20	1.10	68.00 \pm 0.58	0.093 \pm 0.003	1.852 \pm 0.064	10.2 \pm 0.663	6.936 \pm 0.450
30	0.66	70.00 \pm 0.29	0.103 \pm 0.003	2.068 \pm 0.063	9.0 \pm 0.707	6.300 \pm 0.495
30	0.88	67.00 \pm 0.29	0.103 \pm 0.002	2.056 \pm 0.047	8.2 \pm 0.663	5.494 \pm 0.444
30	1.10	56.00 \pm 0.58	0.077 \pm 0.002	1.540 \pm 0.040	4.6 \pm 1.030	2.576 \pm 0.577
Significance		***	***	***	***	***
CD at 5%		1.33	0.023	0.096	3.18	2.24

***-Significance at 0.1%

\pm Values represent the Standard Error

Table 3: The optimal media and hormonal combination for development of embryogenic callus. 50 mg of fresh wt. of callus was inoculated initially. Data recorded after 4 weeks.

Types of Media	Embryogenic response (%)	Fresh callus wt. (gm)	Callus multiplication rate (folds)	Mean no. of embryos	Embryogenic efficiency
B ₅ + 2,4-D (10 µM) + BAP (0.88 µM)	45.00 ± 0.58	0.116 ± 0.008	2.32 ± 0.162	7.2 ± 0.583	3.24 ± 0.262
MS + 2,4-D (10 µM) + BAP (0.88 µM)	90.00 ± 0.58	0.140 ± 0.012	2.80 ± 0.237	18.4 ± 0.510	16.56 ± 0.459
Significance	***	NS	NS	***	***
CD at 5%	2.27			1.78	1.15

NS – Non – Significant ***-Significance at 0.1%

± Values represent the Standard Error

Table 4: Effect of sucrose concentration on multiplication of embryogenic callus on MS + 2, 4-D (10µM) + BAP (0.88µM). 50 mg of fresh wt. of callus was inoculated initially. Data recorded after 4 weeks.

Sucrose conc. (%)	Embryogenic response (%)	Fresh callus wt. (gm)	Callus multiplication rate (folds)	Mean no. of embryos	Embryogenic efficiency
Control	50.0 ± 0.61	0.083 ± 0.024	2.12 ± 0.21	9.2 ± 0.66	4.6 ± 0.33
1	70.0 ± 0.58	0.132 ± 0.006	2.60 ± 0.04	16.2 ± 1.07	11.34 ± 0.75
2	75.0 ± 0.63	0.152 ± 0.016	3.04 ± 0.31	19.8 ± 0.58	14.85 ± 0.48
3	65.0 ± 0.66	0.080 ± 0.012	1.60 ± 0.16	11.2 ± 0.80	7.28 ± 0.52
4	60.0 ± 0.68	0.116 ± 0.010	2.40 ± 0.10	8.2 ± 0.66	4.92 ± 0.39
5	35.0 ± 0.61	0.120 ± 0.015	2.40 ± 0.26	5.8 ± 0.58	2.03 ± 0.20
6	30.0 ± 0.58	0.064 ± 0.010	1.04 ± 0.07	5.0 ± 0.95	1.50 ± 0.28
Significance	***	***	***	***	***
CD	1.78	0.032	0.51	2.26	1.30

NS – Non – Significant *- Significance at 5%

** - Significance at 1% ***-Significance at 0.1%

± Values represent the Standard deviation

Table 5: Effect of subculture duration on multiplication of embryogenic callus on MS + 2, 4-D (10 μ M) + BAP (0.88 μ M). 50 mg of fresh wt. of callus was inoculated initially.

Subculture interval	Embryogenic response (%)	Fresh callus wt. (gm)	Callus multiplication rate (folds)	Dry wt. of callus	Mean no. of embryos	Embryogenic efficiency
2 weeks	65 \pm 0.606	0.132 \pm 0.006	2.64 \pm 0.120	0.020 \pm 0.003	12.4 \pm 0.966	8.06 \pm 0.628
4 weeks	90 \pm 0.592	0.190 \pm 0.006	3.80 \pm 0.125	0.048 \pm 0.003	20 \pm 1.155	18 \pm 1.039
6 weeks	60 \pm 0.577	0.238 \pm 0.011	4.85 \pm 0.211	0.036 \pm 0.004	16.2 \pm 1.033	9.72 \pm 0.620
8 weeks	45 \pm 0.658	0.272 \pm 0.010	5.44 \pm 0.195	0.035 \pm 0.004	13.2 \pm 1.111	5.94 \pm 0.500
Significance	***	***	***	***	***	***
CD	1.33	0.020	0.39	0.0084	2.48	1.69

NS – Non – Significant

*- Significance at 5%

**- Significance at 1%

***-Significance at 0.1%

 \pm Values represent the Standard deviation**Table 6: Effect of ABA in MS medium on maturation of embryos. 50 mg of fresh wt. of callus was inoculated initially. Data recorded after 4 weeks.**

ABA (μ M)	Responding calli (%)	Efficiency of total no. of embryos	Efficiency of formation of scutellar/coleoptillar embryos	Stages of embryos in callus
0.5	65.0 \pm 0.58	31.2 \pm 0.48	5.2 \pm 0.63	Globular mostly
1.5	90.0 \pm 0.58	24.4 \pm 1.56	8.2 \pm 0.48	Globular/Scutellar
2.5	90.0 \pm 0.29	22.2 \pm 0.86	11.0 \pm 0.91	Globular/Scutellar
3.5	99.7 \pm 0.33	30.0 \pm 0.71	20.2 \pm 1.11	Scutellar/Coleoptillar
4.5	99.8 \pm 0.15	31.6 \pm 1.13	19.4 \pm 1.13	Scutellar/Coleoptillar
5.5	95.0 \pm 0.58	28.2 \pm 1.38	15.2 \pm 1.32	Globular/Scutellar/Coleop.
6.5	75.0 \pm 0.58	06.8 \pm 1.11	10.2 \pm 0.86	G/S/C Fused
7.5	70.0 \pm 0.58	10.0 \pm 0.91	6.4 \pm 0.88	G/S/C Fused
Significance	***	***	***	
CD at 5%	1.46	2.39	2.12	

Table 7: Effect of sucrose in MS medium on maturation of embryos. 50 mg of fresh wt. of callus was inoculated initially. Data recorded after 4 weeks.

Sucrose (%)	Responding calli (%)	Efficiency of total no. of embryos	Efficiency of formation of scutellar/coleoptillar embryos	Stages of embryos in callus
2%	75 ± 0.577	20.2 ± 0.753	3.2 ± 0.483	Globular/Scutellar
4%	70 ± 0.289	10.4 ± 0.966	4.0 ± 0.913	Globular/Scutellar
6%	70 ± 0.577	16.4 ± 0.876	11.4 ± 1.329	Scutellar/Coleoptillar
8%	45 ± 0.289	05.4 ± 0.658	3.0 ± 0.577	G/S/C Fused
10%	30 ± 0.577	05.2 ± 0.753	1.6 ± 0.516	G/S/C Fused
Significance	***	***	***	
CD at 5%	1.52	1.77	1.82	

***-Significance at 0.1%

± Values represent the Standard Error

Table 8: Effect of phytohormones in MS medium on germination of coleoptillar embryos. Data recorded after 4 weeks.

Hormonal concentration (µM)		Germination response (%)
BAP	GA ₃	
0.0	0.0	50.0 ± 0.289
5.0	0.0	65.0 ± 0.289
10.0	0.0	50.0 ± 0.289
15.0	0.0	45.0 ± 0.289
5.0	2.5	59.0 ± 0.289
10.0	2.5	40.0 ± 0.577
15.0	2.5	45.0 ± 0.577
5.0	5.0	53.0 ± 0.577
10.0	5.0	45.0 ± 0.289
15.0	5.0	30.0 ± 0.289
Significance		***
CD at 5%		1.18

Table 9: Effect of cytokinin (BAP) in MS medium on multiplication of shoots derived from somatic embryos. Data recorded after 4 weeks.

BAP (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
0.5	10.08 ± 0.833	0.89 ± 0.023	2.52 ± 0.06
1.5	22.41 ± 1.403	1.62 ± 0.0023	5.62 ± 0.13
2.5	37.08 ± 0.796	2.42 ± 0.013	9.27 ± 0.13
3.5	35.00 ± 0.778	2.26 ± 0.017	8.75 ± 0.16
4.5	32.16 ± 0.980	2.47 ± 0.018	8.04 ± 0.09
5.5	30.58 ± 0.716	2.68 ± 0.020	7.64 ± 0.08
Significance	***	***	***
CD at 5%	1.34	0.06	0.32

***-Significance at 0.1%

± Values represent the Standard Error

Maturation of embryos

The growth of embryo beyond scutellar stage was only sporadic and embryos turned green when transferred to light conditions. For maturation and further development of embryos exposure of embryos to ABA or sucrose is essential.

Effect of ABA and Sucrose

Conversion of globular somatic embryos to scutellar and coleoptillar stage of embryos was favoured by addition of ABA (0.5-7.5 μM) in the medium. It was found that maturation of embryos was dependent on concentration of ABA in MS medium that forces scutellar stages of embryos to undergo further development to coleoptillar stages. MS medium supplemented with 3.5 μM ABA showed highest efficiency (20.2 embryos/culture) of distinguishable scutellar and coleoptillar embryos within 6-8 weeks of incubation (Table 6 & Fig. 1e). At reduced levels of ABA (0.5 to 2.5 μM) embryogenic efficiency was comparable but coleoptillar stage embryos were less in numbers. Increased levels of ABA (7.5 μM) showed decreased efficiency and moreover callus turns brown or pale yellow.

Sucrose at lower concentration (1-3%) in MS medium promotes multiplication of globular somatic embryos, higher sucrose concentration (4-6%) promoted the maturation of embryos present in callus. Sucrose at 6% level showed highest efficiency (11.4 embryos/culture) of scutellar and coleoptillar embryos. Concentrations higher than 6% of sucrose in MS medium resulted in decreased maturation rate and browning of callus (Table 7). Scutellar and coleoptillar embryos with shoot primordia were observed on maturation medium. Callus if transferred to light conditions turns green without proper germination of embryos.

Germination of embryos

Callus with mature coleoptillar embryos were selected for germination. It was observed that selection of mature somatic embryos affected the germination rate of somatic embryos. Coleoptillar stage somatic embryos developed shoots and roots when these were transferred to MS medium supplemented with BAP (5 μM -15 μM). Maximum germination frequency of 65% was observed on MS medium supplemented with 5 μM BAP under 16/8 hrs (light/dark) photoperiod and $25 \pm 2^{\circ}\text{C}$ temperature (Table 8). Addition of GA₃ to BAP did not improve the germination frequency. Within 6 weeks complete plantlets with well-developed shoots and roots was observed (F 1f). 65% of coleoptillar stage embryos regenerated to plantlets on germination medium, remaining embryos formed only shoots (Fig. 2a). These *in vitro* shoots were multiplied on multiplication medium using standard procedures. Germinated embryos (now plantlets) were placed in polybags containing soil, sand and FYM in 1:1:1 proportion and placed in the mist chamber for 7-15 days with a RH of 80-90% and temperature $30 \pm 2^{\circ}\text{C}$ for hardening. These were regularly fed with half strength MS medium solution without organics. After 15 days in the mist chamber these plants were shifted to agro net shade house to protect plants from strong sunlight. Under shade house these plants were acclimatized for 1-2 months and were later transferred to the field.

In vitro shoot multiplication

In vitro shoots produced by the somatic embryos were isolated from embryogenic cultures and were multiplied on MS medium supplemented with cytokinin BAP (2.0-3.0 mg/l) under light conditions. A routine subculturing of these *in vitro* shoots after 4 weeks increased its number. Multiplication rate of 8-10 folds was obtained on MS medium supplemented with 2.5 mg/l BAP after sixth subculture cycle (Table 9 & Fig. 2b). In the beginning these *in vitro* shoots were thin but later they picked up growth and were normal in appearance.

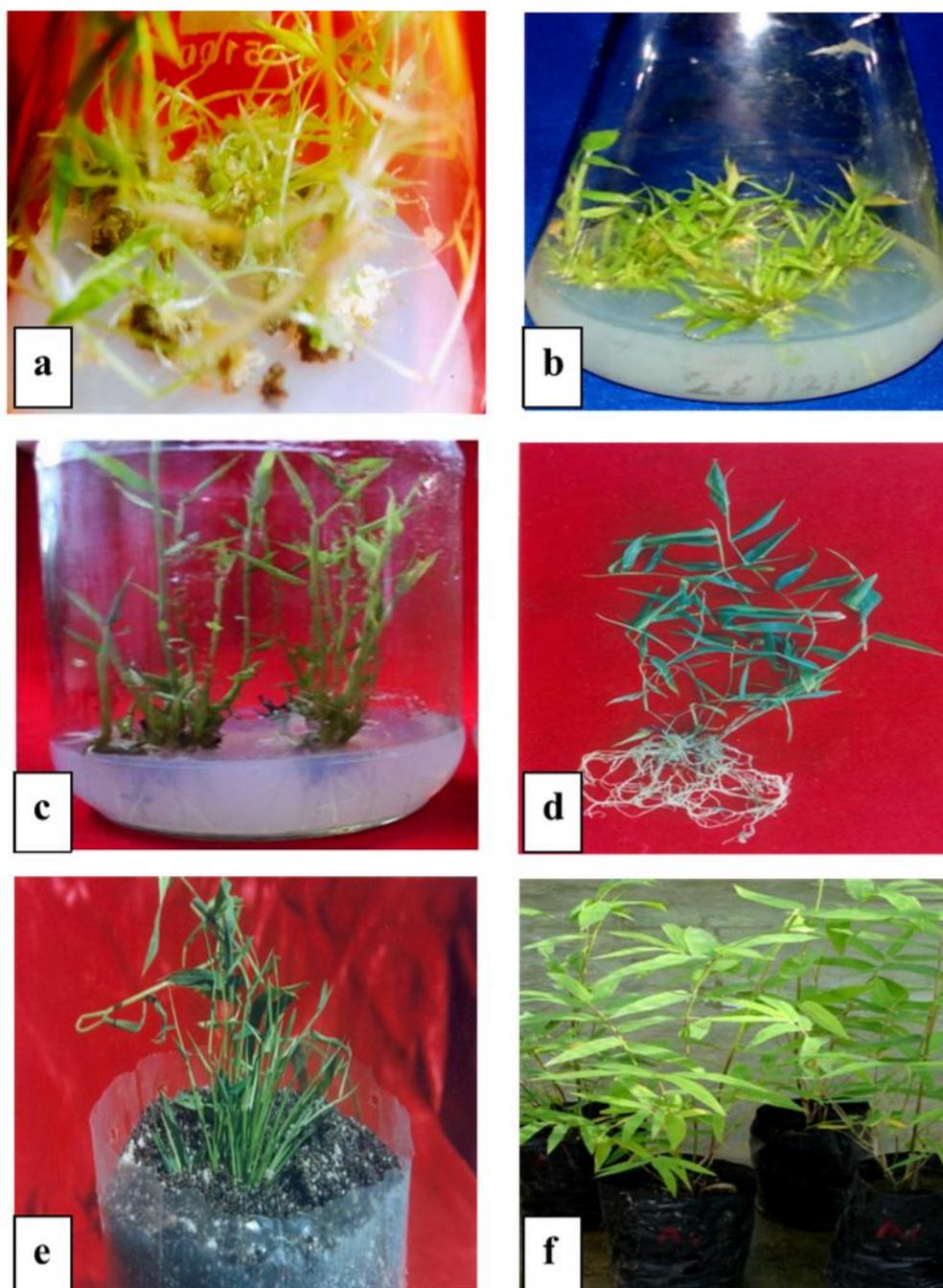


Figure 2. Plantlet regeneration from somatic embryos of *Drepanostachym falcatum*. a) Multiple shoots formation during germination of somatic embryos. b) *In vitro* multiplication of shoots derived from somatic embryos on MS+2.5mg/l BAP. c & d) *In vitro* rooting of shoots derived from somatic embryos on MS+7mg/l IBA. e & f) Hardening and acclimatization of plantlets raised through somatic embryogenesis.

In vitro rooting

In vitro rooting was achieved when *in vitro* raised shoots were transferred on NAA and IBA supplemented MS medium. 90-95% success was obtained in *in vitro* rooting conditions. *In vitro* roots were induced in *in vitro* raised shoots derived from embryogenic calli cultured on MS medium supplemented with auxins (NAA, IBA) in concentration from 1.0-11.0 mg/l. It was found that IBA at 7.0 mg/l produced optimal rooting response of 95-100%, on reducing the level of IBA to 3.0-5.0 mg/l in MS medium rooting percentage decreases to 80%. On further reducing the IBA to 1.0 mg/l rooting response sharply declines to 45%. At increased level of IBA beyond the optimal level 7.0 mg/l it was observed a fall in roots number per propagule. It was observed that maximum number of roots per propagule (12-14) were produced on 7.0 mg/l IBA supplemented MS medium (Fig. 2c & d).

In vitro shoots when transferred on NAA supplemented medium produced a maximum of 98-99% rooting on 3.0-5.0 mg/l NAA with 8 roots per propagule. The optimal NAA concentration was found to be 5.0 mg/l where maximum rooting response was noticed. A decrease in NAA concentration in MS medium showed a fall in rooting percentage as well as decreased formation of average root number. Similarly, at increased level of NAA (11.0 mg/l) rooting percentage falls to 70% with a sharp decline in average root number formation i.e. 4 roots per propagule.

Hardening and acclimatization

85-90% plants survival rate was observed when germinated embryos were hardened and acclimatized in the mist chamber followed by shade house within 1-2 months duration. The four weeks old plantlets that were directly transferred to polybags (containing soil: sand: FYM) without hardening and acclimatization showed 10-20% survival rate that too when transplanted in rainy season. In contrast 90-95% plantlets survival was obtained when plantlets were hardened and acclimatized prior to field transfer. Best results were obtained when 4 weeks old plantlets were transferred to autoclaved vermiculite soaked with half strength MS nutrient medium without organics under mist chamber conditions of 90% RH and $30 \pm 2^{\circ}\text{C}$ temperatures (Fig. 2e). During hardening the shoots elongated, leaves turned greener and expanded. The plants became much healthier after hardening. After one month in the shade house the plants were transferred to polybags containing soil: sand: FYM in 1:1:1 proportion by volume and were irrigated with water (Fig. 2f). In next 2-3 months these plants developed rhizomes and were ready for field plantation.

Discussion

In the present study somatic embryogenesis was induced using juvenile vegetative tissue. Nodal segments and leaves were used as explant for induction of somatic callus. Nodal segments as explant for induction of callus was used in a number of bamboo species like *Bambusa vulgaris*, *Dendrocalamus giganteus* and *D. strictus* (Rout and Das, 1994; Vongvijitra, 1988), *D. giganteus* (Ramanayake and Wanniarachchi, 2003), *Bambusa eludis* (Lin *et al.*, 2004). Leaf sheath or leaf tissue is utilized for the initiation of somatic callus in *Bambusa flexicosa*, *Phyllostachys viridis*, *Bambusa glaucescens* (Vasana, 1985; Hassan and Debergh, 1987; Jullien and Tran Thanh Van, 1994). Responsiveness towards regeneration of particular explants depends on the presence of meristematic cells and juvenility of material as developmental stage of explants do play a vital role in regeneration of somatic callus (Kysely and Jacobsen, 1990). In *Drepanostachyum falcatum* the leaf base has shown embryogenic response than the whole leaf tissue, as it is the zone of meristematic cells due to growth pattern in monocots where basal portion is more meristematic and young than tip portion.

Auxins play a critical role in the induction of embryogenic callus in present study. 2,4-D was effective over a large concentration range and promoted somatic embryogenesis. Use of 2,4-D to induce embryogenic response is reported in a number of bamboo species (Mehta *et al.*, 1982; Huang and Murashige, 1983; Rao *et al.*, 1985; Yeh and Chang, 1986 a, b; Vongvijitra, 1988; Dekkers and Rao, 1989; Tsay *et al.*, 1990; Zamora and Gruezo, 1990; 1991; Kanyaratt, 1991; Wood *et al.*, 1992; Saxena and Bhojwani, 1993; Rout and Das, 1994; Chang and Lan, 1995; Saxena and Dhawan, 1999; Wu Yimin *et al.*, 2000; Godbole *et al.*, 2002, Ramanayake and Wanniarachchi, 2003; Satsangi, 2003; Lin *et al.*, 2004).

In the present case for initiation of callus darkness was preferred over illumination as compactness and embryogenic potential was maintained in dark for long. Similarly, Rout and Das (1994) in *Bambusa vulgaris*, *Dendrocalamus giganteus* and *D. strictus* found that the rate of callus proliferation was better when cultures were incubated in dark.

Occurrence of three types of calli in the present investigation is in consistence with the earlier reports on bamboos (Woods *et al.*, 1992; Saxena and Bhojwani, 1993; Rout and Das, 1994; Jullien and Tran Thanh Van, 1994; Chang and Lan, 1995; Saxena and Dhawan, 1999). Low concentration of cytokinin BAP improved the embryo development. Highest frequency of embryogenesis and somatic embryos were seen on agarified MS medium supplemented with 10 μM 2,4-D and 0.88 μM BAP. Maintenance on multiplication medium consisted of lower concentration of 2,4-D alone or supplemented with BAP/ Kn as is evident from reports on cereals and bamboos and is different from induction medium (Vongvijitra, 1988; Jullien and Tran Thanh Van, 1994; Saxena and Dhawan, 1999) and in cereals (Lu and Vasil, 1982; Nabors *et al.*, 1983; Swedlund and Locy, 1988). In *D. falcatum* three developmental stages of somatic embryos were observed in embryogenic callus as globular, scutellar and coleoptillar under different sets of experiments. Occurrence of three developmental stages of embryos is evident from several reports in monocots (Vasil, 1982; Bhojwani and Raazdan, 1996).

In this study 2% sucrose supported maximum number of globular stage embryos, callus efficiency and multiplication of callus. Woods *et al.* (1992) also reported use of 2% sucrose for callus proliferation and embryogenesis. The carbohydrate concentration and type have been effective in inducing non-competent cells to become competent for embryogenesis preventing precocious germination and promoting secondary embryogenesis while controlling abnormal embryo morphology (Michler and Bauer, 1991).

In the present study four weeks subculture duration was found to maintain maximum embryogenic capacity. Callus subculturing is required frequently for further proliferation and embryo development as frequency of embryogenic callus increases on subculturing. Chang and Lan (1995) reported that subculturing and freshening of media every 8 weeks maintained the viability of the callus. In most of

the bamboos subculture interval of 4-6 weeks is reported to be beneficial for maintenance of embryogenic potential. Maturation was carried out in order to facilitate further histo-differentiation of embryos to coleoptillar stages and proper formation of shoot and root primordias to improve the percentage of germination of embryos. Chang and Lan (1995) in *Bambusa beecheyana* Munro var *beecheyana* reported use of ABA (0.1-2 mg/l) and other osmoticums (PEG, polyamine, mannitol) for further development of embryos. In the present study, on 2-3 weeks of culturing on ABA embryos became rich in starch and other storage products that led to its characteristic hardening and firm organization. Coleoptile appeared from middle of scutella during maturation phase as a smooth and shining tubular structure with a terminal pore. 3.5 μ M ABA is found to support maximum number of scutellar and coleoptillar embryos and is thus chosen as optimum in present study. In bamboos in case of *B. beecheyana* (Yeh and Chang, 1986) ABA when added to medium facilitated conversion of globular embryos to scutellar embryos. In *Bambusa glaucescens* (Jullien and Tran Thanh Van, 1994) ABA has shown to induce polarity of embryos on its addition. Umbeck and Norstog (1979) also found that ABA was essential for organized development of embryoids in barley and pennisetum cultures. It is suggested that the favorable effect of ABA may be due to increase in storage reserves, such as storage proteins, triglycerides and lipids.

Germination was achieved by transferring somatic embryos on to MS medium supplemented with 5 μ M BAP, though germination percentage could not be improved beyond 65% in present case and rest of embryos formed only shoots. BAP is used for germination of ginseng embryos (Arya *et al.*, 1991). In order to increase the frequency of plant recovery, shoots that developed from somatic embryos were micropropagated and 1000 plants have been produced. Similar studies were also reported in *Dendrocalamus strictus* by Saxena and Dhawan (1999) where germination is preceded by micropropagation of shoots. Rao *et al.* (1985) in *D. strictus* reported only 40% germination, 25% converted to shoots, rest 35% died. Embryoids were also obtained in case of cereals by Vasil and Vasil (1981) in *Pennisetum ammericana* that showed only shoot meristem associated with coleoptiles but no root was apparent. Ozias-Akins and Vasil (1982) also observed formation of shoots without root formation during germination of embryos in wheat.

Light conditions enhanced the conversion of coleoptiles to shoot meristem that developed into leafy structure and chlorophyll is developed in them within 2-3 weeks. Light also causes precocious germination as reported in number of cereals (Vasil and Vasil, 1981). Shoots developed from somatic embryos were further multiplied.

Thus induction of somatic embryogenesis and development of somatic embryos leading to plant regeneration is an efficient method for rapid mass multiplication in the present case. The technique developed provided fast means of propagation in two ways 1) through direct regeneration of somatic embryos into plants and 2) Through *in vitro* shoot multiplication from the *in vitro* shoot formed by the somatic embryos. Present protocol of somatic embryogenesis can be used for successful production of plants. Moreover, plants produced from somatic embryogenesis are equivalent to seed raised plantlets as in them resetting of intermast period takes place as reported by Rao *et al.* (1990) and Nadgauda *et al.* (1997). Thus the risk of flowering is reduced which is a major limiting factor in micropropagation.

References

- Arya, I.D.; Arya, S. 1997. *In vitro* culture and establishment of exotic bamboo *Dendrocalamus asper*. Indian Journal of Experimental Biology, 35, 1252-1255.
- Chang, W.C.; Lan, T.H. 1995. Somatic embryogenesis and plant regeneration from roots of bamboo (*Bambusa beecheyana* Munro var. *beecheyana*). Journal of Plant Physiology, 145, 535-538.
- Chittendon, F. 1956. RHS Dictionary of Plants plus Supplement. Oxford Press, 1951.
- Dekkers, A.J.; Rao, A.N. 1989. Tissue culture of 4 bamboo genera. In: Rao, A.N.; Yusoff, A.M. eds, Proc. seminar on tissue culture of forest species. Forest Research Institute, Malaysia and IDRC, Canada, pp. 83-90.
- Gamborg, O.L.; Miller, R.A.; Ojima, K. 1968. Nutrient requirements of suspension cultures of soyabean root cells. Experimental Cell Research, 50, 151-158.
- Godbole, S.; Sood, A.; Thakur, R.; Sharma, M.; Nagar, P.K.; Ahuja, P.S. 2004. Starch deposition and amylase accumulation during somatic embryogenesis in bamboo (*Dendrocalamus hamiltonii*) Journal of Plant Physiology, 162(2), 245-248.
- Hassan, A.; Debergh, P. 1987. Embryogenesis and plantlet development in bamboo *Phyllostachys viridis* (Young) McClure. Plant Cell Tissue & Organ Culture, 10, 73-77.
- Huang, L.C.; Murashige, T. 1983. Tissue culture investigations of bamboo. 1. Callus culture of *Bambusa*, *Phyllostachys* and *Sasa*. Bot. Acad. Sinica, 24, 31-52.
- Jullien, F.; Tran Thanh Van, K. 1994. Micropropagation and embryoids formation from young leaves of *Bambusa glaucescens* 'Golden Goddess'. Plant Science, 98(2), 199-207.
- Kanyaratt, S. 1991. *In vitro* culture of some economic bamboos. Dissertation in Thailand. M. Sc. Thesis, Kasetsart University. pp. 266.
- Kondas, S. 1982. Bamboo biology, culm potential and problems of cultivation. Indian Forester, 108(3), 179-188.
- Lazzeri, P.A.; Hildebrandt, D.F.; Collins, G.B. 1987. Soyabean somatic embryogenesis: Effects of hormones and culture manipulations. Plant Cell Reports, 8, 379-382.
- Lin, C.S.; Lin, C.C.; Chang, W.C. 2004. Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambusa eludis*. Plant Cell Tissue & Organ Culture, 76, 75-82.
- Lu, C.; Vasil, I.K. 1982. Somatic embryogenesis and plant regeneration in tissue cultures of *Panicum maximum* Jacq. American Journal of Botany, 69, 77-81.
- McClure, F.A. 1956. Bamboo in the economy of oriental people. Economic Botany, 10(4), 335-61.
- Mehta, U.; Rao, I.V.R.; Ram, H.Y.M. 1982. Somatic embryogenesis in bamboo. In: Fujiwara, A. (Ed.) Plant Tissue culture Proc. 5th Intl. Cong. Plant Tissue and Cell culture. pp. 109-110.
- Michler, C.H.; Bauer, E.O. 1991. High frequency somatic embryogenesis from leaf tissue of *Populus* spp. Plant Science, 77, 111-118.
- Mrudul, V.; Shirgurkar, S.; Thengane, R.; Insiya, S.; Poonawala, J.; Nadgauda, R.S.; Mascarenhas, A.F. 1996. A simple *in vitro* method of propagation and rhizome formation in *Dendrocalamus strictus* Nees. Current Science, 70(10), 940-943.
- Murashige, T.; Skoog, F. 1962. A revised medium for rapid growth and bio assay with tobacco tissue culture. Physiologia Plantarum, 15, 473-97.
- Nabors, M.W.; Heyser, J.W.; Dykes, T.A.; Demott, K.J. 1983. Long duration, high frequency plant regeneration from cereal tissue cultures. Planta, 157, 385-391.
- Nadgauda, R.S.; John, C.K.; Parasharami, V.A.; Joshi, M.S.; Mascarenhas, A.F. 1997. A comparison of *in vitro* with *in vivo* flowering in bamboo: *Bambusa arundinacea*. Plant Cell Tissue & Organ Culture, 48, 181-188.

- Ohrnberger, D.; Goerrings, J. 1990. Bamboos of the world. International Book Distributors, Dehradun.
- Ozias-Akins, P.; Vasil, I.K. 1982. Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L. (wheat): Evidence for somatic embryogenesis. *Protoplasma*, 110, 95-105.
- Ramanayake, S. M. S. D.; Wanniarachchi, W.A.V.R. 2003. Organogenesis in callus derived from an adult giant bamboo (*Dendrocalamus giganteus* Wall. Ex Munro) Scitific. *Horticulture*, 98, 195-200.
- Rao, I. U.; Rao, I. V. R.; Narang, V. 1985. Somatic embryogenesis and regeneration of Plants in the bamboo *Dendrocalamus strictus*. *Plant Cell Reports*. 4 (4), 191-194
- Rao, I. U.; Rao, I. V. R.; Narang, V.; Jerath, R.; Pillai, K.G. 1990. Mass propagation of Bamboos from somatic embryos and their successful transfer to the forests. In: Rao, I. V.R.; Gnanaharan, R. ; Sastry, C.B. eds, Bamboos current Research. Proceedings of the Int'l Bamboo Workshop, Nov 14-18, 1988, Cochin, India, pp. 167-172.
- Rao, I.V.R.; Rao, I.U.1988. In: Bamboo current Research. Proc. Int'l Bamboo work shop. In: Rao I.V.R.; Gnanaharan, R.; Cherla, B.S. eds., FRI, Kerala & IDRC, Canada, pp. 151.
- Rout, G.R.; Das, P. 1994. Somatic embryogenesis and *in vitro* flowering of three species of bamboos. *Plant Cell Reports*, 13, 683-686.
- Satsangi, R. 2003. Tissue culture studies for improvement in multiplication of *Dendrocalamus asper*. Thesis, Deemed University, Forest Research Institute, Dehra Dun, India.
- Saxena, S.; Bhojwani, S.S. 1993. *In vitro* clonal multiplication of 4-year old plants of the bamboo *Dendrocalamus longispathus* Kurz. *In vitro Cellular Developmental Biology*, 29, 135-142.
- Saxena, S.; Dhawan, V. 1999. Regeneration and large scale propagation of bamboo (*Dendrocalamus strictus* Nees) through somatic embryogenesis. *Plant Cell Reports*, 18(5), 438-443.
- Swedlund, B.; Locy, R.D. 1988. Somatic embryogenesis and plant regeneration in 2-year old cultures of *Zea diploperenis*. *Plant Cell Reports*, 7, 144-147.
- Tsay, H.S.; Yeh, C.C.; Hsu, J.Y. 1990. Embryogenesis and plant regeneration from anther culture of bamboo *Sinocalamus latiflora* (Munro) McClure. *Plant Cell Reports*, 9(7), 349-351.
- Umbeck, P.F.; Norstog, K.1979. Effects of abscisic acid and ammonium ion on morphogenesis of cultured barley embryos. *Bull. Torrey. Bot. Club.*, 106, 110-116.
- Uniyal, S.; Awasthi, A. 2000. Bamboos: Their distribution and Biomass in Bhagirathi Catchment, Garhwal Himalaya. *Indian. Journal of Forestry*, 23(4), 490-495.
- Vasana, N. 1985. Aseptic culture propagation and conservation of bamboo. Graduate School Thesis (M.S. in Agriculture). Kasetesart University, Bangkok, Thailand.
- Vasil, V.; Vasil, I.K. 1981. Somatic embryogenesis and plant regeneration from tissue cultures of *Pennisetum americanum* and *P. americanum* x *P. purpureum* hybrid. *American Journal of Botany* , 68, 864-872.
- Vongvijitra, R. 1988. Bamboo current Research. In: Rao. I.V.R.; Gnanaharan. R.; Cherla, B.S. eds, Proc. Int'l Bamboo Workshop. FRI, Kerela & IDRC, Canada, pp. 148.
- Woods, S.H.; Phillips, G.C.; Wood, J.E.; Collins, G.B. 1992. Somatic embryogenesis and plant regeneration from zygotic embryo explants in Mexican weeping bamboo *Otatea acuminata aztecorum*. *Plant Cell Reports*, 11, 257-261.
- Wu-Yimin,; Bian, H.W.; Wang, J.H.; Huang, C.N. 2000. Establishment of bamboo cell suspension culture and observation of the transplants of tissue culture derived seedlings. *Journal of Bamboo Research*, 19(12), 52-56.
- Yeh, M.L.; Chang, W.C. 1986a. Plant regeneration through somatic embryogenesis in callus culture of green bamboo (*Bambusa oldhamii* Munro) Theoretical. *Applied Genetics*, 73(2),161-163.

- Yeh, M.L.; Chang, W.C. 1986b. Somatic embryogenesis and subsequent plant regeneration from inflorescence callus of *Bambusa beecheyana* Munro var. *beecheyana*. *Plant Cell Reports*, 5, 409-411.
- Zamora, A.B.; Gruezo, S. S. 1990. Embryo cell of bamboo (*Dendrocalamus strictus* Nees). *Philippine Agri.* 73(2),199-206.
- Zamora, A.B.; Gruezo, S. S. 1991. Callus establishment from excised embryo and plant regeneration of five bamboo species *Philippine Journal of Crop Science*. Philippines, 16(1), 834.

Somatic embryogenesis and plant regeneration from the anther callus of *Dendrocalamus latiflorus* Munro

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Abstract

Bamboo cultivars are difficult to create using traditional cross breeding methods. We developed an efficient system to regenerate *Dendrocalamus latiflorus* (tropical giant bamboo) using *in vivo* anther to generate calli in M8 medium supplemented with plant growth regulators at optimal concentrations. To identify the optimal growing conditions for callus induction and shoot differentiation *in vitro*, experiments were carried out using an orthogonal design. The regeneration system had a high rate of callus induction and shoot differentiation. The highest calli induction rate (5.08 ± 0.606 %) was obtained in M8 medium supplemented with 1 mg/L NAA, 0.3 mg/L 6-BA, 15 mg/L PAA, 7.5 mg/L STS, 500 mg/L CH, 100 mg/L proline, 100 mg/L glutamine, 5.4% maltose, and 0.8% agar. Optimal conditions for shoot differentiation (27.8 ± 9.53 %) and subsequent shoot growth were also obtained in M8 medium supplemented with 0.5 mg/L KT, 2 mg/L BA, 0.5 mg/L NAA and 15 mg/L PAA, 7.5 mg/L STS, 500 mg/L CH, 100 mg/L proline, 100 mg/L glutamine, 5.4% maltose, and 0.8% agar. Roots were optimally generated in medium containing the micronutrient and organic components of B5 at 50% concentration, the macronutrient components of MS at 50% concentration, and 3% sucrose, but without any growth regulators. Our study provides an innovative method for bamboo plant regeneration and a useful method for transformation.

Keywords

Bamboo, Anther culture, *Dendrocalamus latiflorus* Munro, Embryogenesis

Abbreviations

6-BA, 6-benzyladenine

KT, Kinetin

NAA, Naphthaleneacetic acid

STS, silver thiosulfate;

CH, caseinized yeast extract

PAA, phenylacetic acid

PGRs, plant growth regulators.

Introduction

Bamboo is a member of the grass family, and includes more than 70 genera and 1200 species worldwide. It is particularly important, both economically and environmentally, to China (Jiang 2002). Bamboos are an integral part of many social forestry projects and plans due to their short rotation cycles, rapid growth, and the possibility that these trees can be harvested sustainably. *Dendrocalamus latiflorus* Munro, known as the “tropical giant bamboo,” is an evergreen bamboo species native to southern China. It is a clumping bamboo with large green culms that can reach up to 20 m in height and 20 cm in diameter. Its succulent young shoots are edible, while the longer mature culms are useful in construction.

Bamboos rarely blossom, and do so in an unpredictable manner, so it is difficult to create marketable bamboo cultivars by using traditional cross breeding methods. Plant tissue culture techniques provide a possible way to induce and select variants of any economical bamboo species. However, the usefulness of culture systems for plant improvement will depend on the ability to regenerate whole plants. In general, bamboos are difficult plants to culture *in vitro*. Alexander and Rao (1968) reported zygotic embryo cultures of bamboo, while callus cultures derived from the leaves and shoot tips of several species are also available (Huang and Murashige, 1983). Furthermore, regeneration of bamboo plants was observed in some bamboo species using callus cultures derived from mature seeds (Mehta et al., 1982; Rao et al., 1985) and young inflorescences (Yeh et al. 1986a; Yeh et al. 1986b; Yuan et al. 2009). Anthers of *Dendrocalamus latiflorus* had regenerated whole plants via somatic embryogenesis (Tsay et al. 1990; Qiao et al. 2010). Nevertheless, a stable and reproducible regeneration technique with high regeneration rate is still lacking for genetic transformation and for the creation of valuable bamboo cultivars.

In this report, we describe an improved medium composition and determined the optimal growing conditions for callus induction and differentiation in the anther culture of *D. latiflorus*. We also thoroughly examined the effects of PGRs on callus induction from anther and the effects of KT, NAA, PAA, and BA on shoot differentiation. The chromosome number and nuclear DNA content of the seedling samples and of the anther-regenerated plants were compared by microscopy and flow cytometry.

Material and methods

Dendrocalamus latiflorus Munro (belonging to the Poaceae subfamily Bambusoideae) was obtained from Zhangzhou, Fujian Province, P.R.China. Florescences or spikelets were collected from field-grown plants. Each spikelet contained 6-8 functional flower buds. Florescences or spikelets were excised and washed for 3 h in running water containing a few drops of detergent, then rinsed in distilled water. Cleaned plant material was then dipped in 70% ethanol for 1 min, and immersed for 15 min in 30% commercial bleach (2.0% sodium hypochlorite final concentration). The samples were finally rinsed 3 times in sterile water. The 1.0-1.5 cm anthers from florescences or spikelets were used as explant donors.

Effect of pretreatment time and growth regulators on callus induction

In order to overcome the difficulty of inducing embryogenic calli from anthers, we used plant growth regulators at various concentrations with different pretreatment times. Qiao (2010) and Mei et al. (1988) used M8 medium for callus induction from anther of *Dendrocalamus latiflorus*. Anthers of *Dendrocalamus latiflorus* were cultured on M8 medium supplemented with 7.5 mg/L STS, 100 mg/L glutamine, 100 mg/L proline, 0.8% agar, and 5.4% maltose. Cultures were treated with BA (0.3, 0.5, or 0.7 mg/L), NAA (0.5, 1.0 or 1.5 mg/L), and PAA (10, 15, or 20 mg/L) for pretreatment

times of 1, 2, or 3 days in an orthogonal array [L₉(3⁴)] experiment. The pH of the medium was adjusted to 5.8 using NaOH (1N) and HCl (1N) and 20-25 ml of medium was dispensed in culturists. One hundred sterilized functional flower buds were plated on each culturist containing 25 ml solid medium.

Effect of plant growth regulators on shoot differentiation

Nodular and compact calli were cultured on the M8 medium supplemented with 7.5 mg/L STS, 100 mg/L glutamine, 100 mg/L proline, 0.8% agar, and 5.4% maltose. Cultures were treated with BA (1, 2, or 3 mg/L), KT (0.5, 1.0, or 1.5 mg/L), and NAA (0.5, 1.0, or 1.5 mg/L), and PAA (10, 15, or 20 mg/L) in an orthogonal array [L₉(3⁴)] study. The pH of the medium was adjusted to 5.8 using NaOH (1 M) and HCl (1 M) and 20-25 ml of medium was dispensed in culturists. Each subculture was conducted on a subculture medium in the light (2,000 lx, 16 h light/8 h dark) at 25 °C for 20 days. In all cases, the media were steam sterilized at a pressure of 1.06 kg/cm² for 15 min.

Rooting of shoots and transplantation

A mixture of agarified MS macronutrients, B5 micronutrients, B5 vitamins and MS (Fe), all at 50% concentration, with 3% sucrose was used for rooting. No growth regulators were added. The medium was gelled with 0.25% gelrite and the pH adjusted to 5.8. Shoots were grown in 35-40 ml of medium in 150 ml glass bottles. Rooted shoots (4-weeks-old) were transplanted to potting mix comprised of equal parts soil, soilrite and organic manure (v/v) for hardening in a greenhouse. In all case, the soilrite was steam sterilized at a pressure of 1.06 kg/cm² for 15 minutes.

Estimation of ploidy level

Chromosome numbers in root tip cells were counted by the hypotonic wall degradation method as described by Chen et al. (1982) with root trips collected from embryo-derived plants. The root tips were sampled in the morning and treated with 0.1% colchicine for 3 h at 4 °C before fixation, hydrolysis, and staining. In addition, a FACSCalibur flow cytometer (Becton Dickinson, USA) was used to compare the genomic sizes by the Otto I method as described by Otto (1990) of both the seedling samples and the anther-regenerated plants.

Statistical analysis

All treatments in this study were repeated at least three times. Percentage data were transformed via arcsine and then analyzed using SPSS version 15. The difference between the means was scored using Duncan's multiple range test (Duncan 1955).

Results

Effect of pretreatment time and growth regulators on callus induction

Explants of anther typically turned brown after 2–3 weeks in culture. Over seven weeks, some calli developed from the surfaces of the explants. The effect of PGRs at different pretreatment times on callus formation is described in Table 1. While all of the nine combinations of NAA, BA, and PAA at all pretreatment times promoted callus induction and development, the M8 medium with 1 mg/L NAA, 0.3 mg/L BA, 15 mg/L PAA and a pretreatment time of 3 days was optimal for callus induction, with $5.08 \pm 0.606\%$ of the explants developing vigorous, granular, and compact calluses. Three types of calli developed from the browning anther explants, all similar to those described in other cultures of cereals and grasses (Wang and Vasil 1982; Peggy and Vasil 1982; Heyser et al. 1985). Some calli were yellowish, soft, and non-embryogenic, and consisted of filamentous cells that produced adventitious roots and bristles (Fig. 1a). The second type were creamy-yellow, granular, compact (Fig. 1b), and consisted of small, generally rounded cells rich in cytoplasm. Cells had prominent nuclei and starch grains from which the embryogenic callus lines were derived. The occurrence of this second type was associated most frequently with the slower-growing original brown callus. The third type were watery, sticky, and translucent calli (Fig. 1c).

Table 1. Effect of PGRs and pretreatment time on callus induction

PGRs(mg/L)			Pretreatment Time(days)	callus induction (%)	Callus growth pattern
NAA	BA	PAA			
0.5	0.3	10	1	2.89 ± 0.215	Most yellowish, compact calli
0.5	0.5	15	2	4.64 ± 0.467	Yellowish, compact calli, and few watery sticky calli
0.5	0.7	20	3	2.14 ± 0.393	Most watery, and sticky calli
1.0	0.3	15	3	5.08 ± 0.606	Creamy-yellow calli
1.0	0.5	20	1	3.73 ± 0.632	Yellowish, compact calli, and sticky calli
1.0	0.7	10	2	3.88 ± 0.175	Most yellowish, compact calli and fascicled buds
1.5	0.3	20	2	1.62 ± 0.322	Yellowish, compact calli
1.5	0.5	10	3	2.41 ± 0.152	Yellowish, compact calli ,and few buds
1.5	0.7	15	1	2.48 ± 0.123	Yellowish, compact calli and few buds

Three replications of 30 explants each. Values followed by the same letter are not significantly different within the same column according to the least significant difference at $P < 0.05$ (Duncan 1955). All data were collected 8 weeks after treatment with PGRs.

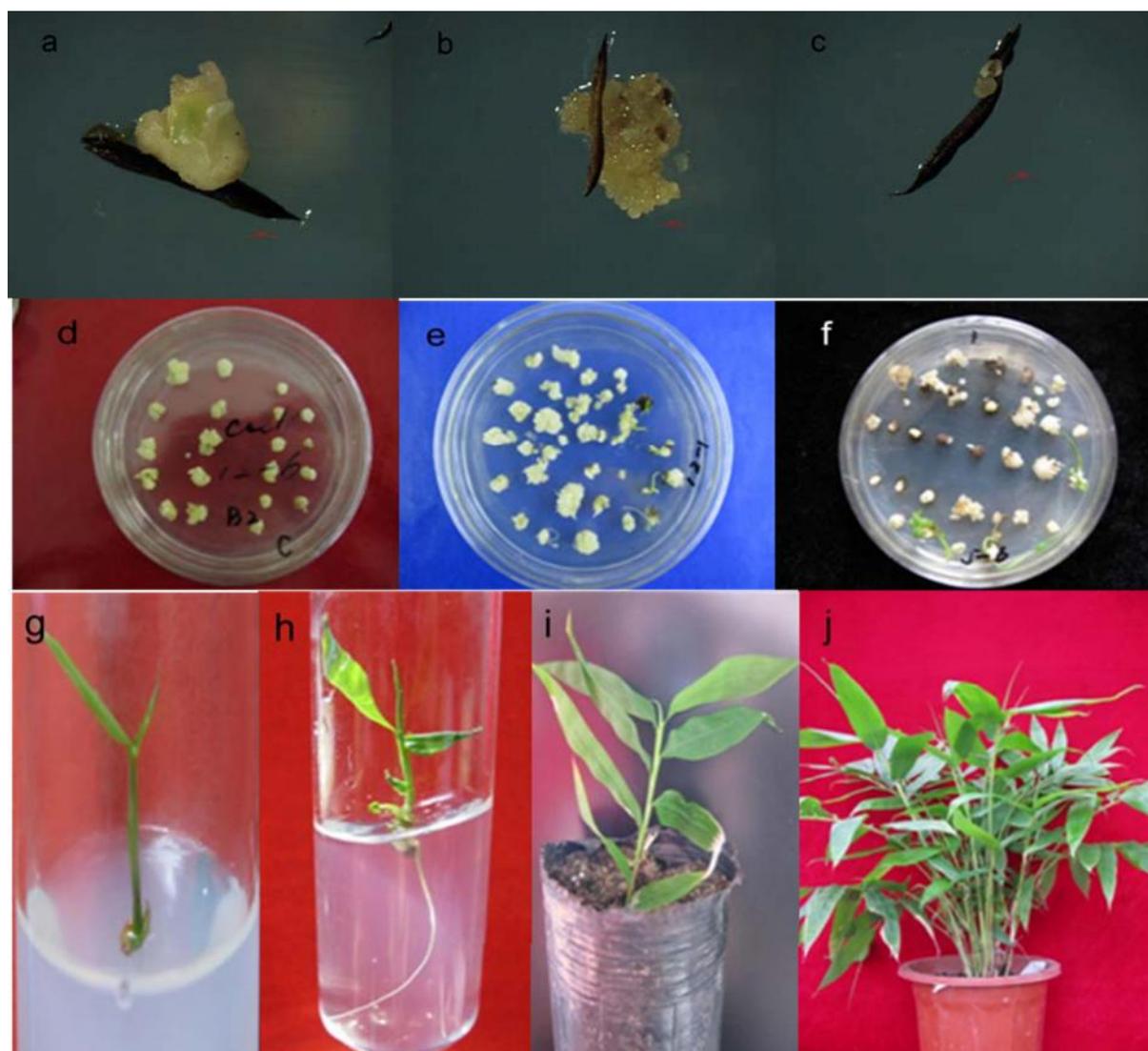


Figure 1. Plantlets regenerated from embryonic tissues of *D. latiflorus*. a. creamy-yellow, granular and compact callus; b. watery, sticky, and translucent callus; c. yellowish, compact, and non-embryogenic callus; d. buds protruding from callus in cluster; e and f. plantlets developed through organogenesis; g. plantlet developed by embryogenesis; h. root induction of shoots; i and j. plantlets after transfer to soil.

Effect of growth regulators on shoot differentiation

The calli were subcultured on fresh media every 2 weeks in the dark to maintain viability. Further subculturing caused most calli to develop clusters of pale-yellow somatic embryos, while a few turned brown.

The effects of PGRs on shoot differentiation and development are presented in Table 2. While all nine combinations of KT, NAA, BA, and PAA promoted shoot differentiation and development, the M8 medium with 0.5 mg/L KT, 0.2 mg/L NAA, 2 mg/L BA and 15 mg/L PAA promoted the highest shoot development, with 27.8% of all young plantlets showing vigorous budding and elongation. As a result, plantlets were regenerated via embryogenesis (Fig. 1d, 1e, 1f) three weeks after treatment with BA, KT, NAA, and PAA. In contrast to previous studies (Rout and Das, 1994; Liang, 1996; Ho and Chang, 1998; Lin and Chang, 1998), we obtained few albino-mutants among the regenerated shoots.

Rooting of shoots and Transplantation

Plantlet rooting was significantly enhanced after two weeks treatment with hormone-free media, resulting in more than 90% rooting. The plantlets with 4-5 cm long roots were transferred to potting soil in a greenhouse (25°C, 2000 lx, 16 h light/8 h dark). Rooted plantlets exhibited up to 95% survival rate (as shown in Fig. 1g, 1h, 1i).

Table 2. Effect of PGRs on shoot differentiation in *D. latiflorus*

KT	PGRs (mg/L)			Shoot differentiation (%)	Callus growth pattern
	NAA	BA	PAA		
0.5	0.1	1	10	11.1 ± 5.09	Light-yellow; few buds
0.5	0.2	2	15	27.8 ± 9.53	Yellow-green; fascicled buds
0.5	0.3	3	20	16.7 ± 3.33	Light-yellow; fascicled buds
1.0	0.1	2	20	13.3 ± 8.81	Yellowish; few buds
1.0	0.2	3	10	24.4 ± 14.53	Yellow-green; fascicled buds
1.0	0.3	1	15	17.8 ± 5.09	Light-yellow; thin buds
1.5	0.1	3	15	17.8 ± 9.62	Light-yellow; fascicled buds
1.5	0.2	1	20	8.91 ± 5.09	Light-yellow; few buds
1.5	0.3	2	10	18.9 ± 5.09	Yellowish; fascicled buds

Three replications of 30 explants each. Values followed by the same letter are not significantly different within the same column according to the least significant difference at $P < 0.05$ (Duncan 1955). All data were collected 3 weeks after treatment with PGRs.

Comparative study on ploidy level

The genomic content of seedling samples and the anther-regenerated plants of *Dendrocalamus latiflorus* were examined by chromosome counting and flow cytometry. Results indicated that among a hundred regeneration plants, only four plants were hexaploid, and the chromosome number of the others was double that of the parents (Figure 2a, 2b, 2c; Figure 3a, 3b, 3c).

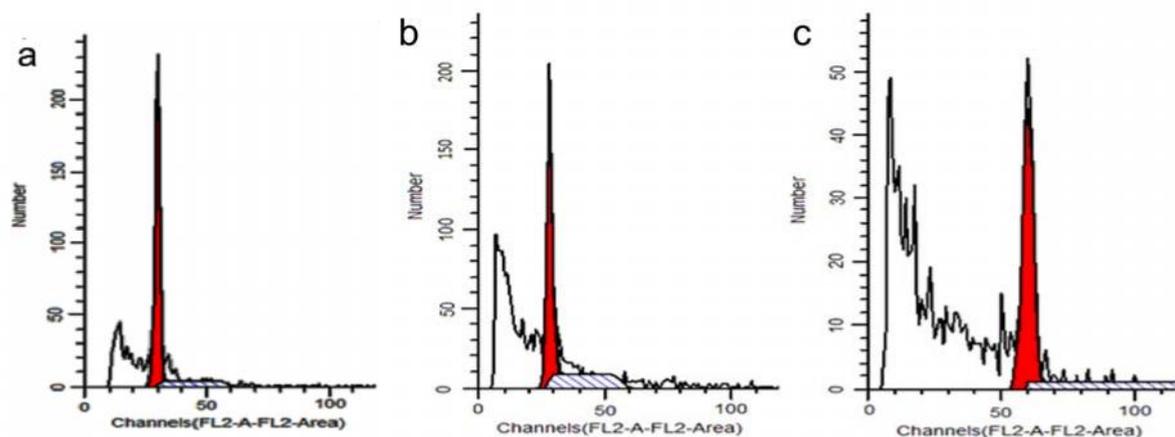


Figure 2. Comparison of fluorescence intensity between seedling-derived and anther-regenerated *D. latiflorus* plants using flow cytometry. Fluorescence intensity is indicative of DNA content. (a) The result of Seedling samples (6x); (b) Anther-regenerated plants (6x); (c) The result of the anther-regenerated plants (12x).

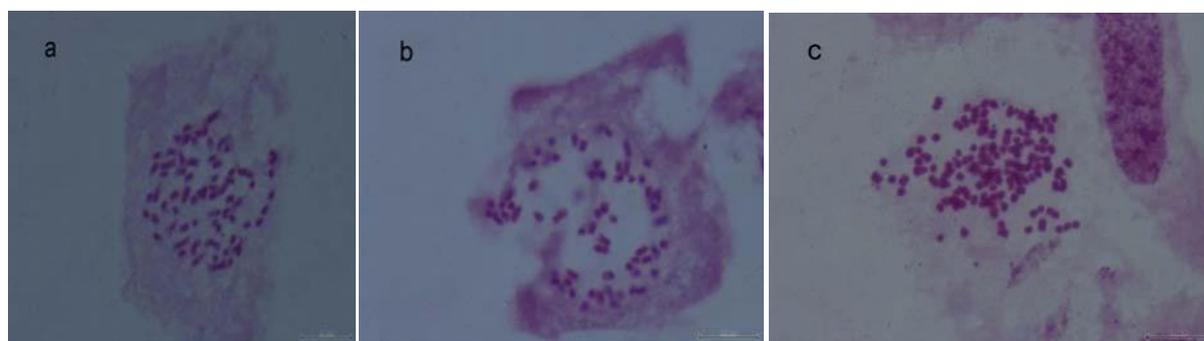


Figure 3. Images of mitotic metaphase chromosomes of the seedling samples and of anther-regenerated *D. latiflorus*. (a) The chromosomes in seedling sample (6x), total number =72; (b) The chromosomes in anther-regenerated plants (6x), total number =72; (c) The chromosomes in anther-regenerated plants (12x), total number >135. Bar =10 µm

Discussion

Dendrocalamus latiflorus has a long juvenility stage and a low seed set in the field, so reproduction is generally difficult by traditional cross breeding methods. Thus, our bamboo anther culture method holds great potential for genetic breeding and for analyzing the effects of different PGRs on organ development.

Cytokinins are essential for in vitro plant tissue culture during organogenesis and embryogenesis in bamboos (Yeh and Chang, 1986a, b; 1987). Lin et al. (2003) found that the auxin NAA acted as a negative regulator for *B. edulis* vegetative shoot proliferation. In our study, we observed that NAA could induce callus initiation for the induction of somatic embryogenesis in bamboos, a result also observed in Mexican weeping bamboo (Woods et al. 1992), *Bambusa beecheyana* (Yeh et al. 1987) and *Bambusa vulgaris*, *Dendrocalamus giganteus* and *Dendrocalamus strictus* (Rout et al. 1994). Liu et al (1996; 2009) suggested that KT and BA were important for callus induction and growth maintenance in *Zoysia japonica* and maize, but were deleterious for callus induction and growth maintenance in the bamboos *Bambusa oldhami* and *Bambusa multiplex* (Huang et al. 1983). In our study, NAA was more potent than the other plant growth regulators tested for *D. latiflorus* anther callus induction and growth maintenance.

The concentration of cytokinin in the culture medium is also known to be critical for shoot differentiation. During shoot differentiation and development, we found that NAA combined with BA effectively improved shoot differentiation.

In summary, we developed a stable and effective regeneration system to create bamboo plantlets from anther of *D. latiflorus* using M8 basal medium and various plant growth regulators. In comparison to early studies of somatic embryogenesis and subsequent plant regeneration from anther of *D. latiflorus* (Tsay 1990), we established an efficient regeneration system with higher regeneration efficiency through both organogenesis and somatic embryogenesis. In addition, our study describes a feasible method for the transgenic improvement of bamboo species.

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References

- Alexander and Rao A.N. (1968). In vitro culture of bamboo embryos. *Cur.Sci* 37, 415.
- Chen R.Y., Song W.Q., Li X.L. (1982). Wall degradation hypotonic method of preparing chromosome samples in plant and its significance in the cytogenetics. *Acta Genetica Sinica*. 9:151-159.
- Duncan D.B. (1955). Multiple range and multiple F test. *Biometrics* 11:1-42.
- Heyser J.W., Nabors M.W., Makinnon C., Dykes T.A., Demott J.K., Kautzmann D.C., Mujeeb-kazi A. (1985). Long-term, high-frequent plant regeneration and the induction of somatic embryogenesis in callus cultures of wheat (*Triticum aestivum*) *L.Z Pflanzenzucht* 94: 218-233.
- Huang L.C., Murashige T. (1983). Tissue culture investigation of bamboo I: Callus culture of *Bambusa*, *Phyllostachys* and *Sasa*. *Bot Bull Acad Sin* 24:31-52.
- Huang L.C., Huang B.L., Chen W.L. (1989). Tissue culture investigations of bamboo IV: Organogenesis leading to adventitious shoots and plants in excised shoot apices. *Environ Exp Bot* 29:307-315.
- Ho C.W., Chang W.C. (1998). In vitro flowering of albino bamboo (*Bambusa oldhamii* Munro.) regenerants derived from an 11-year-old embryogenic cell line. *Acta Hort*. 461:433-438.
- Jiang Z.H. (2002). *Bamboo and Rattan in the World*. Liaoning science and technology press, Shenyang. pp. 3-4
- Liang C.J. (1996). Tissue Culture of *Bambusa oldhamii* Munro, *Dendrocalamus latiflorus* Munro and *Bambusa multiplex* (Lour.). Master Thesis, National Taiwan University, Taipei, Taiwan. pp: 71.
- Lin C.S., Chang W.C. (1998). Micropropagation of *Bambusa edulis* through nodal explants of field-grown clumps and flowering of regenerated plantlets. *Plant Cell Rep*. 17: 617-620
- Lin C.S., Lin C.C., Chang W.C. (2003). In vitro flowering of *Bambusa edulis* and subsequent plantlet survival. *Plant Cell Tiss. Organ Cult*. 72:71-78.
- Liu J.H., Shi J.C. (1996). Studies on selection of valuable somaclonal mutants in silage maize. *Acta Bot Sin* 38:839-842.
- Liu L., Fan X.L., Zhang J.W., Yan M.L., Bao M.Z. (2009). Long-term cultured callus and the effect factor of high-frequency plantlet regeneration and somatic embryogenesis maintenance in *Zoysia japonica*. *In Vitro Cell Dev Biol-Plant* 45:673-680.
- Mehta U.I., Rao V.R., Ram.H.Y.M. (1982). Somatic embryogenesis in bamboo. *Plant Cell Tissue and Organ Culture*. 109-110.

- Mei C.S., Zhang J.Y., Wu G.N. (1988). Improving regeneration rate of anther culture in indica rice (*Oryza sativa* L. subsp. indica). Jiangsu Journal of Agriculture Sciences. 4:45-48.
- Otto F (1990). DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. In: Crissman HA, Darzynkiewicz Z, eds. Methods in Cell Biology, Vol. 33. New York: Academic Press. Flow Cytometry 33, 105-110. pp. 105-110.
- Peggy O.A., Vasil I.K. (1983). Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* (wheat). Protoplasma 117:40-44.
- Qiao G.R., Li H.Y., Jiang J., Sun Z.X., Zhuo R.Y. (2010). Anther culture and plant regeneration of *Dendrocalamus latiflorus*. 45:88-90.
- Rao I.U., Rao I.V.R., Narang V. (1985). Somatic embryogenesis and regeneration of plants in the bamboo *Dendrocalamus strictus*. Plant Cell Rep 4:191-194.
- Rout G.R., Das P. (1994). Somatic embryogenesis and in vitro flowering of 3 species of bamboo. Plant Cell Rep 13:683-686.
- Tsay H.S., Yeh C.C., Hsu J.Y. (1990). Embryogenesis and plant regeneration from anther culture of bamboo (*Sinocalamus latiflora* (Munro) McClure). Plant Cell Rep 9:349-351.
- Wang D., Vasil I.K. (1982). Somatic embryogenesis and plant regeneration from inflorescence segments of *Pennisetum purpureum* Schum. (Napier or Elephant grass). Plant Sci Lett 25:147-154.
- Woods S.H., Phillips G.C., Woods J.E., Collins G.B. (1992). Somatic embryogenesis and plant regeneration from zygotic embryo explants in mexican weeping bamboo, *Otatea acuminata* aztecorum. Plant Cell Rep 11:257-261.
- Yeh M.L., Chang W.C. (1986). Plant regeneration through somatic embryogenesis in callus culture of green bamboo (*Bambusa oldhamii* Munro). Theoretical and Applied Genetics. 73 (2):161-163.
- Yeh M.L., Chang W.C. (1986). Somatic embryogenesis and subsequent plant regeneration from inflorescence of *Bambusa beecheyana* Munro var. *beecheyana*. Plant Cell Rep. 409-411.
- Yeh M., Chang W.C. (1987). Plant regeneration via somatic embryogenesis in mature embryo-derived callus culture of *Sinocalamus latiflora* (Munro) McClure. Plant Sci 51: 93-96.
- Yuan J.L., Gu X.P., Li L.B., Yue J.J., Yao N., Guo G.P. (2009). Induction and plantlet regeneration of *Bambusa multiplex*. Scientia Silvae Sinicae 3:35- 40.

Micropropagation of economically important bamboo- *Bambusa polymorpha*

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Abstract

Bamboo, a group of tall arborescent grasses, has intimately been associated with mankind since ancient times. They occur mostly in natural vegetation in tropical, subtropical and temperate regions and are found in great abundance in tropical Asia. Due to their high economic value bamboo forest cover are fastly declining. In order to get a sustained supply of bamboo, it is necessary to raise them on a mass scale in plantation. Current methods of bamboo propagation rely on Culm cuttings, rhizome division or on seeds. Bamboo propagation by seeds has problems like short viability periods of seeds, flowering after long interval, poor seed set during off season flowering, seed sterility and large scale consumption of seeds by rodents and wild animals. Vegetative propagation has many constraints likes rooting of cuttings is difficult, non availability of propagule in required numbers. Hence it is necessary that non conventional methods likes tissue culture may be used for improvement and mass propagation of bamboos. Tissue culture protocol for micropropagation of economically important bamboo *Bambusa polymorpha* has been described. *Bambusa polymorpha* is a very useful bamboo and is of major importance in rural areas of its natural range. It has outstanding mechanical properties, durability and is popular for house building, used in the production of pulp and paper, can be used for making fiber boards. Nodal segments with single axillary buds were used as source materials for micropropagation. The axillary buds were first washed with 5% cetrimide solution for 5 minutes and then surface sterilization with 0.1% mercuric chloride solution for 10-15 minutes and rinsed 3-4 times with sterile distilled water. Axillary buds cultured on cytokinin (BAP/Kn) supplemented MS medium showed axillary bud breaks in 2 weeks. 2-3 shoots axillary shoots proliferated from the cultured nodal segment containing axillary bud. The proliferated shoots were excised and subculture on MS medium supplemented with BAP where 2-4 fold shoots multiplication was obtained in four week sub-cultured cycle. *In vitro* rooting was obtained on MS medium supplemented with IBA/ NAA. Plants were hardened and acclimatized before field transfer. The field plants developed rhizome and later sprouted new culms.

Keywords

Bamboo, Tissue-culture, micropropagation, *Bambusa polymorpha*

Introduction

Bambusa polymorpha is a very useful bamboo and is of major importance in rural areas of its natural range. It has outstanding mechanical properties, durability and is popular for house building, used in the production of pulp and paper, can be used for making fiber boards.

The life cycle of *B. polymorpha* is estimated to be approximately about 60 years. It has been reported to flower gregariously as well as sporadically and die after seeding. Current methods of this bamboo propagation rely on culm cuttings, rhizome division and on seeds. It is monocarpic in habit. The seeds produced are unreliable and is beset with many problems like short viability periods of seeds, flowering after long intervals, poor seed set during off season flowering, seed sterility and large scale consumption of seeds by rodent and wild animals. Thus, viable seeds are not available in required number. Vegetative propagation also has many constraints like rooting of cuttings is difficult, bulk size of propagule, transportation difficulties over long distances and non availability of propagules in required number. Present investigation was undertaken to develop tissue culture technique of micropropagation of *Bambusa polymorpha*.

Materials and methods

Explant source

Young juvenile shoots were collected from mature clumps of *B. polymorpha*. Nodal segments (containing single axillary bud) of these young shoots were used as explants. The nodal segments were washed with Citrimide (ICI Ltd., India) solution and washed thoroughly with water. Later, these buds were treated with 0.1% solution of Mercuric chloride for 8-15min followed by washing thrice with autoclaved distilled water. These surface sterilized axillary buds were cultured on MS liquid and semisolid medium (Murashige and Skoog's, 1962) supplemented with 5-50 μ M BAP. The cultures were maintained at 25 ± 2 °C under illumination for 16 hours photo-period with light intensity of 2500 lux obtained from white cool fluorescent tubes of 40 watts (Philip).

Axillary Shoot Proliferation

The liquid and agar gelled MS medium, Woody Plant Medium (Llyod and McCown, 1980), and B5 medium (Gamborg *et al.*, 1968) were tested for shoot proliferation from the axillary bud. Different concentrations of cytokinin (BAP) in the basal medium were tested for the maximum shoot proliferation, on both liquid and semisolid medium.

In vitro shoot multiplication

The shoots which proliferated from the axillary buds were excised from the mother explant and were further multiplied by culturing on semisolid and liquid MS medium supplemented with different concentration of BAP and was termed as the first *in vitro* shoot multiplication cycle. After first shoot multiplication cycle, the shoots were cut and separated into groups of 3-5 shoots called the propagule (subculture unit). These propagules were subcultured on fresh semisolid MS media supplemented with cytokinin (BAP) at an interval of 4 weeks for multiplication. The number of shoots in a propagule cultured and derived at the end of subculture cycle is regarded as the rate of multiplication. For each experiment a minimum of 30 propagules (each propagule representing a replicate) were taken.

Rooting of *in vitro* shoots

In vitro rooting of shoots was attempted with shoots produced during *in vitro* multiplication cycles. Effect of IBA and NAA was studied for *in vitro* rooting, both in liquid and agar solidified medium.

After 4 weeks, data was collected regarding the percentage of rooted propagules and the number of roots per propagule. A propagule of 3 shoots was used for each experiment and minimum of 30 replicates were taken for data collection.

Hardening and Acclimatization

The performance of tissue culture raised plantlets was poor when they were transferred directly to the field. Thus, these *in vitro* propagated plants were gradually hardened and acclimatized to outside environmental conditions. Micropropagated plants are generally susceptible to transplantation shock. Therefore, the plants should be properly acclimatized when they are transferred from *in vitro* environment to the soil. For hardening, the plantlets were taken out from the flasks, washed the removed adhered agar and then transferred to autoclaved 250 ml screw cap glass bottle containing 1/3 volume of soilrite. These plantlets were nurtured with half strength MS medium (without organics) twice a week for two weeks and were kept in tissue culture room. After two weeks, these bottles were shifted to mist chamber having related humidity of 80- 90 % with a temperature of $30 \pm 2^\circ \text{C}$. The caps of bottles were removed and plantlets were allowed to remain in the bottles for 3-4 days before they were transferred into a mixture of sand, farmyard manure and soil in a ratio of 1:1:1. In mist chamber, the plants were kept for three weeks and were irrigated with half strength MS nutrients without organics. After hardening in mist chamber, these plants were shifted to shade house for acclimatization. After one month in shade house the plants were transferred to bigger polybags (size 14") containing same soil composition. During this acclimatization the plants were irrigated with tap water. Generally, plants are kept in shade house for two months.

Field Establishment

Hardened and acclimatized plants were directly planted in the field preferably during monsoon and after monsoon season (July to December). Simple silvicultural practices were followed during planted with 5 x 5-meter spacing. The field plants were irrigated every 15 days for first three months.

Results and discussion

Axillary bud break

The seasonal variation in terms of percentage contamination, number and health of proliferated axillary shoots was observed in buds taken as explant. The buds were collected during different seasons, throughout the year, axillary buds collected during winter season (October to January) were best to culture. The nodal segments washed with the solution of 0.1% Mercuric chloride for 8-15 min. yielded 70 % sterilized buds. However, best sterilization results were achieved when the nodal segments rubbed and cleaned with cotton swab dipped in Ethyl alcohol prior to the above treatment. In this case 80% aseptic cultures were obtained. Nodal segments were collected from young primary and lateral branches of mother clumps. 80-90% axillary bud breaks responses was obtained(Fig.1A). Axillary buds breaks was observed 3-8 days of culture on MS medium supplemented with 5-50 μM BAP.

In vitro Shoot multiplication

The proliferated shoots were excised from the explant, cut into clusters of 3-5 shoots and were subcultured on liquid as well as agarified MS medium supplemented with 5-30 μM BAP. The proliferated shoots further multiplied on different BAP concentrations in the MS medium. Optimal *in vitro* shoot multiplication of 3.8 fold was obtained on 10 μM BAP supplemented medium as compared to other concentrations (Table-1).

The multiplied shoots were later cut into propagules of 3-5 shoots each and were again subcultured on MS medium supplemented with 10 μ M BAP (multiplication medium) for further shoot multiplication. On this medium a multiplication rate of 3-4 fold was obtained in 4-week subculture cycle (Fig.1B). Effect of BAP on axillary bud break and shoot proliferation was also observed in *D. strictus* (Nadgir et al., 1984), *B. ventricosa* (Dekkers, 1989), *D. giganteus* and *D. strictus* (Das and Rout, 1991), *D. asper* (Arya et al., 2002), *Drepanostachyum falcatum* (Arya et al., 2008), *D. hamiltonii* (Arya et al., 2009)

Effect of Kinetin either alone or with BAP in the medium was also studied on shoot multiplication of *B. polymorpha*. A reduced shoot multiplication rate was obtained on Kinetin supplemented MS medium as compared to BAP supplemented medium (Table 2). Superiority of BAP over Kinetin has been reported and discussed in relation to shoot proliferation of trees by Bonga and Van Aderkas (1992) and Deora and Shekhawat, (1995).

Table 1: Effect of BAP on shoot multiplication of *Bambusa polymorpha*.

Treatment (MS medium)	No. of shoots inoculated	Average shoot fold	Average shoot length (cm)
Control	3-5	0.0	0.0
5 μ M BAP	3-5	1.73	3.73
10 μ M BAP	3-5	3.86	3.66
15 μ M BAP	3-5	3.10	3.70
20 μ M BAP	3-5	2.53	3.56
25 μ M BAP	3-5	2.53	3.20
30 μ M BAP	3-5	2.51	3.13
S. E. \pm		0.4124	0.6050
C.D. (at 5%)		0.8845	1.2976

Table 2: Effect of Kinetin and Kn + BAP on shoot multiplication (*Bambusa polymorpha*).

Treatment (μ M) in MS medium	No. of shoots inoculated	Average shoot fold	Average shoot length
Control	3-5	0.0	0.00
5 μ M Kn	3-5	1.46	3.43
10 μ M Kn	3-5	2.10	3.53
20 μ M Kn	3-5	2.18	3.63
30 μ M Kn	3-5	2.45	3.06
5 μ M Kn+10 μ M BAP	3-5	1.88	3.60
10 μ M Kn+10 μ M BAP	3-5	4.23	3.33
20 μ M Kn+10 μ M BAP	3-5	3.13	3.43
30 μ M Kn +10 μ M BAP	3-5	1.88	2.93
S. E. \pm		0.2754	0.6668
C.D. (at 5%)		0.5786	1.4010

Rooting of *in vitro* shoots

Attempts were made for both *in vivo* and *in vitro* rooting of *in vitro* raised shoots. *In vitro* rooting of the shoots met with complete failure under all experimental conditions. The shoots turned pale within 3-5 days and eventually died. A 60% to 80% success in *in vitro* rooting was achieved in *in-vitro* raised shoots with different concentrations of IBA and NAA respectively. Rooting was obtained when shoot propagules (3-4 shoots each) were transferred on both liquid and agarified MS medium supplemented with 5-40 μM IBA and NAA(Fig.1C).

In IBA supplemented MS medium best rooting (60%) were achieved at 30 μM concentration where an average of 8.3 roots developed from the propagules of four shoots in 4 weeks (Table 3). Optimal NAA concentration was 20 μM in the MS medium where 83% rooting was achieved in the *in vitro* shoots whereas, there was slight decrease in root number/ propagule (7.66) as compared with IBA. Any increase in NAA concentration in the MS medium (30-40 μM) resulted in reduced rooting percentage and root development from the propagule (Table 4).

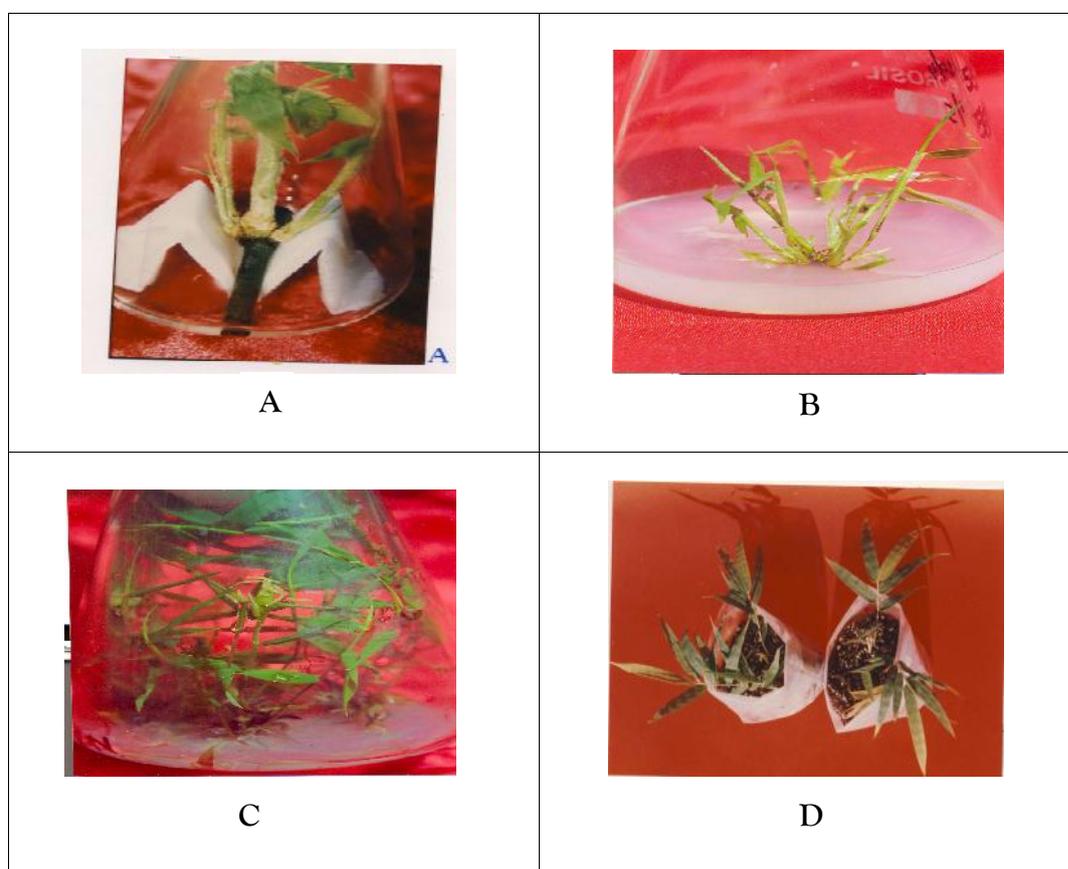


Figure 1: Micropropagation of *Bambusa ploymorpha*.

A. Axillary bud break.

B.: First subculture for *in vitro* shoot multiplication on MS + BAP medium.

C. In-vitro rooting MS + 20 μM NAA.

D. Hardened and acclimatized Tissue Culture plants ready for field transfer.

Root initiation was observed in 1-2 weeks. It was observed that root initiation was slightly early in liquid medium. Generally 7-8 roots developed from new sprouting shoot primordia of the propagule under optimal concentrations of IBA and NAA. During plantlet formation, generally 1-2 shoots of the propagule died and the remaining shoots developed. New shoots also developed from these plantlets simultaneously. Single shoots were also tried for rooting but they never developed roots. The role of auxins in root development is well established and has been reviewed by Torrey (1976).

Table 3: Effect of IBA on *in vitro* rooting of *Bambusa polymorpha* in MS medium, propagules of 3-4 shoots were used.

Treatment	Average root No./ propagule.	Average root length (cm)	Rooting percentage
Control	0.0	0.0	0.0
5 μ M IBA	2.66	5.46	16.60
10 μ M IBA	4.66	5.50	26.60
20 μ M IBA	5.33	5.33	46.60
30 μ M IBA	8.33	6.23	66.60
40 μ M IBA	6.66	6.56	53.30
S.E. \pm	1.4782	1.8488	-
C.D.(at 5%)	3.2208	4.0282	-

Table 4: Effect of NAA on *in vitro* rooting of *Bambusa polymorpha* in MS medium. Propagules of 3-4 shoots were used.

Treatment NAA(μ M)	Average root No./ propagule.	Average root length (cm)	Rooting percentage
Control	0.0	0.0	0.0
5 μ M NAA	4.0	2.40	13.30
10 μ M NAA	5.33	6.56	30.00
20 μ M NAA	7.66	5.86	83.30
30 μ M NAA	4.66	3.66	70.00
40 μ M NAA	4.33	2.03	56.60
S.E. \pm	1.5031	1.1539	-
C.D. (at 5%)	3.2749	2.5141	-

Hardening, acclimatization and field transfer

Tissue culture raised plantlets were gradually hardened and acclimatized prior to field plantation. Hardening of these plantlets was done in the mist chamber whereas acclimatization of the plants was carried out in high-density agronet shade house open from the sides. The tissue culture raised plantlets were taken out from the culture bottle and were transferred into the polybags containing soilrite(Fig.1D). These polybags were kept in the chamber under relative humidity of 80-90 % and temperature of 30°C for one month. These plants were supplied with half strength MS salts twice a week. After mist chamber stage the plants became hardened and were shifted to open shade house conditions for acclimatization to outer environmental conditions. In shade house the plants were transferred into bigger polybags (14") containing sand: soil:FYM in 1:1:1 ratio and where irrigated with water. In next two-month these plants developed rhizome in the shade house. At this stage the plants were field planted. Nearly one hundred fifty plants of *B. polymorpha* were micropropagated and field planted successfully. The field plants developed rhizome and later sprouted new culms. The present protocol is an efficient and rapid method for multiplication of *B. polymorpha* which otherwise take a period of several years for seed production.

References

- Arya, S.; Satsangi, R.; Arya, I.D. 2002. Rapid mass multiplication of edible bamboo *Dendrocalamus asper*. J. Sustainable Forestry. 4 : 103-109.
- Arya, S.; Kant, A.; Sharma, D; Arya,I.D. 2008. Micropropagation of two economically important bamboo: *Drepanostachyum falcatum* and *Bambusa balcooa*. The Indian Forester 134(9):1211-1221
- Arya,S.; Kaur,B; Arya,I.D. 2009 Micropropagation of economically important bamboo *Dendrocalmus hamiltonii* through axillary bud and seed culture. 8th world bamboo congress proceedings, Thailand vol(6):122-130
- Bonga, J.M; Von Aderkas, P. 1992. *In vitro* culture of trees. Kluwer Academic Publishers, Dordrecht.
- Das, P.; Rout, G.R. 1991. Mass multiplication of flowering of bamboo *in vitro*. Orissa Journal of Horticulture 19 (1 and 2): 118-121.
- Dekkers, A.J. 1989. *In vitro* propagation and germplasm conservation of certain bamboo, ginger and costus species. Ph.D. Thesis, National University of Singapore, Singapore.
- Deora, N.S; Shekhawat, N.S. 1995. Micropropagation of *Capparis decidua* (Forsk.) Edgew.-a tree of arid horticulture. Plant Cell Reports 15: 278-281.
- Gamborg, O.L.; Miller, R.A.; Ojima, L. 1968. Nutrient requirement of suspension culture of Soyabean. Experimental Cell Research 50: 151-158.
- Lloyd, G.; McCown, B.H. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot- tip culture. International Plant Propagator's Society. Combined Proceeding, 30: 421-427.
- Murashige, T.; Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.
- Nadgir, A.L.; Phadke, C.H.; Gupta, P.K.; Parasharami, V.A.; Nair, S; Mascarenhas, A.F. 1984. Rapid multiplication of Bamboo by tissue culture. Silvia Genetica, 33 (6): 219-233.
- Torrey, J.G. 1976. Root hormones and plant growth. Annual Review of Plant Physiology, 27 : 435-459.

Origin, Growth and Anatomy of the *In Vitro* formed Rhizome of *Dendrocalamus strictus* Nees.

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Abstract

Although the rhizome system has important functions, it is one of the least understood parts of the bamboo plant. Moreover, an understanding of the form of the rhizome system is prerequisite to understand the clump habit in any bamboo. The diverse manifestations of the rhizome system allow challenging research opportunities for molecular, physiological, biochemical, anatomical, morphological and taxonomical studies. The present knowledge about the anatomy of bamboo rhizome is restricted and rudimentary due to difficulty of precocious rhizome induction under *in vitro* conditions. And also a rhizome production under natural conditions is considered as cumbersome, hard and bulky propagules for carrying out histological studies. Precocious rhizome induction was achieved from caryopses of *Dendrocalamus strictus* (Roxb.) Nees after three weeks of inoculation on MS basal medium supplemented with 5 μ M BAP + 25 μ M NAA + 0.1 μ M GA₃. Developmental pattern, growth and branching behaviour of the rhizome were studied. Anatomical studies revealed the origin of rhizome from the lowermost node of the primary shoot. Longitudinal section of the basal portion of eight day old seedling showed the development of meristematic tissues into a primary thickening meristem of the rhizome having a distinct protoderm and a promeristematic region. Differentiation of leaf primordia was observed after twelve days of culture. The deeply stained apical dome was covered with three to four layers of leaf primordia. Vascular bundles were scattered in the cortical region. The histochemical Periodic Acid-Schiff's test showed large accumulation of starch grains at the promeristematic region during rhizome initiation. The starch grains were sparsely distributed in the region where rhizome primordium differentiated, whereas starch grains were abundantly present in the parenchymatous cells away from the primordium.

Keywords

Auxin, Bamboo, Cytokinin, *Dendrocalamus strictus*, Gibberellin, rhizome

Abbreviations

BAP-N⁶ Benzyl aminopurine, GA-Gibberellic acid, NAA- α -Naphthyl acetic acid, MS -Murashige & Skoog (1962)

Introduction

Bamboos are the giants of the grass family Poaceae (Gramineae) belonging to sub family Bambusoideae. There are more than 1575 species under 75 genera, unevenly distributed worldwide in various parts of the humid tropical, sub-tropical and temperate regions (Gupta, 2008).

Bamboos are conventionally propagated through rhizomes. Two types of rhizomes are present in bamboos, viz. leptomorph and pachymorph. The former type of rhizome is common in bamboos of temperate and cold region such as *Arundinaria*, *Phyllostachys* and *Sasa*. The pachymorph rhizome is common in bamboos of warm, tropical region such as *Bambusa*, *Dendrocalamus* and alpine bamboos such as *Fargesia*, *Sinarundinaria* and *Gigantochloa*. The pachymorph type is actually sympodial and the leptomorph type is monopodial. The present knowledge for bamboo subterranean system is too narrow and rudimentary to throw light on problems in the field of rhizome biology and many aspects about bamboo subterranean system needs to be explored. According to McClure (1966), the rhizome system is one of the least understood parts of the bamboo plant. This neglect has unfortunate consequences, because the rhizome system has important functions in the life of the plant. Moreover, an understanding of the form of the rhizome system is prerequisite to an understanding of the clump habit in any bamboo. The rhizome system assumes, in plants of different species and genera, a number of more or less sharply distinct forms and ways of growth. The diverse manifestations allow challenging research openings for molecular, physiological, biochemical, anatomical, morphological and taxonomical studies. A part of this neglect is because of the difficulty of precocious rhizome induction under *in vitro* condition and also rhizomes produced as per stipulated time in the life cycle of bamboos under natural conditions are considered as cumbersome and bulky propagules for carrying out even conventional propagation.

In the present paper we describe a protocol for precocious direct rhizome induction *in vitro*. Anatomical study of the origin, growth and development of *in vitro* rhizome and formation of plants by using growth regulators was also done. Histochemical analysis to study the formation of rhizome by using Periodic Acid-Schiff's reaction so as to localize starch in the developing cells of rhizome is described.

Material and Methods

Caryopses of *Dendrocalamus strictus* Nees was procured from Forest Research Institute (FRI) Dehradun, India. After dehusking, the seeds were surface sterilized by treating them with 0.1% solution of mercuric chloride for one minute. After three rinsing with sterile distilled water, caryopses were inoculated on nutrient medium consisted of salts and vitamins of Murashige and Skoog (1962) containing 5 % (w/v) sucrose and 0.6% (w/v) agar. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C and 15 psi for 18 minutes. Prior to introducing the seed, tubes and instruments were placed under ultra violet light (60 watt, General Electric, USA) for 45 minutes. The seeds were transferred to culture tubes in a Thermadyne HCl-160 Laminar air flow. All necessary instruments (made of stainless steel) were dipped in 90% ethanol, flamed over a spirit lamp and cooled before use. Cultures were kept in the culture room at $25 \pm 2^\circ\text{C}$ in 16/8 h light/dark regime provided by cool white 40 watt fluorescence tubes ($30\mu\text{molm}^{-2}\text{s}^{-1}$, Philips, India).

The steps used for histological study are as follows:

Fixation- The basal part containing root and shoot of *Dendrocalamus strictus* raised in MS basal medium devoid of growth regulators (control) (4, 8 and 12d) and plantlets raised in MS medium supplemented with BAP + NAA + GA₃ + 5% sucrose (4, 8 and 12 d) of *Dendrocalamus strictus* with newly formed rhizomes were fixed in 4% aqueous solution of formaldehyde (pH 7.0, adjusted by using pyridine) at 4 °C for 24-48 hrs. The fixed material was then transferred to 70% ethyl alcohol.

Tissue dehydration- The materials were dehydrated through alcohol series. It was kept in a passing tube and was passed through a series of solution mixtures.

Infiltration- The dehydrated tissues were immersed in xylene contained in a vial and placed in an oven at 60°C. Molten paraffin wax is slowly added in the vial in a drop wise manner along the walls of the vial at intervals till it infiltrates the tissue and slowly displaces the xylene as it evaporates (Jensen, 1962).

Microtomy and staining- The tissue were left in pure wax for 48h and cast in wax biscuits. Serial sections (8µM-10µM thick) were cut by using a microtome (Thermo Scientific Microm HM315) and placed on a slide coated with Haupt's adhesive [gelatin (1g) + phenol (2g) + glycerine (15ml)].

The sections were dewaxed in xylene, stained with haematoxylin (stains the nucleus of the cell purple-blue) and counter-stained with Erythrosin B (stains the cytoplasm pink).

The sections were then mounted in DPX and viewed and photographed using a Zeiss Primo Star Photomicroscope.

Results

Effects of Auxin and Cytokinin on Induction of *In Vitro* Rhizome

Effects of NAA and BAP supplemented to MS medium on induction of rhizome was studied. Addition of BAP to the culture at concentrations of less than 1ml/l was ineffective in the induction of rhizome. On the other hand, higher BAP levels in the culture medium strongly inhibited the induction of rhizome. Induction of rhizome was enhanced when higher NAA levels in the medium was employed.

In vitro rhizome formation of *Dendrocalamus strictus* Nees is highly sensitive to NAA and BAP. Root formation was strongly inhibited when high BAP:NAA ratio is supplemented in the medium. Lower BAP:NAA ratios in the culture medium resulted in the formation of rhizome but rhizome never developed into culm shoots. Under higher NAA:BAP ratios the formation of abnormally swollen rhizome occurred in the culture medium.

On MS medium supplemented with 25µM NAA + 5µM BAP, well developed rhizomes were observed after three weeks of inoculation. Rhizomes had conspicuous nodes and internodes. Rhizome nodes towards the proximal end of the shoot axis were covered with thick brownish scale leaves while the nodes on the distal end were covered with light green scale leaves. Initially, the growth of rhizome was geotropic, later rhizome tip exhibited apogeotropic growth to form culm shoot. Formation of roots from the node of rhizome was conspicuous.

Effects of NAA, BAP and GA₃ on Growth and Development of Rhizome

The effect of multiple interactions of different concentrations of BAP, NAA and GA₃ was tested on rhizome induction. BAP used singly promoted induction of rhizome. GA₃ was effective in elongation of shoot while NAA induced rooting. Therefore, interaction of varying concentrations of NAA, BAP and GA₃ was tested to study their effects on induction, growth and development of rhizome.

Varying concentrations of NAA was incorporated to MS basal medium containing BAP and GA₃. There was formation of rhizome from the embryonal end showing apogeotropic growth of the terminal region to form a culm shoot on MS medium supplemented with NAA + BAP and GA₃. Rhizomes were covered with brown hairy scale leaves. There was a presence of ephemeral brown coleoptilar sheath attached to the base of primary shoot. The development of rhizome bud from the node of first formed rhizome was also observed. There was a dose dependent significant increase in the length of rhizome when GA₃ is incorporated in the rhizome inducing MS medium containing NAA and BAP. One hundred per cent formation of rhizome was observed in 25µM NAA along with 5µM BAP and 0.1µM GA₃. After eight weeks of culture, rhizome elongated to form culm shoot with well expanded laminae and thick long roots originated from the nodes of the rhizome. Maximum culm shoot formation was also

observed in this combination.

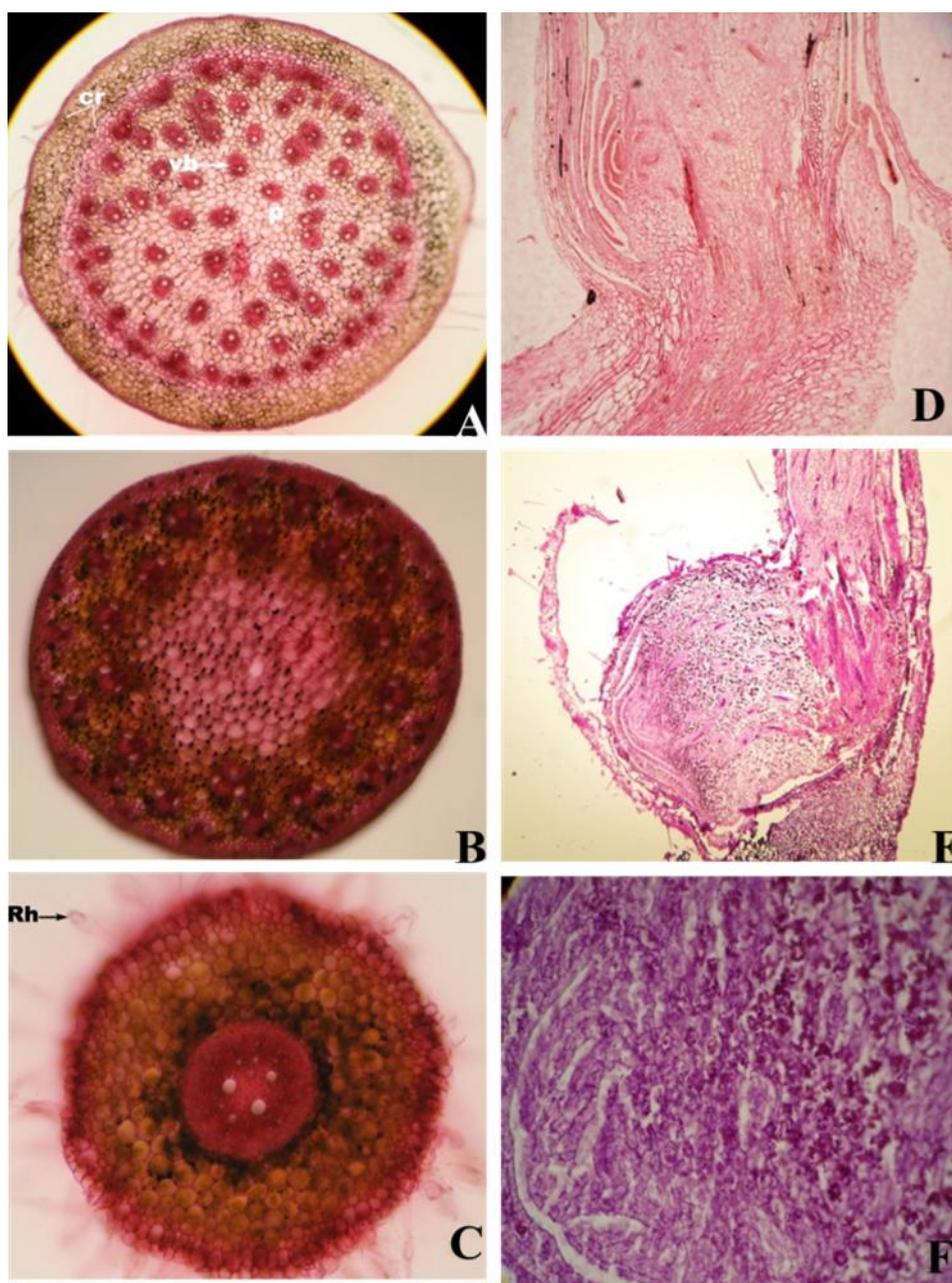


Fig.1 A.T.S. of rhizome with vascular bundles scattered in the central portion with narrow pith. B.T.S. of culm shoots showing scattered vascular bundles at the peripheral region and the large pith ring of non-vascular tissue composed of many layers of parenchymatous cells. C.T.S. of root showing vascular bundles with prominent metaxylem elements occupies central portion of the root along with pith. D.L.S. of the basal portion of 12 d old seedling on MS medium (control) showing bud primordium enclosed by leaf primordia E.L.S. of the basal portion of 12 d old seedling showing the development of rhizome on MS medium containing growth regulators. F.L.S. of 12d old seedling showing distribution of starch grains in the rhizome primordium.

Anatomy of *In Vitro* Formed Rhizome, Culm Shoot and Root

Histological studies were performed to trace the origin, growth, development and formation of rhizome under the influence of various growth regulators. Transverse sectioning of rhizome from four weeks old cultures was performed. In transverse section the epidermis of the rhizome was made up of long and short cells. Beneath the epidermis, the cortex was present and it consisted of six to seven layers of schlerenchymatous cells. Parenchymatous cells were found lying below the stomata as passage for gas exchange. Single layered endodermis demarcated the cortex from the vasculature. Vascular bundles with prominent metaxylem elements were scattered in the central portion with narrow pith (Fig. 1A). Vascular bundles showed conjoint, collateral and endarch condition, large metaxylem elements enclosed phloem. Vascular bundles were V shaped and consisted of xylem and phloem. The xylem consisted of one or two small protoxylem elements and two large metaxylem vessels.

Transverse sections of culm shoot and rhizome were similar in that vasculature of shoot consisted of scattered vascular bundles with large pith. Transverse section of culm shoot showed thin epidermal layer. To the inner side was the actual cortex with scattered vascular bundles at the peripheral region and the large pith ring of non-vascular tissue composed of many layers of parenchymatous cells (Fig. 1B). Vascular bundles were conjoint, collateral and endarch condition. Metaxylem elements were away from the pith and phloem was seen in between the two arms of metaxylem elements.

The root which was cut transversely showed three zoned structure. Outermost single thick epidermis with unicellular root hairs was followed by two layered schlerenchymatous cortex followed by five layered parenchymatous cortex which was demarcated from the innermost vasculature by a single layered pericycle composed of thick walled schlerenchymatous cells. Scattered vascular bundles (exarch) with prominent metaxylem elements occupied central portion of the root along with pith (Fig. 1C). Parenchymatous cells were followed by a single layered endodermis with prominent casparian strips and a layer of pericycle. Two arms of the protoxylem elements terminated in large prominent metaxylem elements. Thick wall cells formed the innermost pith.

Longitudinal section of the basal portion of 4 d old seedling on MS medium revealed the emergence of root and shoot from the caryopsis. There was a vascular connection between the root and shoot system. Vascular bundles were scattered in the parenchymatous portion of the shoot. Longitudinal sections of the basal portion of 8 d old seedling on MS medium showed deeply stained three meristematic regions. Promeristematic regions were protected by cataphylls. Parenchymatous cortical region of shoot showed scattered vascular bundles. Formation of axillary bud primordia flanked by cataphylls was observed. Patches of vascular bundles were also scattered in the cortical region. Longitudinal section of the basal portion of 12 d old seedling on MS medium showed bud primordia with intense staining and enclosed by leaf primordia. Magnified view showed bud primordium with leaf primordia flanked by cataphylls. Procambial strands and vascular bundles were scattered in the cortical region (Fig. 1D).

Anatomical sections of seedling on MS medium supplemented with $5\mu\text{M}$ BAP + $25\mu\text{M}$ NAA + $0.1\mu\text{M}$ GA₃ + 5% sucrose exhibited the initiation of deeply stained rhizome meristem at the base of the shoot and root from the endosperm of the caryopsis. Longitudinal section of 4 d old seedling showed prominent meristematic tissues present below the cataphyll of developing rhizome. Slightly below the apex, there was an over arching of procambial strands. Longitudinal section of the basal portion of 8 d old seedling showed the development of meristematic tissues into primary thickening meristem of rhizome having distinct protoderm and promeristematic region. A prominent axillary bud with intensely stained dome covered leaf primordia and cataphylls was observed. Patches of procambial strand and vascular bundles were scattered in the cortical region. The deeply stained meristematic region of the upper node consisted of an apical dome flanked by leaf primordia. The basal node elongated to form large swollen portion of rhizome. Rhizome originated from the endodermal layer of

the shoot. It was flanked by cataphylls. Patches of procambial strand and vascular bundles were scattered in the cortical region. Longitudinal section of the basal portion of 12 d old seedling showed the differentiation of rhizome primordium covered with leaf primordia. The deeply stained apical dome was covered with three to four layers of leaf primordia. Vascular bundles were scattered in the cortical region. Enlarged view showed distinct deeply stained meristematic rhizome primordium covered with leaf primordia. Procambial strands and vascular bundles were scattered in the cortical region (Fig. 1E).

Histological Studies Tracing the Formation of Rhizome from Caryopses

The histochemical Periodic Acid-Schiff's test was performed to trace the localization of polysaccharide (starch) in the tissue for studying organogenesis in caryopses inoculated on MS medium supplemented with BAP + NAA + GA₃ at different time intervals after inoculation. Longitudinal section of the basal portion of 4 d old seedling on MS medium supplemented with BAP + NAA + GA₃ revealed the emergence of shoot and root from the caryopsis. Magnified view showed the origin of rhizome which was a deeply stained meristematic region between the endosperm of the caryopsis and the base of the shoot. The cell walls and starch grains stained positively with PAS reagent. There was accumulation of scanty starch grains in the cells near the rhizome primordium. Longitudinal section of the basal portion of 8 d old seedling showed two meristematic regions on the nodes of the shoot. Large accumulation of starch grains was observed at the promeristematic region during rhizome initiation. Promeristematic region was covered with cataphyll. Prominent procambial strands were scattered in the cortical region. The cell walls and starch grains stained positively with PAS reagent. The origin of rhizome was traced to the lowermost node of the shoot. Elongated portion of rhizome could be seen as deeply stained meristematic dome covered with cataphylls which was in continuation with the root. Patches of vascular bundles were scattered in the cortical region. Longitudinal section of the basal portion of 12 d old seedling showed the differentiation of leaf primordia. The deeply stained apical dome was covered with three to four layers of leaf primordia. The starch grains were sparsely distributed in the rhizome primordium from where leaf primordia differentiated whereas these were abundantly present in the parenchymatous cells of the cortex of the rhizome (Fig. 1F).

Discussions

In vitro rhizome induction was conducted using plant growth regulators on MS basal medium. Both BAP and NAA at optimal concentration help in growth and development of rhizome. One hundred per cent culture showed formation of rhizome on MS medium containing 5µM BAP and 25µM NAA. Presence of GA₃ in the medium containing NAA and BAP effectively increased the length of rhizome. Similar result was reported by Shimasaki and Uemoto (1990) in which growth of *Cymbidium kanran* rhizome was enhanced by higher NAA: BAP ratios in modified MS medium. Lower NAA : BAP ratios resulted in formation of vegetative shoots from *in vitro* cultured rhizomes. Significantly, new rhizomes were induced from the axils of leaves when shoots were explanted to medium containing higher concentration of NAA. The authors suggested that usually elongation and proliferation of rhizomes occurs when high auxin concentration are present in the culture medium. In the present study, the presence of NAA with BAP in MS medium with high amount of sucrose played a critical role in proliferation of *in vitro* induced rhizomes. The positive effects of auxins and cytokinins on rhizome growth and shoot formation in *Cymbidium forrestii* was reported by Paek and Yeung (1991). Within the range of concentrations of applied plant growth substances, the rhizome meristem maintained its integrity and at the same time reacted to external signals bringing about a change in the morphogenetic pattern, i.e. continual rhizome growth or shoot formation. The authors noted that auxin

promoted rhizome growth and suggested that this pattern of development was primarily due to cell enlargement and accelerated development of axillary meristems. Shoot formation is associated with a change in the growth habit, i.e. a change from a diageotropic growth habit to an orthotropic leafy shoot. In case of potato stolons, BAP has been shown to play an important role in the turning up of the stolon and subsequent development into leafy shoot. Paek and Yeung (1991) suggested that in *Cymbidium forrestii*, the presence of BAP altered the size of cytoplasmic zone of rhizome apex and enhanced leaf development which altered the endogenous hormone balance leading to a change of the diageotropic growth habit of rhizome. Similarly, in the present study the higher growth of culm shoot and inhibition of rooting of rhizome was observed with increase in BAP : NAA ratio in MS medium along with 5% sucrose can be primarily attributed to the presence of high amount of cytokinin BAP.

Plant growth regulators are known to affect normal shoot organogenesis, which can be modified to generate tubers, bulbils, rhizomes and corms. Cytokinins play a critical role in *in vitro* organogenesis as seen in the present study. Cytokinins promote movement of nutrients, a phenomenon known as cytokinin-induced nutrient mobilization. The nutrients are preferentially transported to, and accumulate in the cytokinin-treated tissues. It has been postulated that the hormone causes nutrient mobilization by creating a new source-sink relationship. Nutrients that are translocated in the phloem move from a site of production or storage to a site of utilization. The metabolism of the treated area may be stimulated by the hormone so that nutrients move towards it (Taiz & Zeiger, 2003). It is speculated that in the present study, application of cytokinin (BAP) alongwith other factors caused a nutrient mobilization to the treated caryposes which showed formation of rhizomes.

It is well known that cytokinins promote cell division and cell expansion in plant tissue culture and many studies have reported suitable cytokinins types and their concentrations for each species (Ružić & Vujovic, 2008). Cytokinins feature strongly as activators of cell division in plants both *in vivo* and *in vitro*. Their role in cell division has been marked out and they are known to act in the regulation of both postmitotic interphase (G1) - DNA synthetic phase (S) and post synthetic interphase (G2-S) transitions. During the G1-S transition cytokinins increase the G1 cyclin, cyclin D3. Cytokinins are also important for the regulation of the G2-M transition which is mediated by the activation of CDK proteins (Kakimoto, 2003). BAP treatment can shorten the duration of S-phase through recruitment of latent origins of DNA replication. Kn is involved in the phosphoregulation of the G2-M checkpoint; the major cyclin-dependent kinase (Cdk) at this checkpoint has been shown to be dephosphorylated as a result of cytokinins treatment (Francis & Sorrell, 2001). A drastic reduction in the number of roots and the root length was observed in the cytokinin treated seedlings of *D.strictus*. Similar, inhibition in the formation of roots has also been observed in other species like the orchid *Geodorum densifolium* and in cassava *M. esculenta* (Roy & Banerjee, 2002; Medina *et al.*, 2007). Negative regulatory role of cytokinins in root growth has been well elucidated where the reason cited for the phenomenon is that in presence of cytokinins root meristem cells have a prolonged meristematic phase and eventually undergo additional rounds of mitosis (Werner *et al.*, 2001).

Auxins are typically associated with cell elongation, while auxin and cytokinin act synergistically to regulate the process of cell division. Depending on the ratio of auxin and cytokinin, the organogenesis of roots and shoots is specified and the best evidence for these effects derives from the analysis of *Arabidopsis* mutants, either with altered levels of hormones or altered signaling (Frank *et al.*, 2000).

The action of auxin and cytokinin has been linked from early studies, as cytokinins were first identified by their ability to stimulate, in concert with auxin, cell division in cultured plant cells (Miller *et al.*, 1955). The ratio of cytokinins to auxin is known to determine the type of organs regenerated

from undifferentiated callus tissue *in vitro*: callus placed on media with a high cytokinin to auxin ratio produces mostly roots (Skoog & Miller, 1957). Since these initial discoveries, auxin and cytokinin have been shown to interact in several physiological and developmental processes, including apical dominance, control of cell cycle, lateral root initiation, regulation of senescence and vasculature development (Coenen & Lomax, 1997). The interaction of these two hormones can be synergistic, as is the case for the regulation of the cell cycle, or antagonistic, as is the case for the regulation of axillary bud meristems and the formation of lateral roots. In the present study caryopses inoculated on medium with low cytokinins to auxin ratio promoted induction of rhizome and subsequent development of rhizome into culm shoot.

There are many reports in which interaction of BAP with NAA has led to formation of storage organs. Grari and Backhaus (1987) reported *in vitro* propagation of red squill *Urginea maritima* in which bulblets were induced from bulb-scale culture in the dark on a medium containing MS salt supplemented with a combination of 0.5 μ M or 1.6 μ M NAA and 0.4 μ M or 1.3 μ M 6-BAP. The author noticed that the bulblet could be subcultured to regenerate adventitious shoots in medium containing 4.4 μ M or 13.2 μ M BAP and rooted in medium containing 0.5 μ M or 1.6 μ M NAA. Bulb-scale explants when subcultured on fresh medium containing both NAA and BAP regenerated new bulblets which were easily rooted. In the present study also, it was observed that on increasing concentration of BAP to 5 μ M and 25 μ M NAA in MS medium containing 5% sucrose, there was an increase in percentage and number of rhizome induction. It also promoted growth of culm shoots which ultimately rooted and form plantlets.

Gibberellins (GAs) are a class of phytohormones that impact various aspects of plant growth and development (reviewed in Fleet & Sun, 2005). For more than fifty years, GAs have been known for their dramatic impact on plant stature. Inhibition of GA biosynthesis results in dwarfism (Ninnemann *et al.*, 1964), whereas exogenously applied gibberellic acid promotes internodal stem growth (Brian *et al.*, 1954). Recent evidence suggests that GAs also play an important role in lateral root development. Mutants defective in GA biosynthesis (Berova & Zlatev, 2000) or signaling (Busov *et al.*, 2006) were found to have enhanced lateral root formation. Maheshwari *et al.* (1980) noted an interaction of GA₃ and IAA in the growth of excised *Cuscuta* shoot tips *in vitro*. GA₃ induced a marked elongation of shoot tips of *Cuscuta chinensis* culture *in vitro*. The response of hormones was dependent on exogenous carbohydrate supply. The growth response progressively decreased if GA₃ was given at increasingly later times after culturing, but the decreased growth response could be restored by the application of IAA to the apex. Explant deprived of GA₃ gradually lost the ability to transport IAA basipetally, but this ability was restored by auxin application. The results were explained on the basis that the growth of *Cuscuta* shoot tip *in vitro* required at least both an auxin and gibberellins and in the absence of gibberellins the culture shoot tip explants lose the ability to produce and / or transport auxin. Also Adhikari and Bajracharya, (1978) suggested that gibberellins play an important role in combination with auxin, in the initiation of root formation in *Pisum sativum* cuttings.

In the present study incorporation of GA₃ (0.1 μ M) along with optimal concentration of BAP and NAA enhanced the percentage of cultures showing rhizome formation. The stimulatory effect of GA₃ on the development of plants is well known, as it has been found to promote cell division and also GA₃ exerted its influence in rhizome development by inducing formation of intermediary nodes and subsequent development of culm shoots from these nodes in the rhizomes. GA₃ (0.1 μ M) acted in synergism with BAP and NAA in the process of *in vitro* rhizome formation. Hence the application of NAA at 25 μ M +5 μ M BAP+0.1 μ M GA₃ is recommended for optimum induction of rhizome and

development of rhizome into culm shoot. Experiments have demonstrated the existence of synergistic, antagonistic and additive interactions between cytokinins and auxin suggesting a complex web of signal interactions. These two group of growth regulators cross talk on the molecular level (Coenen & Lomax, 1997).

Due to a variety of reasons, the bamboos are difficult if not complex materials to work with and hence the paucity of knowledge on many basic aspects, including anatomy (Esau, 1965; 1977; Fahn, 1967; Cutter, 1971). This paper report on certain anatomical characters on origin, growth and development of rhizome of *Dendrocalamus strictus* (Roxb.) Nees.

In bamboo rhizome consists of nodes and internodes. From the nodes adventitious roots develop with root hairs as outgrowth of the epidermis for the uptake of water and mineral elements. However, the adventitious roots develop not only from the rhizome, but also from the culm base, which bears another root system and performs the same functions as the roots from the rhizome. The bamboo culm has distinct nodes and internodes. The usually empty inside is called medullary cavity and the walls surrounding are called culm walls. The developing bamboo wall is divided beginning outside into: bamboo outer-skin, bamboo pulp, and bamboo-inner skin. But from an anatomic point of view it is divided into epidermis, derma, ground tissue, vascular bundles and cavity wall (Wen & Zhou, 1984; Fang, 1989).

The gross anatomical structure of a transverse section of bamboo culm internode and rhizome is determined by the shape, size, arrangement and number of the vascular bundles. They are clearly contrasted by the darker colored sclerenchymatous tissue against the paren- chymatous ground tissue (Liese, 1998). At the peripheral zone of the culm the vascular bundles are smaller and more numerous and in the inner parts larger and fewer. Within the culm wall the total number of vascular bundles decreases from bottom towards the top, while their density increases at the same time. The culm tissue is mostly parenchymatous and the vascular bundles which are composed of vessels, sieve tubes, companion cells and fibres (Liese, 1998).

Similar to above the findings, in the present study the transverse section of *Dendrocalamus strictus* culm shoot revealed that the epidermis of the rhizome is made up of long and short cells. Beneath the epidermis lies the hypodermis which consists of two to three layers of sclerenchymatous cells. They are substituted under the stomata by parenchymatous cells as passage for gas exchange. The vascular bundle of rhizome consists of xylem and phloem. The xylem consists of one or two small protoxylem elements and two large metaxylem vessels. The phloem contains large sieve tubes with companion cells. The sieve tubes are characterized by numerous sieve elements on the lateral walls.

The rhizome has a smaller diameter and shorter internodes than the culm. The anatomical structure of *D. strictus*, as revealed in transverse section, is basically similar to the culm. Distinct differences, however, exist regarding a thick cortex, random orientation of vascular bundles, poorly developed fibre strands, absence of a pith ring, and only a small pith cavity in rhizome.

In the present study the origin and development of rhizome was traced by using anatomical technique as described in the material and methods. In rhizome inducing medium promeristematic region was identified after 4 d of culture. Rhizome primordium could be observed after 8 d of culture from the axil of emergent cotyledon. After 12 d of culture in rhizome inducing medium, the rhizome primordium flanked by leaf primordia were formed. This marks the origin and direct continuation of rhizome from the epicotylar end of hypocotyl.

Sharma (1976), described the origin and development of the rhizome of *Dioscorea deltoidea* Wallich, which is similar to the origin and development of bamboo rhizome as seen in the present study. According to him, the rhizome is perennial and digitately branched. It is cylindrical, woody and scaly. It bears adventitious roots emerging out of the scale leaves. The pointed rhizome apices are covered with scale leaves (prophylls). The first plumular leaf subtends a perennial bud. Its axillary position is established by the presence of leaf trace away from the plumular axis. Later on an accessory bud is initiated between the perennial bud and subtending scale leaf. Abnormally a bud may arise in the axil of emergent cotyledon if growth of the plumule is retarded. From the hypocotyl the primary thickening meristem extends beyond the cotyledonary node into the highly stunted first plumular internode and to the base of the perennial bud. This marks the origin and direct continuation of rhizome from the epicotylar end of hypocotyl. The primary thickening meristem extends further into the hypopodium (shoot axis below the insertion of prophyll) and to the base of new perennial bud. Everytime this step is repeated and an extremely short segment is added on to the rhizome. Thus rhizome is formed by the hypopodia of successive shoots, with the first rhizome segment normally consisting of the hypocotyl and basal plumular internode. The sympodial nature is shown by the position of vascular bundle of each prophyll opposite the axis bearing it. The primary thickening meristem is confined to the rhizome, following the perennial bud closely. Due to rapid secondary growth, the rhizome attains its current thickness just behind the apex.

In the present study rhizome primordium was initiated from the lowermost node of the shoot which further developed into rhizome and culm shoot on rhizome inducing medium. However, on MS medium devoid of growth regulator only axillary bud meristems flanked by catphylls could be observed. This finding is in accordance with the description of perennial grasses as described by McIntyre (1967). He reported that in perennial grasses of rhizomatous habit, rhizomes and tillers are of similar origin in so far as both are initiated from axillary buds on the parent shoot. They differ, however, in that rhizomes are normally produced from basal buds whereas tillers arise from buds at higher nodes on the stem. The axillary buds on the shoot are potentially capable of developing either as tillers or as rhizomes and their path of development are determined to a considerable extent by environmental conditions. It is noted however, that tillers are normally produced from buds at higher nodes on the shoot than those which develop as rhizomes and when bud activity is reduced by unfavourable condition it is generally the bud nearest the base of the shoot, i.e. at the coleoptile node, whose growth is inhibited. Thus the pattern of bud development is apparently determined by an interaction between the environmental conditions and certain other factors which are related to the bud's position on the shoot (McIntyre, 1967).

In relation to the shoot apex of rhizome, according to Santos and Silva (1997), the monocotyledons often presented a one-layered tunica while the region of the corpus comprised various cellular strata, which exhibited divisions at all levels. In *Scleria*, a cellular layer consisting of the tunic and two or three cellular strata comprising the corpus were also observed in the promerismatic region. Similarly, longitudinal section of the *D. strictus* rhizome showed prominent apical dome covered with leaf sheaths. The apical dome of rhizome showed upper one tunica layers composed of rectangular cells which has intensely stained nuclei. Tunica enclosed inner two-three corpus layers composed of small randomly dividing cells. Apex was covered by leaf primordia.

For studying the localization of starch in rhizome induction MS medium supplemented with NAA, BAP and GA₃, histochemical examination was done by PAS. On 4 d of inoculation, the longitudinal section of seedling exhibited starch grains in parenchymatous cells near the base. After 8 d of culture, starch grains in large aggregates were observed in the parenchymatous cells in the peripheral region of

the basal node. By this time, rhizome had already initiated from the lowermost node of the seedling. It was only after 12 d of culture that cells in the cortical region of the rhizome exhibited starch grains in clusters while peripheral region showing conspicuous depletion of the starch grains due to development of rhizome on MS medium supplemented with NAA, BAP and GA₃. Similar to our findings, Thorpe and Murashige (1968) performed microscopic histochemical examinations of cultured tobacco callus and observed a strong correlation between starch accumulation and shoot initiation in tobacco callus cultures. They observed that the tissue which was destined to produce shoots had an abundance of starch granules of various sizes. They further observed that the tissue not forming organs had accumulated considerably lesser starch. Mangat *et al.* (1990) reported that the accumulation of starch and its subsequent disappearance from the cells of developing shoot primordia and the subjacent tissue suggests that starch was used both during organ initiation and its later development. The degradation of starch resulted in formation of glycolytic intermediates, the subsequent oxidative catabolism of these intermediates yielded high amount of ATP which then became available for cellular metabolism.

References

- Adhikari, U.K. ; Bajracharya, D. 1978. Interaction of gibberellic acid and indole-3-acetic acid on root formation in pea (*Pisum sativum* L.) epicotyl cuttings. *Planta*, 143, 331-332.
- Berova, M. ; Zlatev, Z. 2000. Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). *Plant Growth Regulation*, 30, 117-123.
- Brian, P.W.; Elson, G. W.; Hemming, H.G. ; Redley, M. 1954. The plant growth promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. *J. Sci. Food Agric.* 5, 602-612.
- Busov, V.; Meilan, R.; Pearce, D.W.; Rood, S.B.; Ma, C.; Tschaplinski, T.J. ; Strauss, S.H. 2006. Transgenic modification of *gai* or *rgll* causes dwarfing and alters gibberellins, root growth, and metabolite profiles in *Populus*. *Planta*, 224, 288–299.
- Coenen, C. & Lomax, T.L. 1997. Auxin-cytokinin interactions in higher plants: old problems and new tools. *Trends Plant Science*, 2, 351-356.
- Cutter, E. 1971. *Plant Anatomy: Experiment and Interpretation*. Addison-Wesley, London.
- Esau, K. 1965. *Plant Anatomy*. John Wiley & Sons, New York.
- Esau, K. 1977. *Anatomy of Seed Plants*. John Wiley & Sons, New York.
- Fahn, A. 1967. *Plant Anatomy*. Pergamon Press, Oxford. Gilliland, H.B. 1971.
- Fang, W. 1989. A contrastive anatomy of certain domestic bamboo timbers. *J. Bamboo Research*, 8, 1-11.
- Fleet, C.M. ; Sun, T.P. 2005. A DELLAcate balance: the role of gibberellin in plant morphogenesis. *Curr. Opin. Plant Biology*, 8, 77–85.
- Grari, R.E. ; Backhaus, R.A. 1987. *In vitro* propagation of Red Squill, *Urginea maritima* Baker. *Plant Cell Tissue Organ Culture*, 10, 65-71.
- Gupta, A.K. 2008. National Bamboo Mission: A holistic scheme for development of Bamboo Sector in Tripura. *Indian Forester*, 134, 305-324.
- Jensen, W.A. 1962. *Botanical Histochemistry: Principles and Practice*. W.H. Freeman & Co., San Francisco, London.
- Liese, W. 1998. *The Anatomy of Bamboo Culms*. INBAR Technical Report. International Network for Bamboo and Rattan, Beijing. 204.
- Maheshwari, R.; Shalini, C.; Veluthambi, K. ; Mahadevan, S. 1980. Interaction of gibberellic acid and indole-3-acetic acid in the growth of excised *Cuscuta* shoot tips *in vitro*. *Plant Physiology*, 65, 186-192.
- Mangat, B.S.; Pelekis, M.K. ; Cassells, A.C. 1990. Changes in the starch content during organogenesis

- in *in vitro* cultured *Begonia rex* stem explants. *Physiology Plant*, 79, 267-274.
- McClure, F.A. 1966. *The Bamboos - a Fresh Perspective*. Harvard Univ. Press, Cambridge, Massachusetts.
- McIntyre, G.I. 1967. Environmental control of bud and rhizome development in the seedlings of *Agropyron repens* L. Beauv. *Canadian J. Botany*, 45, 1315-1326.
- Miller, C.O.; Skoog, F.; Von Saltza, M. H. ; Strong, F. 1955. Kinetin, a cell division factor from deoxyribonucleic acid. *J. American Chemical Society*, 77, 1392.
- Murashige, T. ; Skoog, F. 1962. *A revised medium for rapid growth and bioassays with tobacco tissue cultures*. *Physiologia Plantarum*, 15, 473-497.
- Santos, G.O. ; Silva, E.A.M. 1997. Growth and development of rhizome of ginger (*Zingiber officinale* R.). *Arq. Biol. Technol.* 40: 651-656.
- Sharma, O.P. 1976. Anatomy, origin and development of the rhizome of *Dioscorea deltoidea* Wallich; *Proc. Indian Acad. Sci.* 84, 50-55.
- Shimasaki, K. ; Uemoto, S. 1990. Micropropagation of terrestrial *Cymbidium* species using rhizomes developed from seeds and pseudobulbs. *Plant Cell Tissue Organ Culture*, 22, 237-244.
- Skoog, F. ; Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. Exp. Biol.* 11, 118-131.
- Taiz, L. ; Zeiger, E. 2003. *Plant Physiology*. 3rd edn. Sunderland, M.A. Sinauer Associates, Inc.
- Thorpe, T.A.; Murashige, T. 1968. Starch accumulation in shoot-forming tobacco callus culture. *Science*, 160, 421-422.
- Wen, T.H.; Zhou, W.W. 1984. China bamboos: Vascular bundles in anatomy. *J. Bamboo Research*, 3, 1-4.

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Temperate bamboos in ornamental horticulture: differentiators and spillover effects into the 21st century.

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Abstract

To understand the valorization chain of ornamental bamboos in ornamental horticulture, the historical foundations and specific differentiators that made mass production and marketing of ornamental bamboos possible are key. Based on a long tradition, these differentiators comprise the implementation of research results into propagation and production methods, together with modern (and very demanding) methods of marketing and branding with an increasing focus on clumping (non-invasive) bamboos. Such quality standards are much more demanding than for bamboo production in traditional forestry, but the same differentiators that drive ornamental bamboo, can lead to positive spillover effects, with applications of the developed technologies for mass propagation and genetic improvement for the 21st century in forestry and agroforestry with bamboo.

Outline of the paper

The production and trade of bamboo in ornamental horticulture has been increasing rapidly in the past decades. Sales have been rising year after year. The market for ornamental bamboos in Europe has increased by a factor of at least ten compared in the past 15 years, and an estimated 5 million bamboos are produced and sold today, not only as garden plant, but also for terraces and balconies and for public greening. More and more growers produce bamboo in larger numbers and of a quality that is much better than ever. Whereas bamboos were sold almost exclusively in garden centers earlier during limited periods of the year, top quality bamboos are now supplied year-round into markets and distribution channels that have been created by the ornamental industry for foliage and flowering plants in the past. This has required a complete rethinking of the supply and logistics chain of bamboo for a year-round production of top quality plants, in pace with the demanding developments in horticultural industry.

A quantification of the valorization chain of ornamental bamboos from real economic data is virtually impossible, neither in Western Europe nor in the USA. In trade of bamboo products, the plants are no separate category. Within horticulture, with cut flowers and vegetables as major products ornamental garden plants is only a minor part. And within this sector, bamboo and ornamental grasses constitute only a minor part of garden and orangery plant production. Partial market data are available but give no insight in the development of the sector. A qualitative approach, rather than a quantitative may be more valuable.

To understand the valorization chain of ornamental bamboos in ornamental horticulture, focus can be placed on the historical foundations and specific differentiators that made mass production and marketing of ornamental bamboos possible. Based on a long tradition, these differentiators comprise the implementation of research results into propagation and production methods, together with modern (and very demanding) methods of marketing and branding with an increasing focus on clumping (non-invasive) bamboos. Such quality standards are much more demanding than for bamboo production in traditional forestry, but the same differentiators that drive ornamental bamboo, can lead to positive spillover effects, with applications of the developed technologies for mass propagation and genetic improvement for the 21st century in forestry and agroforestry with bamboo. This analysis will draw for a large part on the research & development strategies and experiences of Oprins Plant of the past 25 years.

Ornamental horticulture in the West

The current trend, a wave of interest in bamboo as ornamental plant for gardens in Western Europe and to a lesser extent in the USA, is a revival rather than a new trend. Its foundations go a long way back. Horticulture and landscaping have been dominant factors of European culture mainly in the four past centuries. From the 17th century onwards there was a huge increase in collections from Asia and the New World, hotspots of botanical diversity.

Bamboos, as giant grasses, although already known in Europe from ancient times as Indian Reeds, attracted particular attention. The first known introduction of temperate bamboo into Europe (England) was the remarkable black bamboo *Phyllostachys nigra* around 1827 (timeline comparison: Belgium became an independent nation in 1830). It was reintroduced in France in 1846, and since then collectors have introduced various temperate and tropical bamboos into Europe (Houzeau de Lehaie, 1906, 229-230).

At the turn of the century, moving into the 20th century, there were many public gardens with bamboos all over Europe, from Scotland to the Black Sea, and many more private collectors, as can be read in “*The introduction, acclimation and culture of bamboos in Western Europe and esp. in Belgium*” (Houzeau de Lehaie, 1907, N°9&10, page 227). The most beautiful specimen were found in the

botanical gardens of Italy (according to H.d.L.), and at that time the bamboo collection in the botanical garden of Edinburgh had over 40 species of bamboo, more than Kew. The Bambuseraie in Prafrance was one of the many private gardens. It had been established in 1855 by Eugène Mazel, and came into the hands of the Nègre family in 1902.

Actually history is always a good lesson for those who think that taxonomy, or science and technology started only yesterday. Bamboo has been used in technology long BCE in the Orient, but also in the West it was used in cutting edge innovations in technology. For example, in 1854 German Henricg Globel used a carbonized bamboo filament placed inside a glass bulb. He thus invented the first true light bulb. Edison had experimented with light bulbs from the 1870's onward and around 1880 he improved his light bulb until it could last for over 1200 hours using a bamboo-derived filament. In 1909 the Brazilian Santos-Dumont built an aeroplane (Demoiselle) made with linen and bamboo.

The 19th century was also a golden age for bamboo taxonomy, with scientific descriptions of many genera and species from all over the world. The genus *Bambusa* was described by Schreber in 1780 (herbaceous bamboo *Olyra* was described by Linneaus in 1759) but the majority of genera was described in the 19th century. Ohrnberger's *Bamboos of the World* (1999) also has a list of 'Generic Names in Chronological Order'. Names of botanists like Ruprecht, Trinius, Munro, Gamble, Jussieu, Bentham, Kunth, Franchet are still very well known. *Arundinaria* was described by Michaux in 1803, *Phyllostachys* by Siebold and Zuccarini in 1843 and *Fargesia* in 1893 by Franchet.

In the early twentieth century, Jean Houzeau de Lehaie (Mons, Belgium) published the first bamboo journal *Le Bambou, son Etude, sa Culture, son Emploi*. The opening paragraphs clearly describe the motivation (in French, English and German):

“Before creating the present periodical, we considered carefully if it would not be better to ask from one of the influential horticultural papers, to insert our notices under a special title. But we feared, on one side that the paper should not reach the persons of different nations whom concern bamboo ; on the other side, that the necessarily little room granted to this one underfamily should nearly drown our notices among other articles.

So we resolved to issue a periodical bulletin – probably monthly – totally kept to the study of Bamboo on every way: scientific, horticultural and commercial and to the general questions thereto belonging. Our aim is the facility for botanists and lovers of Bamboo of communicating their studies and desiderata and exchanging their observations”.

Le Bambou, published from 1906-1908, is still a very rich source of information on the various collections and bamboo lovers in Europe and of the growth of bamboo in Belgium. It documents the intense communication between 'lovers of Bamboo'. In this period many books were already available: *A Monograph of the Bambusaceae* by Colonel Munro in 1866, *Les Bambous* by A. and C. Rivière in 1879, *Japanese Bamboos and their introduction into America* by David Fairchild in 1903, *The Bamboo Garden* by Freeman Mitford in 1896, and later *Les Bambusées* by Camus in 1913. Bamboo collectors, bamboo as ornamental for gardens, bamboo networks, journal and newsletters are not new at all.

At L'Ermitage, Houzeau de Lehaie cultivated well over 100 different types of bamboo and described various species of *Phyllostachys*. His efforts should be viewed in a general horticultural framework in Belgium. In 1968 Robert De Belder gave his impression on the development of horticulture in Belgium as follows (Adriaenssen, 2005):

“The nineteenth century was definitely the Golden Age for horticulturists in Belgium. Even more than in other European countries, many people were concerned with horticulture and were cultivating all sorts of exotic plants. At that time, an impressive number of species and varieties, usually tropical and subtropical ones, were introduced into the country. People kept these plants under glass and every large house in the country-side had its greenhouse: a hothouse, a temperate house and an orangery.

We had in the nineteenth century several keen and famous botanists and horticulturists; Names like Louis Van Houtte, Ambroise Verschaffelt, Van Hulsten, Morren, Bijls and Donckelaer are still well known in the horticultural world of today. An amazing number of periodicals were also published in Belgium during that century. They were publications of the highest standing, with good lithographic plates, comparable with the best ones in this country. Let us mention just a few: Flore des Serres et des Jardins de l'Europe, L'Herbier de l'Amateur de Fleurs, Journal Horticole, La Belgique Horticole, Reveu de l'Horticulture Belge and Illustration Horticole. Unfortunately, these lovely publications were too ambitious, and had to be discontinued. However they are still of the highest documentary interest for the study of horticulture”.

On his worldwide trip, David Fairchild (1947) was particularly charmed by Belgium:

“Belgium is full of horticultural interest, for the Belgians as a people are interested in plants. If they were not, one would not find everywhere all sorts of fruit trees espaliered against the walls of their houses. If you have ever tried to espalier a tree against a wall and every spring and every summer pruned back its hundreds of branches, or pinched out the buds which were not in the right place, you will be in a position to understand the Belgium patience in matters of plant culture. They seem to love to garden. They do not seem to be so restless as Americans, they love to stay at home and they build the romance of their lives right about them where they are, instead of trying to get it by gadding about over the surface of the earth....

*M. Charles Vuylsteke has spent his life in a comfortable but modest house just off the main street of Lochristi. Behind his dwelling are the orchids houses, where, for over fifty years he has spent most of his waking hours, for he began young with a passion for orchids. He cross-pollinated a species of *Odontoglossum* from the mountains of tropical America with *Cochlioda nutziana*, an insignificant looking orchid from Peru.... The result was a beautiful new hybrid. From that time on he has been hybridizing orchids and sending the flowers to the great cities of Europe, where he charges his own price for them. He became one of the best-known men in the horticultural world, at least in that part which is interested in orchids. Never in my life have I seen such beautiful masses of orchids in bloom, and the handsomest of all were the *Odontiodas*, which had originated through the skill of this quiet, unostentatious man. When one thinks of the pleasure his orchids give, and of how they are worn at banquets and balls by the most beautiful women of society, is it surprising that M. Vuylsteke found a great satisfaction in having been the means of bringing such beauty into existence? He has the quiet lasting satisfaction of having created something, rather than the evanescent one of having merely seen something.”*

Creating something new for a lasting satisfaction is deeply encoded in our genes. The twentieth century however was a dark period for Europe with World Wars I and II and the economic crisis of 1930, with a major impact on horticulture. De Belder:

“The Belgium amateur was mainly interested in greenhouse plants, unlike his British colleague, who collected all kinds of hardy plants in his garden. Perhaps as a result of this, practically nothing is left in Belgium of that glorious period. Devastation during two world wars, the economic crisis of 1930 and the evolution in taste and fashion are responsible for this situation.

*Most of the nurseries that provided the Belgian and foreign amateurs with plants were located at Ghent. This town is still an important nursery centre. However, the collections have disappeared and only commercially grown plants are produced: *Ficus elastica*, *Clivia*, *Sansevieria*, *Azalea indica* and *tuberous begonia's*”.*

Robert De Belder's quote shows not only regional differences (and similar stories could be told for other countries as well), but points to 1) the devastations of large collections in the 20th century, 2) the revival of ornamental horticulture but with focus on a very limited number of commercial plants.

Things have changed however, and in the forty years following Robert De Belder's 1968 address to the Royal Horticultural Society (of which he was vice-president), we have witnessed an explosive growth of plant collections and awareness, and the applications of fundamental research for mass production and genetic improvement of ornamental plants.

Botanists, plant collectors and plantsmen

Only in a limited number of collections, mainly botanical gardens, collections of bamboo survived the world wars (Prafrance, Kew,) and only from the seventies onwards we witness an important increase in the interest in bamboo as an ornamental plant in Europe with the World Bamboo Congress at Prafrance in 1988 and the establishment of various national bamboo societies in the 1980's. Exchanges between plant collectors and botanical gardens (both public and private) intensified as well. Through connections between Belgium and Japan, the garden of Hemelrijk (private domain of Jelena and Robert De Belder) obtained about 69 different bamboo species, and Harutsugu Kashiwagi, the later director of the Fuji Bamboo Garden with over 450 species, came to Hemelrijk as a student (H. Van Trier, pers. comm.; Borin, 1995). To be sure, such exchanges were not only important for bamboo, but for other plant groups as well.

The commercial side for bamboo was initially driven by a limited number of larger players, with as distinguishing feature the entrepreneurial drive of Yves Crouzet, Wolfgang Eberts and Jan Oprins and others. However, it is also due to the enthusiasm of many collectors and gardeners that the current trend in bamboo sales finds its origin. Evolving from a limited number of species to the explosive growth of number of bamboo species into horticulture and horticultural trade has largely been the work of plant enthusiasts and collectors. It is here also that the 19th century spirit of collection and showcasing is found once more. One of the notions is that of a *plantsman*. David McClintock (Raphael, 1979) gave a simple definition:

"A plantsman is one who loves plants for their own sake and knows how to cherish them. This... concept... may include a botanist: it certainly includes a host of admirable amateurs who may not know what a chromosome looks like or what taxonomy means, but they know the growing plant, wild or cultivated, first-hand. To my mind they are the cream of those in the plant world, a fund of invaluable first-hand information."

Building on traditions, Europe has some past and present notable plantsmen, like David McClintock and Jelena and Robert De Belder; the De Belder's biography (Adriaenssen, 2005) is a must read for any plant lover. In the bamboo world *plants(women)* like Houzeau de Lehaie, Gaston Nègre, David McClintock, Peter Addington, Max Riedelsheimer, Susanne Lucas, Jacques Van Dooren and Jos Van der Palen, are well known to those who witnessed the development of bamboo (and many names could be added, also from the USA). In many famous societies they play an important role. David McClintock was not only president of The Bamboo Society, the Heather Society and the Wild Flower Society, but also vicepresident of the International Dendrological Society, which was founded by Robert and Georges De Belder. Susanne Lucas served long as ABS's president, founded the World Bamboo Organisation and is a board member of the International Plant Propagators Society.

Their roles, in view of the valorization chain of bamboo, include collecting and disseminating genotypes, networking and evangelizing or including bamboos in important trade shows. One of Belgium's oldest organizations on ornamental plants organizes the Floralties of Ghent every five years, a world-class event, where Jan Oprins now serves as its director. In 1906 Houzeau De Lehaie had convinced the organizers to include bamboo in the Floralties of 1908, but unfortunately spring was too

early to have good foliage. The production methods have now allowed for excellent bamboo quality year round, so that in the most recent edition bamboos dominated the Floralties and obtained the second prize in “loveliest ornamental grasses”.

The role of collectors and plantsmen is not that of a scientist or botanist and very often this has led to incongruences. For example in *The Bamboos*, published on the occasion of the international congress on bamboo by the Linnean Society in 1996, a valuable contribution of David McClintock was not deemed valid for publication (It was then published in Dutch translation in the *Newsletter of the Belgian Bamboo Society*; McClintock, 1996). But their role remains decisive and their observations and conclusions help decide which plants can be successful for our gardens. David Fairchild’s (1947) continues:

I am sure there are scattered through Belgium many such plantsman as this orchid hybridizer (Charles Vuylsteke), but only one other was I fortunate enough to see, Houzeau de Lehaie who has gathered around his ancestral château at Mons a rare collection of bamboos. He has acquired these mainly by correspondence and through his acquaintance with the directors of botanical gardens all over Europe. He has set them out in the excellent soil of his garden wherever he had room for them, and through that knowledge one gets from growing plants one's self, he has become an authority on those species which are hardy in Europe. The bamboos are, of all plants, perhaps the most difficult to classify, for they bloom very infrequently, sometimes waiting forty years before doing so, yet the systematic classification of a species must be based on the characters in the flowers. If one were to try to classify the bamboo material found in any of the gardens of Europe, from the specimens of dried leaves, such as are generally all that one finds in the herbaria, one would make a sorry mess of one's classification. In fact, the bamboos are in a mess, scientifically speaking. Houzeau de Lehaie has done, perhaps, as much as anyone to clear this up, so far as the hardy species are concerned, by quietly studying the growing plants of his hobby and observing their relationships, and the world has had to turn to him for advise.

It is very often the case that botanists have a lesser working knowledge of the plants proper they work with (or at least another kind of knowledge) than plantsmen, but that does not mean that there is no hope for botanists. They can develop “a feeling for the organism”, title of a biography of Nobel Prize winning plant scientist Barbara McClintock, who unveiled the dynamic nature of the genome through her studies of genetics of corn (Fox Keller, 1987).

Plantsmen, botanists, growers, plant collectors... have greatly contributed to a growing awareness about bamboo as ornamental plants. To such list taxonomists like Thomas Soderstrom, Lynn Clark and Chris Stapleton may be added, or bamboo scientists like Floyd Alonso McClure, Walter Liese, Jules Janssen. *The Bamboo Book – a comprehensive guide to this remarkable plant, its uses and its history* by David Farrelly became an instant classic. *The horticultural bamboo species of Japan* (Okamura and Tanaka, 1986), *Colored Illustrations of Bambusoideae in China* (Wen, 1993), *A Compendium of Chinese Bamboo* (Shilin et al., 1994) and *American Bamboos* (Judziewicz et al., 1999) became important references for bamboo enthusiasts and growers, often combined in one person.

Scientists, plantsmen, architects, designers and writers all contributed to the awareness of bamboo in the West. Europe had been building its own traditions with many books published in the last two decades, in various fields. Bamboo has become an important brand name and a household name, which has also been important in difficult times. When for example all *Fargesia murielae* plants died in the 1990’s due to mass flowering, this had little or no effect on the growing production and sales of bamboo.

Applications of research in ornamental horticulture

The development of novel propagation and production methods

Having built its own bamboo tradition and narratives, a second major differentiator in Europe was the reduction of ornamental plant production to just a few commercially interesting species as noted by Robert De Belder. This has perhaps been the major driving force for the rapid development of ornamental horticulture in The Netherlands and Belgium with the application of fundamental research in horticulture. The two major parts are methods for mass production (greenhouse technology and micropropagation) on the one hand, and for genetic improvement (breeding, molecular markers, plant tissue culture techniques and genetic transformation) on the other.

In my view it is important to address these issues since for the 21st century there can or will be important spillover effects: technologies developed and perfected in ornamental horticulture may be used for developing methods for both mass propagation and genetic improvement of bamboos in agroforestry and forestry for tropical and temperate regions.

In the past 50 years insights in plant physiology were translated into efficient greenhouse technologies (control of humidity, water, light, CO₂, temperature...), the application of plant growth regulators (auxins for rooting of cuttings, control of flowering and ripening by ethylene, cytokinins for increased branching in fruit trees, or the use of growth retardants to control plant shape and quality, are just a few examples), the use of efficient fertilization methods (inert soils, hydroponics, slow release fertilizers...) and methods for pest and disease control. These greenhouse technologies became a major driving factor for the increase in production and quality, the Green Revolution in horticulture. A good production planning and supply chain are, on the one hand, the result of automation (e.g. of climate control), but on the other also a prerequisite for automation in quality control, sorting and moving plants.

Nevertheless, the most distinguishing feature of ornamental horticulture has not been the production methods, but the methods for propagation of indoor and outdoor ornamental plants. The whole sector of foliage and flowering plants has been driven largely by the development of micropropagation methods (the use of plant tissue culture methods for plant propagation). It became possible to produce millions of plants from selected genotypes in well-controlled conditions, leading to high quality and low cost plants, and this completely transformed the valorization chain and the horticultural business and markets. Expensive orchids became cheap plants, available to anyone.

In the 1970's the plant tissue culture laboratories of P.C. Debergh in Ghent and of R.L.M. Pierik in Wageningen became two of the world's leading schools for tissue culture research, and developed basic propagation protocols for *Ficus*, *Gerbera*, bulbous plants, ferns, Araceae, Maranthaceae, Bromeliaceae and many other plants. These protocols were adopted and adapted by several private laboratories and this rapidly led to the production of millions of plants. They also provided lab space for companies to develop their own protocols as in the case of bamboos. Their contacts and influence opened doors worldwide, and in the early nineties bamboo co-operations emerged with Thailand (Gavinlertvatana and Prutpongse, 1991) and with India (TATA Energy Research Institute, on somatic embryogenesis of *Dendrocalamus* species).

Today these vibrant centers of Ghent and Wageningen are still major hotspots of plant biotechnology with a combination of fundamental research and commercial spinoffs. In Ghent the first genetically transformed plants were produced (Van Montagu, 2011), which initiated the explosion of plant molecular biotechnology for the 21st century.

In 1990 the world production of micropropagated plants was roughly estimated to be 500 million units (Debergh, 1994) of which the majority were ornamental plants. In that period the production in Belgium was about 28 million (Debergh et al., 1990) on a total production of 212.5 million in Western Europe (Pierik, 1991). Two decades ago, Belgium was world leader in the micropropagation of ornamental trees, agricultural crops and herbs and second (after The Netherlands) for pot plants and garden plants, which illustrates the central role of the Low Countries in the development of ornamental

horticulture. In the last two decades micropropagation of ornamentals has certainly doubled with many labs around the globe (actually orchids are the main product from tissue culture worldwide). With a worldwide rise in number of plant tissue culture laboratories (In the 1980's Asia had around 150 commercial plant tissue culture operations and many more plant tissue culture labs in universities and research institutions; Gavintlerwatana and Prutpongse, 1991), companies in Western Europe had to specialize and automate. In 2010 the production in Belgium has more than doubled and 8 laboratories produce 65 million plants per year and five of these labs produce over 5 million plants each (Dhooghe et al., 2011). Over 50 million of these are pot plants and orchids, and over 10 million of the plants produced are garden trees and shrubs, perennial plants, including ornamental grasses and temperate bamboo. From Dhooghe et al. (2011):

When one looks more closely to the classification in plant families, the largest production of plants are species of the Bromeliaceae family (37.7%), followed by the Orchidaceae (17.4%) and Ericaceae (15.4%). The top ranking of the Bromeliaceae regarding production numbers is not unexpected since two world-leading breeding companies of Bromeliaceae are located in Flanders. Although the percentages of some other plant families are relatively minor, the impact on the world production might be high. For example, the majority of the world's micropropagated winter-hardy bamboo and some genera of the Maranthaceae originate from Flemish companies.

Seven stages

Annually, over 1 million temperate bamboos are produced through micropropagation in Belgium, and as for most ornamentals the techniques used are axillary branching, which mimicks the natural process of division in bamboos. Axillary branching induces some rejuvenation (with a seedling-like vigorous growth) but it is the preferred method since the danger of genetic or epigenetic modifications are much smaller compared to organogenesis or somatic embryogenesis, where intermediate callus formation is needed. The latter methods are more useful for forestry, not for horticulture.

It is no exaggeration to say that every lab worldwide has at some point experimented with bamboos in tissue culture. In many countries various groups have developed efficient methods for mass production of tropical bamboos for forestry (for various references Fernandez and Gielis, 2003). In some cases commercial production of temperate bamboos was relatively successful, e.g. at Thai Orchids Labs (Prutpongse and Gavintlerwatana, 1991) and Piccoplant (Germany). Various other labs had one or more species of temperate bamboo, mainly species of *Sasa* or *Pleioblastus*. But all in all the success for temperate bamboos was quite limited. The lack of success is an indication that it is not easy to develop promising research results into successful production systems. Even today, in the scientific literature there are only a few papers with successful protocols. With successful is meant: starting from selected mature plants, with good and stable multiplication rates and transplanting success in the greenhouse between 95 and 100%. In additions, rooting in the greenhouse and re-growth after potting should be fast and reliable, independent of season or other influences. It is a fact that the bar in ornamental industry is considerably higher than in academic research, where still many papers are published with e.g. a low success rate for rooting. Such protocols are simply useless for commercial purposes in horticulture (Gielis and Oprins, 2002). Various recently published papers report findings that have been explored in private laboratories.

Apart from the fact that scientific research did not yield expected results, the integration of tissue culture into a complete ornamental production chain presents its own difficulties in bamboo. Micropropagation has completely changed the markets and valorization chains in ornamental horticulture. Up till then the whole production chain was done *intra muros* (mother plants, cutting, liner and saleable plant production in one single company), but from the seventies, the production chain was cut up into pieces with specialized tissue culture laboratories, young plant growers (from

tissue culture plants to liners) and growers for saleable plants. In the whole ornamental value chain this has led to mass production of plants at extremely low prices, a few cents or less in some cases. As a rule of thumb, the cost of hardened tissue culture plants is less than 10% of the final sales price (and decreasing).

There were some special issues to be dealt with when integrating bamboo micropropagation into any successful scheme for plant production for the ornamental market. Unlike foliage plants, with a long tradition, bamboos were found only in specialized trade. To open new markets, beyond garden centers, for mass sales of low cost bamboo plants of high quality required going against the trend of ornamental horticulture, namely to keep everything in house, from selection to production and marketing of saleable plants, not tissue culture plants.

Classically, micropropagation is limited to four stages (Debergh and Read, 1991) with Stage 0 the *ex situ* conservation of elite plants, Stage I, the initiation stage, involving all subcultures until a constant and stable multiplication rate is obtained. In Stage II about a 1000 plants per species enter the production line and multiplication rates vary according to species from 3-10 every 3-4 weeks depending on the species (in practice multiplication is kept below 6). Stage II is the preparation of tissue culture plants for transplanting into the greenhouse, for hardening and rooting.

In the classical scheme of tissue culture laboratories, the tissue culture phase terminates at Stage III, but in regard to marketing of plants one can also distinguish subsequent stages: (1) Stage IV, the transplantation stage with the end product a rooted plantlet in trays, (2) Stage V, the production of liners, either for production of saleable plants or for use as micro-motherplant, and (3) Stage VI, the production of saleable plants. This distinction (seven stages) is important if the complete chain of production is integrated in a single company, since this determines the added values. In a mass scale production chain micropropagation, hardening, the development of liners, and the growth to saleable plant is then fully integrated.



Figure 1 Hardening of micropropagated bamboos and the production of liners

As an additional advantage it has allowed to assess the quality up to the final stages of production. It is also important to study out the long-term effects: while it is true that the micropropagated plants are rejuvenated, with more and smaller culms per pot (which is a major advantage from a logistics and marketing point of view) and grow initially like seedlings with more culms. They follow the natural

development from seedling to mature plants. Recent independent long-term observations in German gardens (Schütte, 2011) indicate that these plants are very vigorous, like seedlings:

“I have planted Lab-Fargesia’s in various gardens and I have never observed any negative effect of their origin. On the contrary, these plants, starting from small initial plants, always surprise by their enormous vitality and vigorous growth.”

These observations on BambooSelect™ plants (see 4.4) showed that the adult plants grew true-to-type, without negative effects, also not of flowering. Although flowering has been a major concern in the trade, in 15 years of commercial bamboo production through tissue culture with a production of well over 10 million plants, we have never encountered flowering in *Fargesia*.

To assess the long term stability and genetic fidelity at the molecular level we have used various types of molecular markers: Random Amplified Polymorphic DNA (RAPD) markers (Gielis, 1995), Amplified Fragment Length Polymorphisms (AFLP) makers (Gielis et al., 2002), methylation sensitive AFLP (MSAP) markers to assess epigenetic effects (Gillis et al., 2007) and measurements of ploidy levels (Gillis et al., 2007). In none of the cases we could find any indication of changes in the DNA or epigenetic changes related with methylation (Smulders and de Klerk, 2011), using the most sensitive contemporary methods. Of course, this does not mean that there are no effects, but they will most likely be much more subtle than can be found with today’s sensitive methods. Many of the cultivars in bamboo (e.g. color variations in *Phyllostachys*) are due to the effect of transposable elements, but this requires extensive research.

Rational Plant Tissue Culture

The final optimization and integration in a complete production scheme required yet another step. Tissue culture bamboos need to root faster and in a reliable way (i.e. 95% or more) and this high level needs to be achieved throughout the seasons and years. Although prices of tissue cultured *Fargesia*’s were already very competitive compared to foliage plants, lower production prices are always important for the price elasticity and the sales margin of the final products. Production of 50000 bamboos for one customer for one specific delivery on one specific day is no longer uncommon and this requires a very precise planning, from lab to greenhouse. This optimization was achieved through the study of the dynamics of phytohormones in tissue culture.

In general, micropropagation and tissue culture of plants is still much more art and skill than science.

There is, however, a great need for a rational decision methodology in commercial plant production. Rational Plant Tissue Culture involves the efficient determination and dynamic analysis of auxins and cytokinins in plants and media. This allows us, to some extent, to look inside the plant and understand and predict its behavior and here we are at the forefront of developments in plant tissue culture in general.

To achieve this goal, we have developed Ultrahigh Performance Liquid Chromatography (UPLC) methods coupled with simplified methods for sample preparation and measurements, and mass spectrometry MS, and combined this into a high-throughput system for fast and accurate determinations of phytohormones in the service of the micropropagation industry. Due to the superior separation, in combination with robustness, UPLC-MS/MS is considered as the most accurate and reliable method up to now. This method can be used to quantify all plant growth hormones, not only cytokinins, and can be developed into high-throughput systems. These analytical methods allow for high-throughput measurements of cytokinins, auxins, gibberellins, abscisic acid and much more. Some of the results for cell suspension cultures are presented in Prinsen et al., 2012 (this congress).

From the perspective of commercial micropropagation this rational approach has developed into a decision-making method to improve the efficiency of the whole plant production process through monitoring of quality during the production cycles, allowing for considerable cost reductions. For example, using this approach we have been able to shorten the multiplication, rooting and hardening

cycles, with an initial cost reduction of up to 25-28% for various species. A further cost reduction has been achieved since now *Fargesia* plants can be hardened in trays with 104 plants, compared to the trays of 54 plants used before. This increase in efficiency brings further automation and robotics within reach.

The liners (hardened and rooted plants) are more vigorous, and can develop into saleable plants for the ornamental market in less than four months. Since micropropagation is the first step in the whole production chain, everything downstream in the chain, from plant production to marketing and sales, benefits from this optimization. It allows an efficient integrated production planning needed for the demanding ornamental horticultural markets of today.



Figure 2: Growing saleable plants at Lacijns

The new insights in bamboo physiology have opened the door to drastically improve the quality of bamboo. For example, it has now become possible to produce bamboos, which can withstand longer periods without watering. When bamboos are sold in supermarkets, plant care or specialized personnel are not guaranteed and the risk of plant receiving little water in dry weather periods is considerable. In full sun bamboo leaves rapidly curl and become grayish in prolonged dry conditions, but our experiments indicated that with proper care, bamboos shipped from greenhouse to customer withstand 25 days without watering. After more than 3 weeks measurements of the photosynthetic capacity showed that the leaves were as healthy as control plants. Such strategy also opens the door, hopefully, for introducing bamboos as indoor plants. True bamboo for indoor use would be a major step, and some are in the product pipeline (Figure 4).



Figure 3: Results from experiments of 25 days without watering with control on the right

In any case, bamboo is a strong brandname: *Pogonatherum paniceum*, for which efficient methods of somatic embryogenesis were developed in the 1980's under the erroneous name of *Phyllostachys viridis* (Hassan & Debergh, 1987), became a market success under the name Baby Bamboo or House Bamboo, by Derooze Plants, one of Belgium's leading tissue culture labs. Another success story with a bamboo brand name is Lucky Bamboo, *Dracaena sanderiana* (also known as Belgian Evergreen).

Quality and Branding

In addition to propagation and production methods, also marketing and distribution have been completely changed compared to one decade ago. Two decades ago plants were offered sporadically at high prices, with limited availability. One decade ago, the production had increased dramatically, both because of the number of growers and the cultivated area per grower. Today, millions of bamboo plants find their way to the market through different distribution channels. Many of these channels are still classical (collectors, garden centers, landscapers), but sales have been increasingly successful through dedicated channels like supermarkets and wholesalers for garden center groups, or open market places like Groendirect where various cash & carry companies buy plants. The opening of these markets and channels is the direct result of the vision of mass propagation with micropropagation as the central technology.

There is still an increasing demand for high quality bamboos. The production of top quality bamboos should be organized in such a way that they are available in large numbers and at any time throughout the year. In the past, and as we speak in 2012, availability can highly fluctuate in years with harsh winters. Using greenhouse technology or production centers in warmer regions (Spain, Italy and Portugal for Europe) are necessary.

In these modern markets high quality means that all dried leaves have to be removed, green leaves should be of excellent quality (no spots, no dried leaf tips), and the pots should be extremely clean. Labels or pots used depend on the client. Integrating propagation, production and sales, requires an intensive monitoring system, in which each batch of bamboos produced in the lab is labeled with unique identification codes, which are used in the whole production and marketing system. This is necessary for any planning, but also allows feedback from sales to propagation.

To broaden the markets and develop new channels for bamboo sales (high volume – low prices) has also required another change, namely the naming of bamboo. From plants with (varying) latin names, today bamboos are marketed and sold under trendy brand names and a general brand BambooSelect™ (www.bambooselect.com and www.bambooselect.us). For this brand specific labels and a marketing

campaign with brochures were developed. A decisive step towards a consumer market has been the development of trade names like “*New Umbrella*” and “*Green Hedge*”, “*Red Panda*”, “*Asian Wonder*”, “*Great Wall*”, “*Green Panda*” (also known as “*Rufa*”), “*Blue Panda*” and “*Green Screen*” for various *Fargesia* selections¹⁰; “*Black Jade*” and “*Green Perfume*” for *Phyllostachys nigra* and *atrovaginata* respectively, and *Sunshine* for *Bambusa* “Alphonse Karr”.



Figure 4: BambooSelect™ “*Sunshine*” for indoor use

From a market and marketing point of view, the ongoing debate about precise naming of bamboo is simply a nightmare. The many name changes in temperate bamboos (“To be or not to be *Fargesia*, *Sinarundinaria*, *Borinda*, *Thamnocalamus*....”) and the discussions among taxonomists can only create confusion and add nothing of value. For the horticultural markets clarity is necessary. As long as one knows the origin of the plant (e.g. *Great Wall* is a selected *nitida* type), with a specific DNA fingerprint (AFLP™ markers, stored in an open database), one can always trace back any plant. The origin of these selections is with plantmen in Belgium, The Netherlands and Germany and rigorous selection at Oprins Plant Labs. Everyone familiar with the finesses of plant tissue culture understands that *in vitro* selection is crucial to successful products downstream.

¹⁰ Selections of *F. murieliae* (NU & GH), *F. Jiuzhaigou* (RP), *F. scabrida* (AW), *F. nitida* (GW), *F. 'Rufa'* (GP), *F. papyrifera* (BP) and *Fargesia robusta* (GP)



Figure 5: “Green Hedge” (left) and “Blue Panda”

This has also led to the strategy of growing from a pure plant producer to developing intellectual property rights, e.g. with BambooSelect™ and its best clones (IlexSelect™ for hollies is next in line). These include not only proprietary technology for propagation and production (Gielis et al., 1999), but also the commercial side with branding. This complex of technologies and strategies, based on fundamental research, will have various spillover effects in the next decades in agro-forestry and forestry with bamboo.

Into the 21st century: positive spillover effects

Valorization of “horticultural” technology to address major challenges

Botanist David Fairchild not only wrote about horticulture and his travels, but he was also the manager for the Department of Plant Introduction program (United States Department of Agriculture USDA). Fairchild (1947, p.57) writes about an impressive collection of non-native bamboos, growing at the USDA’s plant introduction station in Savannah, Georgia:

“The vast possibilities of bamboo have interested me for years...The one hundred and twenty-five species of bamboo growing there represent what I presume is the largest collection of these useful grasses in the world today. The meager government support which it receives reflects, perhaps, the almost universal ignorance of the Western World with regard to the possibilities of these, the largest of all the grasses.”

Those who see the value of bamboo as ornamentals invariably are drawn to the potential for agriculture. The Savannah collection would become the source for interesting agricultural research into the possibilities of growing bamboo in temperate climates as a source of biomass. The results and techniques were encouraging, as were experiments elsewhere in temperate zones, but it was not until the 1990’s that interest in bamboo as a source of biomass was awakened, both in the US, for example at the University of Washington (with a 1997 Pacific Northwest Agro-forestry Workshop in Port Angeles, Washington) and in Europe, with the Bamboo for Europe project.

Funding of the European Union for this *Bamboo for Europe* project allowed nine partners to work together on research on possibilities and restrictions on bamboo for agriculture and forestry in Europe,

with the establishment of new plantations for research in Portugal, Spain and Belgium. It was found that bamboo can be used as a source of biomass without major extra investments in the sectors of bio-energy and wood industry (boards and panels). Bamboo becomes then an agricultural plant, not forestry, with some specific advantages. Existing harvesting machinery in agriculture can be used directly for mechanical harvesting of bamboo (Gielis 2001; Temmerman et al., 2005; Potters et al., 2012; Schutte, 2011).

It is however, impossible to start any serious large-scale plantation scheme of temperate bamboos without mass propagation methods that have been developed and perfected in horticulture for temperate bamboos. It is impossible to turn the current bamboo resources into forestry liner production to supply enough cheap planting materials, without micropropagation.

A comparison with *Ficus benjamina* seems appropriate: nobody in the tropics in his or her right mind, would even consider propagating a strangler fig tree via tissue culture; it has little value and can be propagated most easily via cuttings. Yet, ALL *Ficus* plants produced in Europe today (approx. 100 million per year) are produced via tissue culture; in fact, micropropagation has replaced propagation via cuttings completely.

Whenever micropropagation methods can be developed successfully into an efficient production method, they will inevitably replace other methods of propagation, not only in ornamental horticulture (foliage plants, bulbs, orchids, ornamental trees and shrubs), but also in food sector (berries, nuts, rootstocks for fruit trees, palms, olives.....) and the forestry sector. This is certainly not new (Debergh and Zimmerman, 1991), but an ever-growing process, although some methods intermediate between plant tissue culture and classical propagation have been developed, for example photoautotrophic (sugar free) micropropagation systems (Watanabe et al., 2000; Kozai et al., 2004), the use of micropropagated plants as mother plants for classical vegetative propagation techniques, including macroproliferation (Kumar, 2012).

The 21st century is the century of biotechnology, with plant tissue culture as one of the key ingredients. While developments in tissue culture and micropropagation have been quite impressive, the challenges are immense, of a different order of magnitude. For plantation and reforestation purposes hundreds of millions of bamboo transplants are needed annually in the future. This is only a few percent of the total need for transplants, and estimates range from 25-40 billion plants per year to be produced (Kozai, 2005):

“In order to solve these global issues in the 21st century, we are requested to develop a concept, a methodology and an industry to produce billions of plants every year, not only for food, feed and environmental conservation, but also for alternative raw materials to produce bio-energy, bio-degradable plastics and many other industrial products. By using plant-derived products, we can minimize the environmental pollution and the use of fossil fuels and atomic power. It has been predicted that in the forthcoming decades, demands for transplants will rise sharply in the pulp, paper, timber, energy, plantation, horticulture and furniture industries and in the desert rehabilitation for environment conservations. A large number of high quality transplants, woody and herbaceous horticultural plants, are also needed every year for people living in cities to improve their quality of life or green amenities. The same is true for medicinal plants.”

The production of genetically uniform and high quality transplant material through micropropagation is the start of a transformation that is ongoing in horticulture, agriculture and forestry. Protocols and methods have been developed worldwide for various genera and species of forestry bamboos and many have been tested in forestry, albeit on a relatively small scale (Yang and Hui, 2010). Somatic embryogenesis, esp. of tropical bamboos (Mehta et al. 1982; Rao et al., 1991) has proven an efficient, fast and cheap method for mass production of bamboos for forestry, including the potential for synthetic seeds (Gielis et al., 1999). In Europe it is far too costly to produce forest bamboos for the tropics and, consequently, production has to be done locally.

Time-keeping of media preparation and transfer times of these experiments, allowed for simulating the efficiency of mass production of *Bambusa balcooa* plants, comparing both axillary branching and somatic embryogenesis. Economical evaluation of the methods allowed for comparing costs in Europe versus South Asia and showed a drastic cost reduction of SE compared to axillary branching (Gillis et al., 2007) and the necessity for local production (in this case at Bambu Nusa Verde, Indonesia; Peeters, 2011). The optimal plant production chain could still involve the production of embryogenic calli in Europe, and the regeneration of embryo's, multiplication and hardening of plants can be done locally. As they produce at low cost and can act in regional markets autonomously, this production chain allows for mass production of tropical bamboo plants at prices within the scope of forestry (Peeters, 2011).

One of the main challenges is to combine this knowledge and deploy the developed technologies in various parts of the world to establish sustainable plantations for biomass and wood of bamboo, adapting it to local situation and demands. Some of the building blocks have been developed, but to integrate these into highly efficient methods for mass propagation as in horticulture requires further steps. Toyoki Kozai (2005) writes:

“Micropropagation is one of the plant tissue culture technologies for producing a large number of genetically superior and pathogen-free transplant in a limited time and space. However, the widespread use of micropropagated plants is still restricted because of its high production costs, mostly attributed to its low growth rate and a significant loss of plants in vitro by microbial contamination, poor rooting, low percent survival at the ex vitro acclimatization stage and high labor costs.”

In contrast to in horticulture we have already tackled and solved most of these issues for ornamental bamboos, so our long-term experiences from horticulture, with extensions to agriculture (in temperate zones) and forestry (tropical zones), should certainly be useful and welcome in competitive markets of forestry where micropropagation *“is still an experimental or small scale production stage”* (Yang and Hui, 2010).

Research challenges for the future

There are still a lot of open challenges for micropropagation of bamboo. The control of somaclonal variation due to transposable elements is one important area for horticulture and somatic embryogenesis for temperate bamboos for example has hardly been successful. Clonal fidelity and long term follow up of the growth is of prime importance (Gillis et al., 2007; Negi and Saxena, 2010; Agnihotri et al., 2010). Even better for the future is that relations of certain molecular markers to development and relation to certain characteristics has been established (Rai et al., 2011; Bhattacharya et al., 2011; Rai et al., 2012).

Beside propagation and mass scale production, one of the main challenges for plant research and biotechnology is the genetic improvement of bamboo. Despite some initial trials in hybridization (Zhang Guangchu, 2002; for other references see Gielis, 1995), and the possibility to induce flowering into tissue culture, we are still far from any breeding program. So far, various research programs for the genetic transformation of bamboo have only yielded transient expression, or when a more stable integration is obtained in cell cultures, regeneration of plants is the bottleneck (Ojita et al., 2011; Sood et al., 2011; own unpublished results).

Since every disadvantage has also its advantage bamboo is probably one of the few cultivated plants that are still in their natural, wild state. Other crops have been domesticated for a long time or recently; extensive breeding programs have been developed and many crops can be genetically engineered (Van Montagu, 2011). Bamboo, on the contrary, is a completely green and natural plant, with a sufficiently large natural genetic variation. It will require concerted efforts of a research consortium to unravel this genetic variation, using molecular markers or high-throughput sequencing.

The use of molecular markers alone will be insufficient. Part of the natural variation is, in my opinion, strongly related to transposable elements (compare color and culm shape variations in many forms and cultivars of bamboo) and thus difficult to study, but the use of state-of-the-art methods like AFLPTM based transposon display (De Keuckeleire et al., 2004) and sequencing (Diao et al., 2006; Zhou et al., 2011) will be of great help.

At present, however, our knowledge of the natural genetic variation of bamboo is extremely limited and fragmented. The use of molecular marker has yielded only a few clear-cut, practical results (Gielis and Oprins, 2009; Rai et al., 2012; BPG, 2012), yet they have become indispensable tools in research, with potentially practical results: the use of AFLPTM in Chinese clumping bamboos shows a geographical grouping, rather than supporting existing taxonomies and the AFLPTM fingerprint can be used to predict cold hardiness of genotypes (Gielis and Oprins, 2009). Despite the fact that we hardly know anything about the genetics of bamboo, it may become possible to close the gap with other plants with a targeted study of the genome of bamboo using state-of-the-art methods (high-throughput sequencing, bioinformatics, synteny (Gielis, 1995; Gui et al., 2010), transposable elements ,....).

High-throughput methods are available also for studies in bamboo physiology, for example in the dynamics of phytohormones (Prinsen et al., 2012), biogenic volatile emissions (Melnichenko et al., 2012) and potentially useful secondary metabolites (Van Hoyweghen et al., 2010). While studies have been performed on microbiomes in the rhizosphere, our understanding of the endophytes in bamboo and their ecology, which may play essential roles in plant physiology, has barely started (Moshynets et al., 2012). Bamboo tissue culture provides many opportunities as a basic method in research.

One major challenge however, is to bridge the gap from research to practice. There is a great need for, as Prof. Walter Liese calls it: Facts and Figures. Bamboo is a wonderful plant, but it has no magical powers for mitigating climate change (Düking et al., 2011), nor does bamboo yield 300 tons of wood per year in any sustainable way, as is often heard and read. A research community focused on 1) understanding and improving bamboo through physiology and molecular biology, combined with 2) a drive to generate real fact and figures is most needed.

There are many opportunities and challenges ahead to attain a better understanding of this wonderful natural resource, in which plant tissue culture is the key technology. Research on molecular and physiological aspects of bamboo flowering, where we have the possibility of induction of flowering *in vitro* (Nadgauda et al., 1991), should definitely be a number one target for an international research consortium, along with research on genetic transformation. Equally important will be the possibility for genetic transformation of bamboo, to further broaden its natural variability adapted to specific conditions. If we can begin to understand and control flowering, or be able to genetically transform bamboo, we hold the keys to making bamboo into one of the most valuable plants for mankind in the next centuries. Highly efficient mass propagation systems work, and markets are eagerly waiting.

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References

- Adriaenssens D. (2005) Jelena and Robert De Belder. Laconti, Brussels.
Bamboo Phylogeny Group (2012) An updated tribal and subtribal classification of the bamboos

- (Poaceae: Bambusoideae). This congress.
- Bhattacharya S., Ghosh J.S., Sahoo D.K., Dey N., Pal A. (2010) Screening of superior fiber-quality-traits among wild accessions of *Bambusa balcooa*: efficient and non-invasive evaluation of fiber developmental stages. *Ann.For.Sci.* 67: 611-620
- Borin G. (1995) Bamboos in Belgium: a long but interrupted tradition. *European Bamboo Society Journal*. EBS Annual General Meeting, Meise, Belgium, May 6, 1995: 21-26.
- Camus E.G. (1913) *Les Bambusées*. Monographie, Biologie, Culture, Principaux usages. Publisher Paul Lechevalier, Paris.
- Chapman (Ed). (1997) *The Bamboos*. Linnean Society, London.
- Colonel Munro (1866) A Monograph of the Bambusaceae, including descriptions of all species. *Transactions of the Linnean Society Vol XXVI*.
- Clark L. (1995) Bamboo systematics today. *European Bamboo Society Journal*. EBS Annual General Meeting, Meise, Belgium, May 6, 15. 40-46.
- Clark L. (1997) Bamboos – the centerpiece of the grass family. In: Chapman *The Bamboos*. Linnean Society Symposium Series N°19 Academic Press: 237-245.
- De Keukeleire P., De Schepper S., Gielis J., Gerats T. (2004) A PCR-based assay to detect hAT-like transposon sequences in plants. *Chromosome Research* 12: 117-123.
- Debergh P.C., Zimmerman R.H. (1991) *Micropropagation: Technology and Application*. Kluwer Academic Publishers.
- Debergh P.C., Read P.E. (1991) *Micropropagation*. In: Debergh P.C, Zimmerman R.H. (1991) *Micropropagation: Technology and Application*. Kluwer Academic Publishers. 1-13.
- Dhooghe E., Van Hylebroeck J., Geelen D. (2011) Ornamental plant tissue culture industry in Flanders (Belgium). *Proc. 7th International symposium on in vitro culture and horticultural Breeding*. Ghent, 18-22 September 2011.
- Diao X., Freeling M., Lisch D. (2006) Horizontal transfer of a plant transposon. DOI: 10.1371/journal.pbio.0040005
- Düking R., Gielis J., Liese W. (2011) Carbon flux and carbon stock in a bamboo stand and their relevance for Mitigating climate Change. *Bamboo Science and Culture* 24: 1-7.
- Fairchild D. (1947) *The World Grows Round My Door*. Charles Scribner's Sons, New York
- Fairchild D. (1903) *Japanese Bamboos and their introduction into America*. USDA Bureau of Plant Industry Bulletin 43.
- Freeman-Mitford A.B. (1896) *The Bamboo Garden*. McMillan, London.
- Farrelly D. (1984) *The Book of Bamboo*. A comprehensive guide to this remarkable plant, its uses, and its history. Sierra Club Books San Francisco.
- Fernandez E., Gielis J. (Eds.) (2003) *Compendium of bamboo research on Bamboo 1970-2003*. EU Bamboo Thematic Network.
- Fox Keller E. (1983) *A feeling for the organism: life and work of Barbara McClintock*. W.H. Freeman and Company. New York.
- Gavinlertvatana P., Prutponse P. (1991) Commercial Micropropagation in Asia. In: Debergh P.C, Zimmerman R.H. (Eds.) *Micropropagation: Technology and Application*. Kluwer Academic Publishers.
- Gielis J. (1995) Bamboo and Biotechnology. *European Bamboo Society Journal*. EBS Annual General Meeting, Meise, Belgium, May 6, 15. 27-39.
- Gielis J., Woods S., Woods J., Oprins J. (1999) Micropropagation, synthetic seeds and germplasm storage of bamboos. US Patent letter 6677154, granted 2004.
- Gielis J., Oprins J. (2002) Micropropagation of temperate and tropical woody bamboos: from biotechnological dream to commercial reality. *Bamboo for Sustainable Development*. INBAR Proceedings N°7. VSP Publishers: 333-344.
- Gielis J., Peeters H., Gillis K., Oprins J., Debergh P.C. (2001) Tissue culture strategies for the genetic improvement of bamboo. *Acta Horticulturae* 552: 195-203.
- Gielis J. (2001) Future possibilities for bamboo in European agriculture. *Journal European Bamboo Society*, Meise, Belgium, June 23-24, 2001: 24-32.
- Gielis J., Oprins J. (2009) Identifying new *Fargesia* Introductions and Predicting their Cold Tolerance using AFLP markers. *WBC VIII Proceedings*, September 16-19, 2009 Bangkok, Vol 6: 56-67.
- Gillis K., Gielis J., Peeter H., Dhooghe E., Oprins J. (2007) Somatic embryogenesis from mature

- Bambusa balcooa* Roxburgh as basis for the mass production of elite forestry bamboos.
- Godbole S., Sood A., Thakur R., Sharma M., Ahuja P.S. (2002) Somatic embryogenesis and its conversion into plantlets in a multipurpose bamboo, *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. *Curr Sci* 83: 885-889.
- Gui Y.J., Zhou Y., Wang Y., Wang S., Wang S.Y., Hu Y., Bo S.P., Chen H., Zhou C.P., Ma N.X., Zhang T.Z., Fan L.J. (2010) Insights into the bamboo genome: syntenic relationships to rice and sorghum. *J. Integr. Plant Biol.* 52(11): 1008-15. doi: 10.1111/j.1744-7909.2010.00965.x.
- Hassan E.H., Debergh P.C. (1987) Embryogenesis and plantlet development in the bamboo *Phyllostachys viridis* (Young) McClure. *Plant, Cell, Tissue and Organ Culture* 10(1): 73-77. Corrigendum 1988 *PCTOC* 12(1): 93.
- Houzeau de Lehaie, J. (1906-1908) *Le Bambou, son Etude, sa Culture, son Emploi*. Mons, Belgium
- Judziwicz E., Clark L.G., Londono X., Stern M.J. (1999) *American Bamboos*. Smithsonian Institute.
- Komatsu Y.H., Batagin-Piotto K.D., Brondani G.E., Gonclaves A.N., Almeida M.D. (2011) In vitro morphogenic response of leaf sheath of *Phyllostachys bambusoides*. *J Forest Res* 22: 209-215
- Kozai T. (2005) Introduction. In: Kozai T., Afreen F., Zobayed S.M.A. (2005) Photoautotrophic (sugar-free) micropropagation as a new micropropagation and transplant production system. Springer Verlag.
- Kozai T., Afreen F., Zobayed S.M.A (2005) Photoautotrophic (sugar-free) micropropagation as a new micropropagation and transplant production system. Springer Verlag.
- Liese W. (1985) *Bamboo: biology, silvics, properties and utilization*. Schriftenreihe der GTZ, N° 180
- Liese W. (2009) Bamboo as carbon sink: Facts or fiction? WBC VIII Proceedings, 16-19 September 2009, Bangkok. Vol 3: 71-86.
- McClintock D. (1996) Bloei bij winterharde bamboos. *Newsletter Belgian Bamboo Society* 13: 5-11
- McClure, F.A (1966) *The bamboos – A fresh perspective*. Harvard University Press., Cambridge, Mass. USA.
- Melnychenko A.N., Rosenstiel T.N. Biogenic volatile organic compound emissions from bamboo: Exploring patterns of diversity across species.. In: *Proceedings IXth World Bamboo Congress, Antwerp*.
- Mehta U, Rao IVR, Ram HYM (1982) Somatic embryogenesis in bamboo. *Plant Tissue Culture. Proc. 5th Intl Cong Plant Tiss Cell Cult* pp 109-110
- Mehta R, Sharma V, Sood A, Sharma M, Sharma RK (2011) Induction of somatic embryogenesis and analysis of genetic fidelity of in vitro derived plantlets of *Bambusa nutans* Wall. using AFLP markers. *Eur J Forest Res* 130: 729-736
- Moshynets E.V., Brunet J., Shpylova S.P., Bogaerts L., Kordium V.A., Potters G. (2012) Visualisation and identification of endophytic bacterial communities of *Phyllostachys* and *Fargesia*. In: *Proceedings IXth World Bamboo Congress, Antwerp*.
- Nadgauda R.S., Parasharami V.A., Mascarenhas A.F. (1990) Precocious flowering and seeding behaviour in tissue-cultured bamboos. *Nature* 344, 335 - 336 (22 March 1990).
- Nadha H.K., Kumar R., Sharma R.K., Anand M., Sood A. (2011) Evaluation of clonal fidelity of in vitro raised plants of *Guadua angustifolia* Kunth using DNA-based markers. *J Med Plants Res* 5: 5636-5641
- Negi D., Saxena S. (2010) Ascertaining clonal fidelity of tissue culture raised plants of *Bambusa balcooa* Roxb. using Inter Simple Sequence Repeat (ISSR) markers. *New Forests*. Vol. 40: 1-8.
- Negi D., Saxena S. (2011) In vitro propagation of *Bambusa nutans* Wall. ex Munro through axillary shoot proliferation. *Plant Biotechnol Rep* 5: 35-43
- Okamura H., Tanaka Y. (1986) *The horticultural bamboo species of Japan*, Kobe
- Orhngerger D. (1999) *The Bamboos of the World*. Elsevier, The Netherlands
- Ogita S, Kashiwagi H, Kato Y (2008) In vitro node culture of seedlings in bamboo plant, *Phyllostachys meyeri* McClure. *Plant Biotechnol* 25: 381-385
- Ojita S., Kikuchi N., Nomura T., Kato Y. (2011) A practical protocol for particle bombardment-mediated transformation of *Phyllostachys* bamboo suspension cells. *Plant Biotechnology* 28:43-50
- Peeters M. (2012) Bamboo Tissue Culture to support plantation development. *Proc. Of the International Bamboo Seminar: strategy and challenges of bamboo and potential non-timber forest products, management and utilization*. Bogor, Indonesia, 23-27 November 2011.

- Pierik R.L.M. (1991) Commercial micropropagation in Western Europe and Israel. In: Debergh P.C, Zimmerman R.H. (Eds.) Micropropagation: Technology and Application. Kluwer Academic Publishers. 155-165.
- Potters G., Schutte F., Van Goethem D., Denollin S., Samson R., Gielis J. (2012) Bamboo as a Crop in Western Europe – A SWOT Analysis. *Acta Horticulturae* (in the press).
- Prinsen E., Van den Akker S., Bormans P., Peeters H., Gielis J. (2012) Cytokinin dynamics in cell suspension cultures of *Bambusa balcooa* Roxburgh using UPLC-ESI/MS/MS. Proc. of IXth World Bamboo Congress, Antwerp, April 10-13, 2012.
- Prutpongse P., Gavinlertvatana P. (1992) In vitro micropropagation of 54 species from 15 genera of bamboo. *Hort. Science* 27:453-454.
- Rai V., Ghosh J.S., Pal A., Dey N. (2011) Identification of genes involved in bamboo fiber development. Genedoi:10.1016/j. gene.2011.01.004
- Rai V., Ghosh J.S., Dey N., Pal A. (2012) A genomic approach to identify genes expressed during fiber development in *Bambusa balcooa*. In Proc. IXth WBC Congress, Antwerp.
- Rao R.I.V., Aziah Mohd. Y., Rao A.N., Sastry C.B. (1990) Propagation of bamboo and rattan through tissue culture. IDRRC Bamboo and Rattan Research Network.
- Raphael S. (1979) A Plantsman defined. *The Plantsman* Vol I Part I, (June 1979).
- Rivière A., Rivière C. (1879) *Les Bambous*. Paris.
- Schutte F. (2011) IKEBANA Brochure, University of Antwerp.
- Schütte J. (2011) Fargesien aus Laborvermehrung. *Bambus Journal* (EBS-Germany), 2011, N°3: 17.
- Shilin Z., Naixun M., Maoyi F. (1994) A compendium of Chinese bamboo. China Forestry Publishing House.
- Smulders M.J.M., de Klerk G.J. (2011) Epigenetics in plant tissue culture. *Plant Growth Regulation* 63: 137-146.
- Sood A., Ahuja P.S., Sharma M., Sharma O.P., Godbole S. (2002) In vitro protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. *Plant Cell Tiss Org Cult* 71: 55-63
- Sood P., Bhattacharya A., Sood A. (2011) Problems and possibilities of monocot transformation. *Biologia Plantarum*, Volume 55, Number 1, pp. 1-15(15)
- Temmerman M., Van Belle J.F., Delcart J., Gielis J., Brias V. (2005) Bamboo as a source of bioenergy. Centre Wallon de Recherches Agronomiques, Gembloux, Belgium.
- Ueda K. (1960) Studies on the physiology of bamboo with reference to practical application. *Bulletin of the Kyoto University Forests*, N°30.
- Van Hoyweghen L., Karalic I., Van Calenbergh S., Deforce D., Heyerick A. (2010) Antioxidant Flavone Glycosides from the Leaves of *Fargesia robusta*. *Journal of Natural Products* 73(9): 1573-1577.
- Van Montagu M. (2011) It is a long way to GM Agriculture. *Annu. Rev. Plant Biology* 62: 1-23.
- Watanabe Y., Sawa Y., Nagaoka N., Kozai T. (2000) A new micropropagation system for *Pleioblastus pygmaeus* Nakai. In: Proceedings of the International Symposium by The Royal Project Foundation, Chiang Mai, Thailand, 2-4 August 2000, 94-101
- Wen T.H. (1993) *Colored Illustrations of Bambusoideae in China*. Tapei, 175 illustrations.
- Yang Y, Hui C. (2010) China's bamboo: Culture/resources/cultivation/utilization. INBAR Technical Report N°33.
- Zhang Guangchu (2002) A Manual of bamboo hybridization. INBAR Technical Report N°21. VSP Publishers.
- Zhou M.B., Liu X.M., Tang D.Q. (2011) Transposable elements in *Phyllostachys pubescens* (Poaceae) genome survey sequences and the full-length cDNA sequences, and their association with simple sequence repeats. *Genet Mol Res* 10: 3026-3037

VOLUME 2

BAMBOO DESIGN INNOVATIONS, ARCHITECTURE AND MODERN TECHNOLOGIES

Session 1. Architecture

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Low carbon construction using Guadua bamboo in Colombia

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Abstract

Guadua Angustifolia Kunth (Guadua) is a tropical species of bamboo endemic to South and Central America and widely used as a mainstream material for construction in Colombia. Its rapid rate of biomass production, renewability, high level of CO₂ fixation and storage, wide diameter, long-length, and durability are distinctive and highly desirable features which can benefit the new built environment

Research interest in Guadua construction increased significantly after many Guadua-constructed buildings withstood or suffered only minor damage during an earthquake which reached 6.2 on the Richter scale in 1999, resulting in the standardization of Guadua in the seismic-resistant Colombian code (NSR-10). However, Guadua buildings constructed in the Americas and other parts of the world, whilst considered to be sustainable, are not fully characterised in terms of the preparation, use and disposal of Guadua. Furthermore, workability, building durability and the construction process have not been specifically documented and evaluated.

The structure, properties and availability of Guadua are described in this paper. A case study on a recently built holiday house is presented illustrating the architectural, structural, environmental and technical performance of a Guadua building. This paper presents the construction process, discusses difficulties encountered during the building life cycle and highlights the need for similar assessments. It is concluded that with the aim of achieving a low carbon construction system using Guadua bamboo, challenges regarding manufacture, bio-deterioration, integration with conventional systems, and environmental impacts must be addressed.

Keywords

Bamboo, *Guadua*, Construction, Case study

Abbreviations

BRE: British Research Establishment

FAO: Food and Agriculture Organization of the United Nations

INBAR: International Network for Bamboo And Rattan

MPa: Megapascals

Non Wood Forest Product (NWFP)

NSR-2010: Norma Sismo Resistente Colombiana, 2010.

Introduction

Bamboo

Commonly referred to as a giant grass, bamboo is an Angiosperm plant from the Poaceae –Gramineae family (grasses) which propagates rapidly by the expansion of underground rhizomes. Unlike trees, bamboos have only one growth stage where the culm (stem) seen in Figure 1a reaches its full height, diameter and wall thickness during the first four to six months of life. Subsequently, consolidation of tissue occurs by secondary thickening until maturity. The fast elongation of its culm is a result of the simple structure of fibres, conductive tissue and parenchyma units (Figure 1b) arranged axially along the stem which allows cellular differentiation and rapid flow of nutrients. As shown in Figure 1a, the continuity of the culm is interrupted by diaphragms that transversely connect the vascular system. The cortex (outer part) is rapidly consolidated with a high content of lignin and silica, providing protection for the load bearing tissue during growth. No growth occurs in the radial, longitudinal or tangential direction in the following years.

Depending on its rhizome structure, tropical and temperate bamboos are classified as sympodial and monopodial. Both present distinctive anatomical and morphological features which determine their physical and mechanical properties (density, strength, bending behaviour, shrinkage and splitting) and thus their end uses. Sympodial bamboos in tropical areas have been widely used in construction and monopodial bamboos in subtropical regions are mainly used for less demanding applications. For example, the sympodial species *Guadua Angustifolia Kunth* (guadua) grows in South and Central America and is suitable for construction. In contrast the monopodial *Phyllostachys heterocycla pubescens* (moso) is grown in Asia and is more suitable for parquet, furniture and decorative applications (Liese 1998). The worldwide distribution of bamboo is summarised in Figure 2.

Bamboo resources have been recently listed as a Non Wood Forest Product (NWFP) and wood substitute at FAO's last Forest Resource Assessment (FAO 2010). From a total bamboo forest of about 1 % of the world's land area (31.5 million ha), moso covers about 70% of the total 3.7 million ha of bamboo forest in China (Yiping et. al. 2010), while guadua resources in Colombia account only for some 51.5 thousand ha (Riaño et. al. 2002).

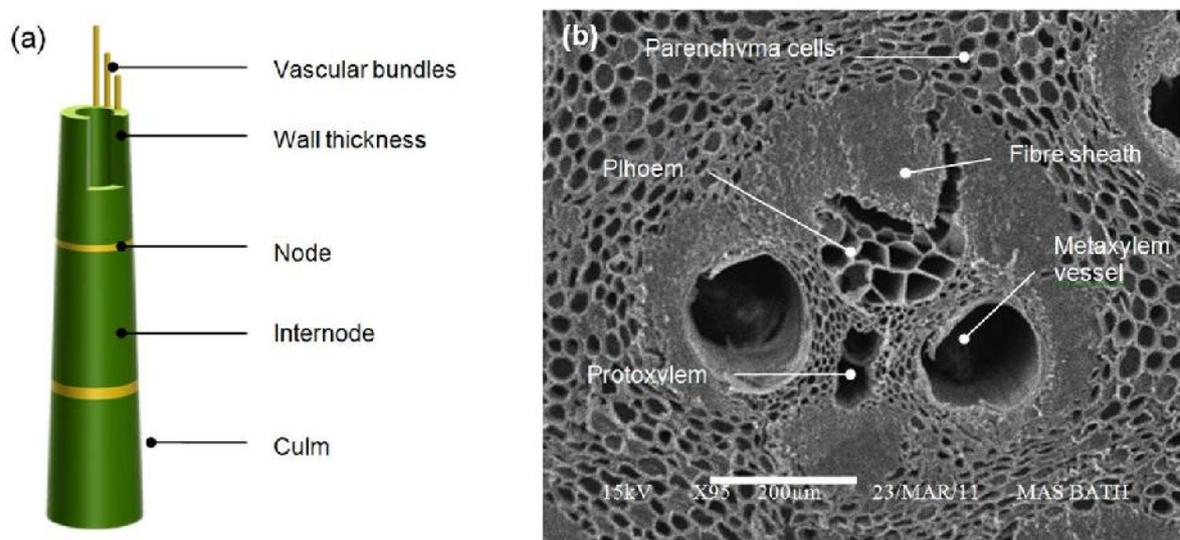


Figure 1 (a) bamboo culm, (b) vascular bundle (*Guadua Angustifolia Kunth*)

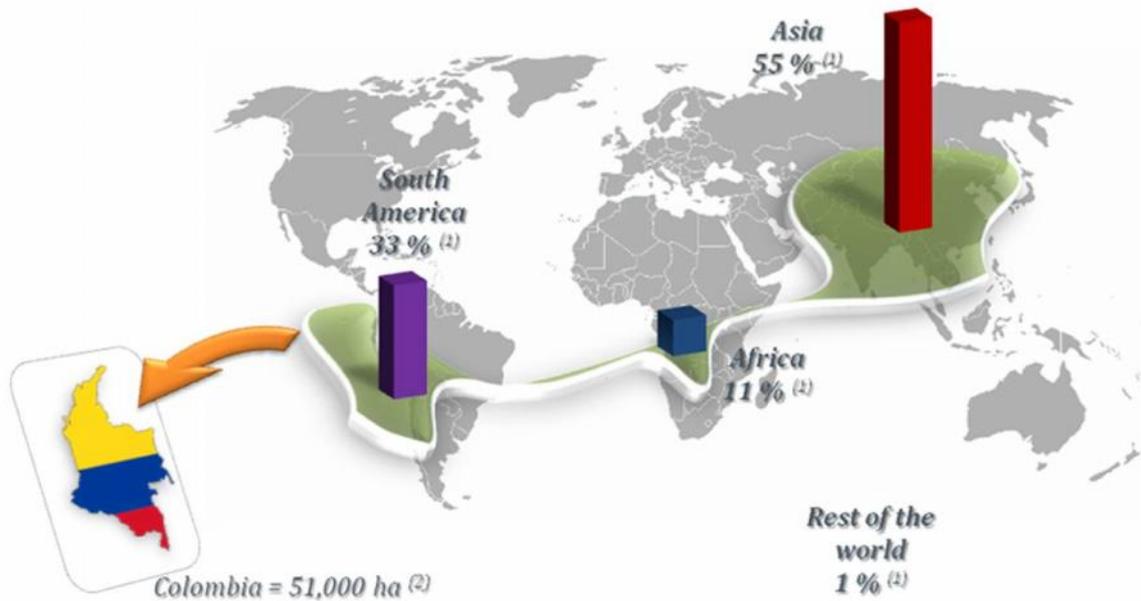


Figure 2. Worldwide distribution of bamboo. 1 (FAO 2010), 2 (Riaño et. al. 2002).

Guadua

Guadua angustifolia Kunth (Guadua) from the subtribe Guadinae is a tropical species of bamboo endemic to South America widely used as a mainstream material for construction in Colombia. It commonly grows at altitudes between 500 and 1500m, in the temperature range 18 to 24° C and relative humidity 80 to 90% in regions with precipitation varying from 1200mm to 2500mm per year (Riaño et. al. 2002). This sympodial bamboo can grow at a maximum rate of 21cm per day to an average height of 25m in the first 6 months (Figure 3a). Its base diameter can reach 22cm, becoming mature between the fourth and fifth year (Figure 3b). These features, along with a specific gravity between 0.7 and 0.8, high strength-to-weight ratio, higher durability than other bamboos, ease of use, abundance and low cost, has contributed to its use in construction applications in Colombia.

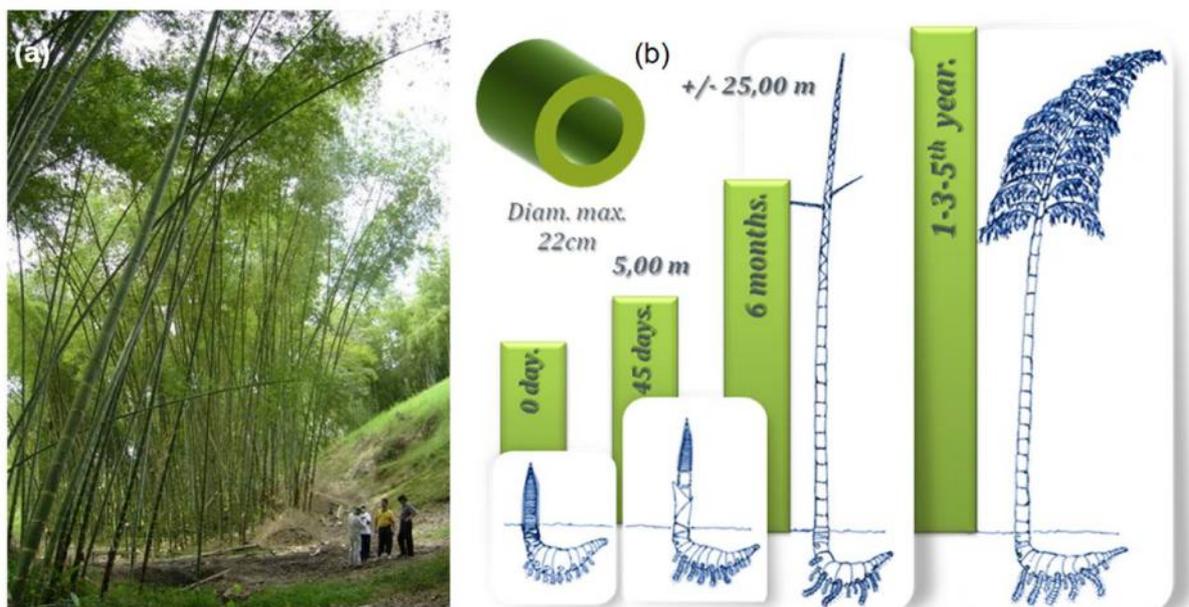


Figure 3 (a) Guadua plantation in Colombia, (b) Growth of Guadua culm

Guadua's structural integrity was dramatically tested in 1999 in Colombia during an earthquake (6.2 on the Richter scale) that devastated a vast area of the coffee growing region (zona cafetera). Most of the buildings entirely built out of Guadua survived with minor structural damage whilst conventional concrete buildings collapsed and almost 60% of all buildings fell down. Since this episode, interest in research on the mechanical response of Guadua and its suitability for construction has resulted in its standardization in the seismic-resistant Colombian code (NSR-10) for (a) dwellings of one and two stories and (b) residential, commercial and institutional buildings (NSR 2010). The first system (a) has been historically present in Colombian architecture and is based on an inexpensive Guadua structural wall framing system with a plastered finish known in Spanish as 'bahareque encementado' (plastered cane). In response to the undervaluation of Guadua as a construction material, architects such as Oscar Hidalgo, Carlos Vergara and Simón Vélez have pioneered a process for pushing the mechanical response of the material to its limit, producing full scale cantilevered roofs and long span structures (Hidalgo 2003). Figures 4a, b and c show the construction process for a building that combines systems (a) & (b) in a three storey holiday house in Colombia.



Figure 4. Construction process of a Guadua building (a) Framing (b) Walling (c) Finishing

Following the regulations of the Colombian building code (NSR-10) practitioners, engineers and architects, including the first author, have participated in the construction of pedestrian bridges, large scale commercial and institutional buildings, urban dwellings and holiday homes. An example of a lightweight Guadua structure that follows these regulations is presented as a case study in this paper. Despite being designed as a framed structure with columns and beams, it also uses non-structural plastered cane for some walls.

Case Study

Location

Project Bohio was built in Colombia in a river valley between the Central and Eastern ranges of the Andes mountain system close to Villeta, a town 80 km away from Bogotá D.C. Colombia is located on the extreme northwest of South America, on the border with Panamá and adjacent to the Atlantic and Pacific oceans (Figure 5a). This geographical situation, together with its position near the Equator provides Colombia with a tropical climate. Bohio is located at an altitude of about 900 m.a.s.l. and its average temperature is 25°C.

The zone where the Bohio project was developed is located on a geological fault considered to be a medium earthquake risk within the Colombian building code (NSR-10) due to constant telluric and volcanic activity in the Pacific Ocean along the Ring of Fire.

Architectural Design

The design process for Bohio follows three main requirements from the owner:

- to conceive a bungalow type holiday house that utilizes only local and natural materials reducing environmental impact
- to avoid an urban style of appearance
- to integrate the building with the surrounding tropical landscape.

These requirements were achieved by evoking a vernacular style of architecture found in Colombia. Basic social, traditional and cultural concepts were integrated into the design which interacts with the environment by the intensive use of natural materials. As a result, a strong sense of appropriateness and architectural identity was attained. The project was implanted high on the side of a valley to gain the views over a river canyon (Figure 5b).

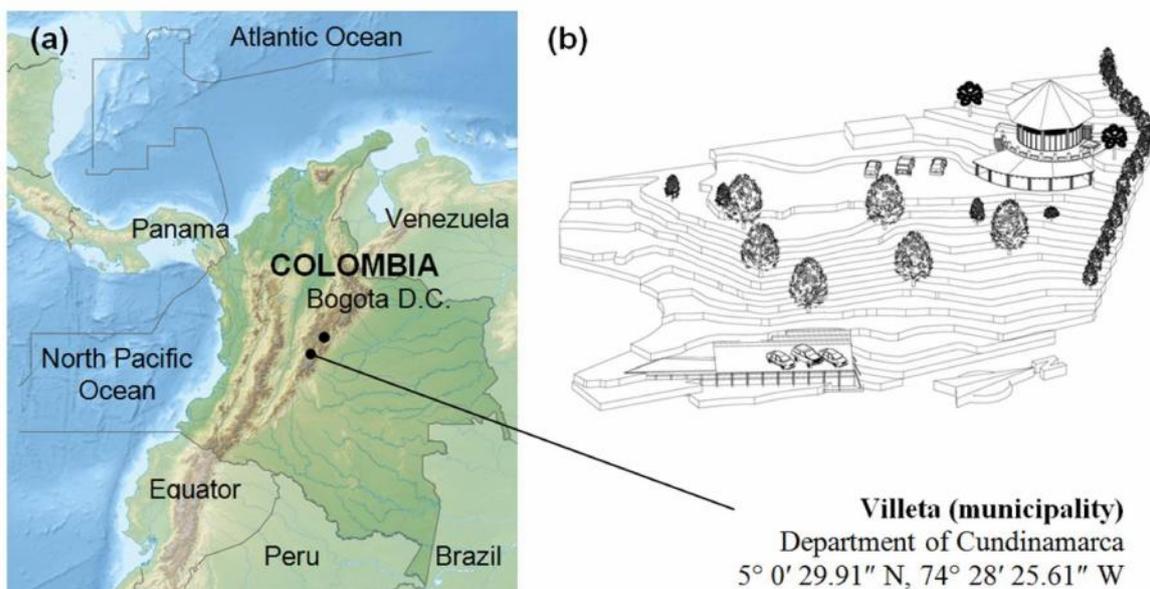


Figure 5 (a) Bohio's geographical location (b) Bohio's situation on the terrain.

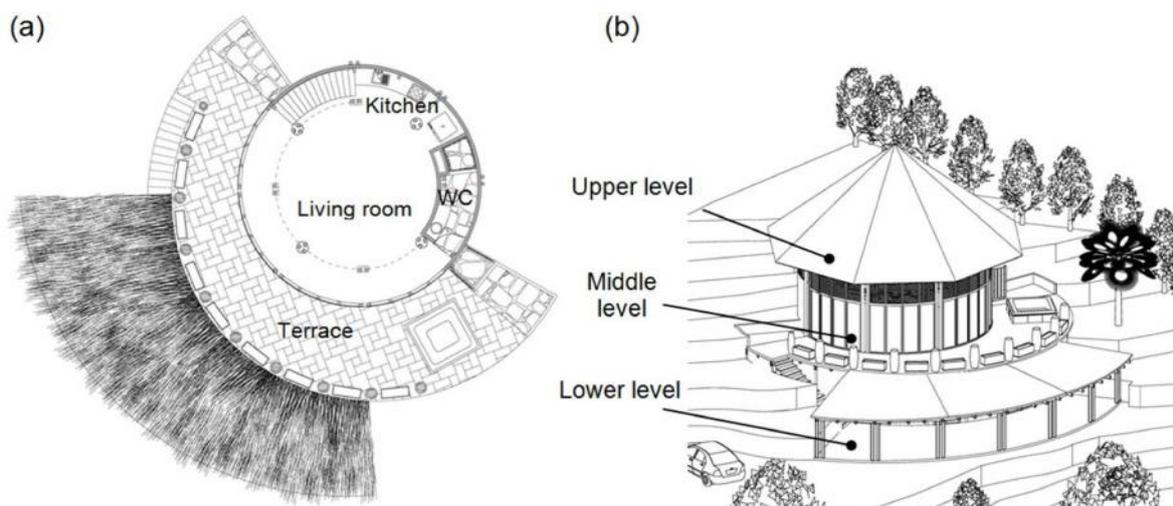


Figure 6. (a) Principal floor plan of Bohio. (b) Three dimensional view of Bohio.

As shown in Figure 6 a & b, this sloping plot allowed a three-level arrangement of the building to be constructed which resulted in separation into three functional zones: a private suite at the top level, a social area in the middle and an auxiliary accommodation and services area on the bottom floor. The middle zone contains an open kitchen and the living room which can be extended to the front terrace and serves as a transition to the lower level where guest, laundry and storage rooms are located. This distribution resembles the spatial hierarchy found in vernacular buildings where the building core contains the main zones (private and social) and auxiliary rooms are located in an external concentric zone.

A conical roof covers the two-storey circular superstructure while the lower level is covered by the projection of the ground floor slab over the basement, making room for an ample terrace and three separate rooms underneath (two for guests and one for storage and laundry). Adjacent to these rooms a sheltered terrace provides protection from the incident sun throughout the year and generates a fresh corridor ideal for hanging hammocks and setting up terrace tables.

The low profile of the basement (2.40m) contrasts with the tall superstructure (8.00m) but both are balanced by their size in the horizontal plane. Extended zones of shadow and generous openings for lighting and ventilation were key factors for assuring internal comfort levels. The thermal mass of the materials used in the building decreases from bottom to top where due to the hot humid climate, high density and high specific heat capacities are not needed.

Materials

As usual in bamboo construction the substructure is made out of concrete, isolating the bottom part of the bamboo superstructure from the soil and high moisture levels. In Bohio's case the structure above ground is completely built out of Guadua and composed of structural frames connected by steel bolts to the floors and roof and using diagonal components to concentrate the loads on the vertical load-bearing elements.

Bamboo-Guadua was selected as the mainstream structural material and comprises about 70% of the whole building. Timber is used for flooring, straw for the roof, fired bricks for the external walls and reinforced concrete for foundation and slabs. Between the 4th and 6th year when maturity is reached, Guadua is harvested for construction purposes. Its culms are subject to a preparation process before use which includes curing on stand for 20 days, preservation by an open tank method (Jayanetti et. al. 1998) for 5 days in a 5% solution of boric acid in water and commonly air dried to balance its moisture content with the construction environment to a maximum value of 19% (NSR 2010).

Structural design

Bohio' structure (Figure 7) can be divided into three parts: the basement (substructure), the superstructure and the roof.

The basement is basically a solid box with a continuous retaining wall which contains the abutting ground. Its base is made out of reinforced concrete, and its walls of fired clay bricks strengthened with concrete piles to restrain lateral deflections. The foundation of the superstructure (Figure 8) consists of four main and four secondary concrete isolated footings interconnected by ground concrete beams. Three-flanged reinforced concrete pedestals emerge above ground from these footings to connect with three Guadua columns using bolts.

Four bamboo columns composed of three elements set the edges of the main square frame and there are four additional two-component Guadua columns which define a secondary square which sits within the primary square but rotated by 45°. Each edge of the squares is connected by Guadua beams

forming an octagon in the first and second levels of the structure. A radial arrangement of bamboo joists supports the timber decking of the first floor, while the roofing system lies on the top of the superstructure. An exterior frame system holds the facade and gives some extra support to the roof. The roof joist converges to the centre from the eight sided structure beneath, reaching a height of 8.00m above the ground floor.



Figure 7. Bohio's structural and functional zones.



Figure 8. Superstructure of Bohio project. Smaller images show (top) the floor, (middle) roof and (bottom) three-flanged reinforced concrete pedestals

Mechanical considerations

In general, the design of a bamboo building is considered as a semi-rigid system for structural purposes. Following the NSR-10 regulations, the allowable stresses for Guadua with a moisture content of 12 % in bending, tension, compression parallel to the grain, compression perpendicular to

the grain and shear were 15, 18, 14, 1.4 and 1.2 MPa respectively. MOE ($E_{0.5}$) average was considered to be 9,500 MPa at the same moisture content. Live loads for this project were calculated to be 180 kg/m² and dead load of the roof to be 80 kg/m². Located in zone 2, as defined by NSR-10, wind loads were estimated at 22m/s = 80km/h. The values for seismic loads for the location of the project are $A_a=0.15$, $A_e=0.17$, $A_v=0.20$, $A_d=0.06$.

Foundations

Built out of reinforced concrete, the foundation soil beneath is prepared by laying down bedrock and concrete where the 1.00 x 1.00 m footings are cast. Square piles of 0.40 x 0.40 m axially emerge from the footings and form the three-flanged concrete pedestals that are connected to and support the Guadua structure. Horizontal holes are left during casting to fix the bolts that attach the columns of Guadua, and steel bars rise from the footings and emerge from the pedestal by 1m to allow continuity of load bearing. In order to attach steel bars to the ends of the Guadua columns one internode cavity is filled with mortar and the bars are bonded into the mortar.

Joints

Structural elements in construction with Guadua are fastened together with 3/8 inch bolts. These transverse bolted connections are located close to or through the nodes, however, due to the dimensional irregularity of the material it cannot be guaranteed. The hollow internodes are filled with cement mortar (10 MPa) to restrict displacement parallel to the grain and prevent crushing. To accommodate the bolts ½ inch holes are drilled into the Guadua. A rubber washer is located beneath the steel washer and nut. Nails and screws are used as non-structural fasteners.

Roof

The main components of the roof cladding (Figure 9a) are a corrugated steel sheet sandwiched between straw bunches with and a polyethylene membrane between the steel sheet and the inner layer of straw. Support is provided by Guadua-roof joists that converge in the centre of the conic roof. Parallel Moso bamboo roof battens arranged in the horizontal plan along the eight triangular sections of the roof tie together the straw bunches. The space left underneath by the 45 degree inclination of the roof gives room for an attic which is subdivided into a two-storey triangular set of bunks.

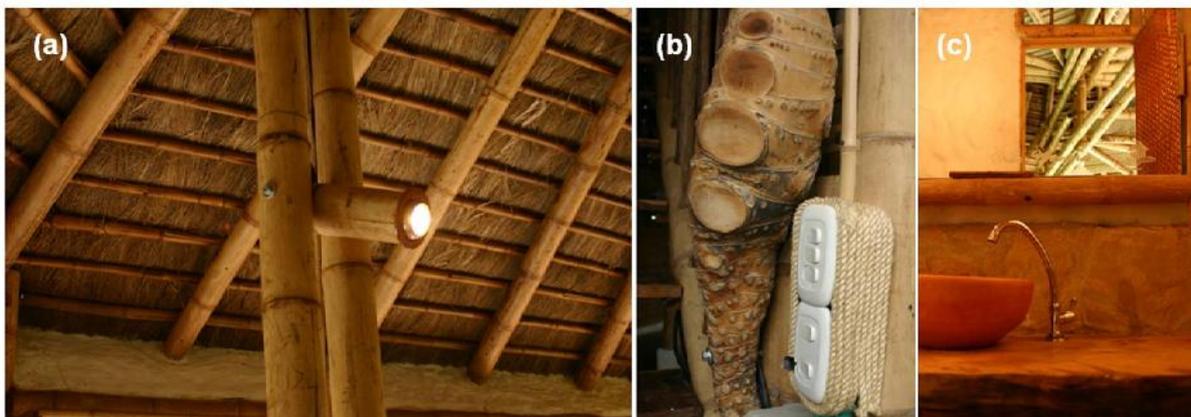


Figure 9. Roof and finishing details

Installations

The piped water supply is centralized and mainly lies underground. At the superstructure level the two bathrooms are vertically overlapped which facilitates the concealment of the pipes conducting of the

water supply. Sewage water conducted by similarly concealed pipes is treated in-situ in a septic tank by an anaerobic process. The electric installations are spread across the building with rubber flexible cable (3 x 12 AWG). The absence of cladding leads to aesthetic issues due to the difficulties of embedding cable inside the Guadua or hiding it in a skeleton-like structure. Where possible cable was covered by half bamboo canes as shown in Figure 9b.

Internal features (Finishing)

Figures 9b & c show internal features where the design and selection of materials follow Bohio's architectural concept. Clay fired accessories for bathroom basins (Figure 9c), a wooden staircase, fitted furniture and gas lamps were inspired by vernacular architecture and regional traditions. Rattan fibres are specified for door faces and bed headboard, sisal cords for covering exposed installation pipes. The completed building is presented in Figure 10.



Figure 10. Bohio's project.

Discussion

Currently, Guadua construction is characterized by a degree of uncertainty and depends on the quality of handicraft. A bamboo building can be designed in detail according to standards and construction codes which give guidelines for structural design and the selection of materials. However, during the construction process the natural variability of the material requires experienced craftsmen to deal with the different diameters, internode lengths and the tapering characteristic of bamboo. Also, expertise in carpentry is required for making cuts and connections. Verticality and horizontality are not easy to achieve. For instance, window frames were built out of Guadua in the uppermost level of Bohio to achieve some weather protection by design, but factors such as variability, the presence of nodes, the constant swelling and contraction and the round surface of the material, hindered the construction process and presented functionality issues. The bio-deterioration of the material presents another challenge for the protection of external building elements, such as columns, cladding or railings. Open porous coatings must be used and exhaustive maintenance performed every 4 to 6 months. Another demanding issue to consider in construction with Guadua is the arrangement of internal installations

which cannot be hidden inside the culms and are usually exposed, leading to aesthetic and functional conflicts.

Conclusions

Project Bohio is an exemplary building constructed with bamboo *Guadua Angustifolia* Kunth that complies with the regulations of the Colombian building code for the design of *Guadua* structures. However, this standardized building system is relatively new and challenges such as the handcraft building process, the bio-deterioration of the material and its integration within conventional systems must be revised. Assessments of buildings such as the one presented in this paper are needed to evaluate the pros and cons of this building system. Once all the aspects have been thoroughly evaluated and environmental impacts addressed, a low carbon construction system using *Guadua* bamboo will be achieved.

Acknowledgments

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References

- Comisión asesora Permanente para el regimen de construcciones sismo resistentes, *Reglamento Colombiano de construcción sismo resistente: NSR-10*. Ministerio de Ambiente, Vivienda y Desarrollo Territorial, República de Colombia, Bogotá D.C., Colombia, (2010).
- FAO. *Global Forest Resources Assessment 2010, Main report*. Forestry Paper 163, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, (2010).
- Hidalgo, O., *Bamboo: The gift of the gods*. D'vinni Ltda, Bogotá D.C. Colombia, (2003).
- Jayanetti, d. L., and Follet, P. R., *Bamboo in construction, An introduction*. TRADA Technology Limited and International Network for Bamboo and Rattan (INBAR), (1998).
- Liese, W. *The anatomy of bamboo culms*. Technical Report No. 18, International Network for Bamboo and Rattan (INBAR) New Delhi, India, (1998).
- Riaño, N.M., Londoño, X., López, Y. and Gómez, J.H., Plant growth and biomass distribution on *Guadua angustifolia* Kunth in relation to ageing in the Valle del Cauca – Colombia. *Bamboo Science and Culture: The Journal of the American Bamboo Society*, 16(1), pp 43-51 (2002).
- Yiping, L., Yanxia, L., Buckingham, K., Henley, G. and Guomo, Z. *Bamboo and climate change mitigation*. Technical Report No. 32, International Network for Bamboo and Rattan (INBAR) Beijing, China, (2010).

Guadua Angustifolia Kunth is an alternative to metals and synthetic fibers in order to build comfortable urban furniture including Hi-Tech wind turbine for powering ultimate technology L.E.D. street lamp.

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Abstract

This paper examines the advantages of using bamboo, *Guadua angustifolia* Kunth, in the field of city outdoor equipment and energy production.

Deploying bamboo as cities' urban furniture will make an easy demonstrative example. So, thanks to being in touch with citizens and potential users, *Guadua* can be taken seriously as a building material in those places where it has never been used before.

This paper explores GAK as an alternative to metals and composites in the field of wind turbines and slender structures for the city.

Mechanical properties, estimated durability and the ability for sequestrate CO₂ makes *Guadua angustifolia* Kunth the best choice for replacing steel, aluminium and other CO₂ producers and energy consumers building and technology materials.

Keywords

guadua, city, bench, lightlamp, energy, windturbine,

Abbreviations

G.A.K.: *Guadua angustifolia* Kunth

Introduction

Different excuses difficult the choice of natural wood resources, in favour of synthetic materials: Properties variability, quality acceptance, fatigue and wear resistance, fire behaviour, price, profitability. The properties of those synthetic materials, like metal, concrete and polymers, are thought to be accurately determined. Furthermore, metals' mechanical properties are isotropic.

Although fatigue and rigidisation occurs at natural materials, they have proved for centuries long term durability; meanwhile synthetic materials are rarely that old.

Guadua angustifolia Kunth, G.A.K., as a wood resources for building shows excellent properties, even better than European woods. Mechanical behaviour, CO₂ sequestration levels, easy growing management, biodiversity and water quality improving properties. But G.A.K. Is not yet being deployed at Europe. To those reasons previously explained for all wood resources, the lack of local examples with G.A.K.

So local examples must be done to show GAK as a great choice for building material.

This paper uses a urban furniture example to be used by people every day. So citizens, professionals and constructors can easily accept G.A.K. as a great structural choice.

Wind city turbine for renewable energy production. Bench and bike park for citizen use.

Extreme structural requirements, wear and fatigue is among a useful tool for citizens.

Requirements for urban furniture designed

In behalf of tax payers, urban furniture should be easy enough to be built and repaired "priceless".

Citizen needs and placement conditions can be very different according to a global application so, industrialized flexible design concepts must be applied.

Places to relax, rest, socialize, feel protected, are principal needs that city furniture should cover.

The project includes: lamp, wind turbine, bench, trash bin and parking for 6 bikes.

A design for easy building is the first step to reduce consumption.

It is also easy to realize that many other designs and scales are possible and ready to achieve. (Figure 1)

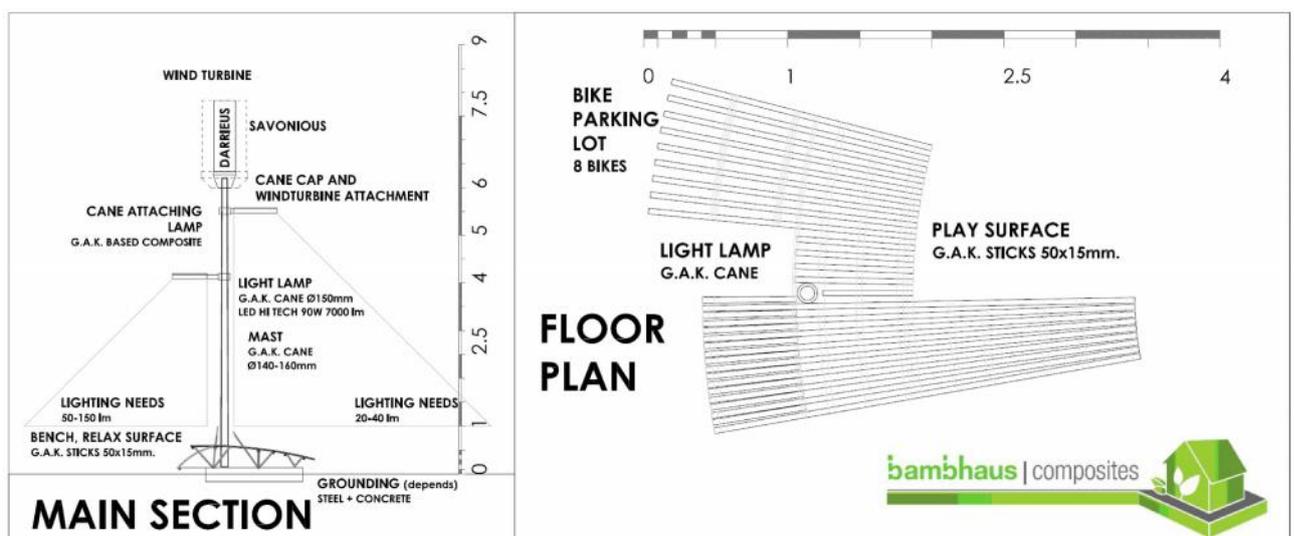


Figure 1. The urban furniture designed.

Wind resource in the cities and electricity production

The wind resource in the city is quite different from that one in open field, as it is affected severely by the near environment. Several factors must be taken into account before installing a wind turbine, whatever the type it is: Some of them are the wind characteristics, energy extraction, fatigue loading, electrical network connection, acoustic emission, aesthetics...

First factor to consider is the wind resource. Classical models describing the atmospheric boundary layer such as Solari's or Von Karman's will not be applicable anymore in urban environment. In spite of that, the wind will be very turbulent, and the medium speed reduced in almost every place. Particular attention deserve street canyons, where higher wind speeds can be expected. As an example we can cite the rivers bridges, particularly if they are surrounded by hills. In areas with a strong dominant wind direction, street canyons can be used to increase wind power draw, but in every other place, main attention must be focused in using the aforementioned turbulence.

To know in advance the amount of energy that a particular urban area can supply, a model of that area is needed in addition to a statistical description of the wind in the area in the last 5 - 10 years. Future development of the city must be considered also to avoid unnecessary spent of resources in places where wind will be brought down in a near future; this includes tree growth. For such a complex environment, state of art is CFD modelling. Specialized software should be used to anticipate the type and size of wind turbine to be installed in a certain location. Adapted models can be used and adjusted by means of measurements in several characteristic points in the area.

It can be anticipated that changing direction is the main factor to deal with. When not installed in urban canyons, this parameter and the high turbulence must be the design drivers in order to obtain the maximum energy of the wind and, nonetheless, fatigue loading in the structure. Wind turbines have the requirement to be light and flexible to better absorb the wind speed changes, and fatigue is a critical issue in their structure design, mainly affecting to the blades and drive train design.

Aspects such as acoustic emission are of primary attention in cities, where a continued noise due to wind is not acceptable. Wind turbines with low noise rating must be installed, and carefully tested in adequate wind tunnels to guarantee that their installation will not be a problem for the comfort of the citizens.

Finally, a more opened approach can be given to the aesthetical design of the wind turbine, as probably the efficiency of the machine is not that important as in areas with strong, constant wind. That allows to the aerodynamic designer more freedom to adapt the design to the architectural requirements.

Wind turbine choice and energy production

The driver concept in the wind turbine design from the aerodynamic point of view is its ability to capture wind energy. The comparison between different machines is achieved through the C_p , which stands for Coefficient of Power. The maximum C_p achievable is 0.59 times the wind energy in a certain area. This limit takes its name after the works of Albert Betz and thus it is known as Betz's limit. Some other authors discuss the adequacy of it for vertical wind turbines, as the starting hypotheses are somewhat questionable. After its definition and knowing the wind statistical distribution in direction and intensity, and knowing the maximum C_p achievable, the calculation of

the amount of annual energy that can be expected from a certain design in a certain place is simple.

When calculating wind energy, it is important to note that energy draw is proportional to the cube of wind speed. That, by considering that the cube of the media is lower than the media of cubes, would suggest that designs must stand a high wind speed better than be designed for lower winds to take the most of it. A problem arises, that aerodynamic design is completely different for both cases and only certain attention can be paid to high wind design when searching low wind use.

As stated before, the wind statistical description is paramount in the energy calculation. As an example, we will use a wind measurement at $z=10$ m in a variety of Spanish cities. Weibull distribution is adequate for wind description and so in has been used in these calculations:

$$C_p = 0.5 \cdot \rho \cdot V^3 \cdot C_p$$

$$AEP = []$$

With these equations and a typical C_p of 0.31 for a Darrieus type, at sea level, and with the data measured in the airports of three cities in three different environments during ten years, the following figures arise:

Mountain-surrounded: 945 kW·h

Seashore, strong winds: 236 kW·h

Seashore, mild winds: 983 kW·h

These calculations are based in wind turbines that can stand very high winds while still working, and not the type with cut-off at a typical 15 m/s. (figure 2)

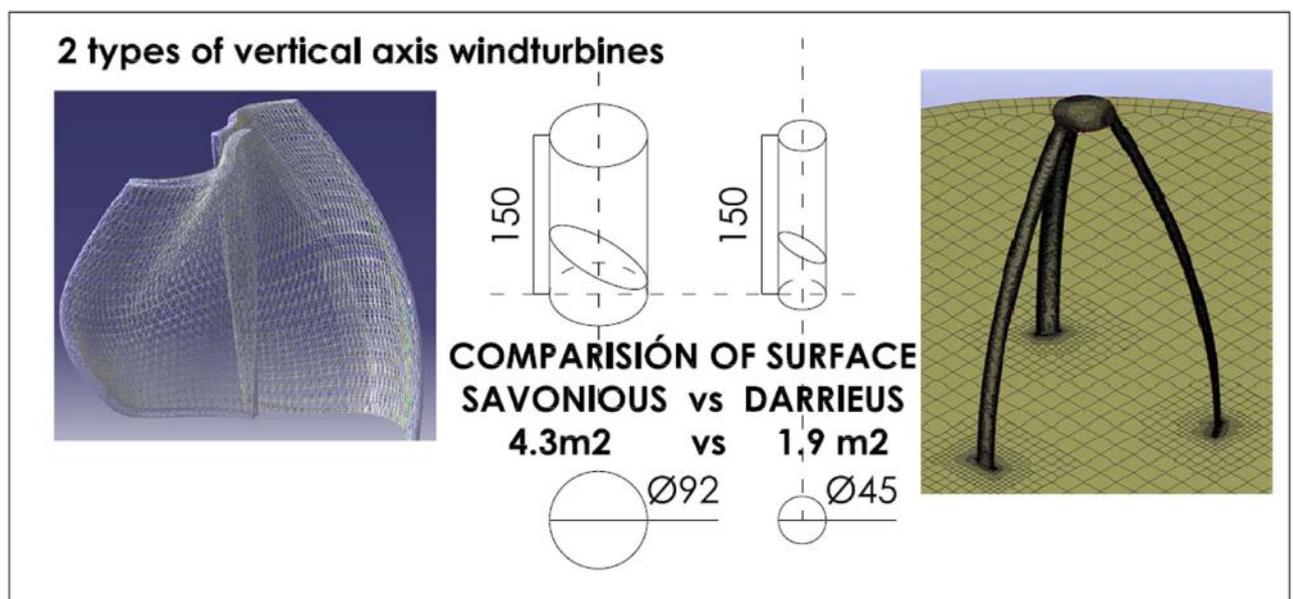


Figure 2. Darrieus wind turbine.

The wind power generator

Due to the constant wind direction change, the selection of the wind turbine concept is key for a maximum energy extraction. The constant yawing of a horizontal, classical wind turbine is not acceptable as the performance would be seriously affected. Instead of that, a vertical axis wind turbine is preferred because of its ability to capture wind direction changes. Besides, they are intrinsically more quiet due to the fact that the wing tip is not the faster part of the blade. Although mathematical

modelling in some cases can be quite complicated, the higher efficiency makes the effort worthwhile: The more we invest in engineering the more profitable the result will be.

Two concepts are next evaluated: The Darrieus and the Savonius. A Savonius type VAWT (Vertical Axis Wind Turbine) is a simple drag driven mechanism, while a Darrieus is almost the most complicated aerodynamic machine that can be built, due to a permanent transient state of the air foils of the blades. Being a lift-driven machine, its C_p is much higher than that of the Savonius.

The requirements of the turbine are the following: Medium power of 300 W during some hours of the night and light, turbulent wind most of the time. While a Darrieus will work at high rotational speed, a Savonius will work at less than half the rpm. Also, the cut-in wind speed is much higher in a Darrieus type, because the Reynolds number seriously affects the aerodynamics of the air foil. Still, the wind machines and in particular air foil based ones, tend to loss efficiency when their size is small, and for Reynolds numbers under 50.000 their Lift/Drag ratio drops drastically.

That said, a Darrieus will work a small amount of hours in the year, but its energy draw will be higher, for the same size, as a Savonius. Therefore a connection to electrical network or a powerful battery is necessary to make the system operative. A Savonius type is not that complicated at all: It will produce less energy, but it will also rotate in the most light wind producing some watts and a wrong design will not prevent it from working, although with a poor performance. The most adequate concept, particularly for urban applications where no wind canyons with a dominant direction exist, is the Savonius type, if no additional considerations are made.

Mathematical modelling of a Savonius type is not an easy work, although some work has been done in past decades. At least a couple of calculation models exist, one of them dating from Wilson-Lissaman in 1974, the other coming from the modern CFD calculation models. As the first one is an analytical model, it is more adequate for a parametric design, that adequately driven by a genetic algorithm or similar means permits improving the design to the maximum, using mathematical concept design rather than big calculation machines as those required for CFD. When a proper design is achieved, the application of CFD seems more adequate for the validation of that model and refinement of details such as acoustic model emission. A C_p of 0.20 is a correct approach for the performance of these turbines.

A Darrieus type admits more different modelling, namely stream-tubes, lifting line, vortex, VLM and again CFD modelling, extremely complicated in all cases due to the transient nature of the problem. As an example, 41 hours of a powerful workstation were needed to get a proper result in a single working point of a turbine. Particularly, lifting line modelling would permit applying the same genetic optimization technique, while also CFD modelling is desirable for the final aerodynamical design phase. Numbers for C_p of 0.31 for fixed blades and 0.52 have been achieved for this design. A model like this is more suitable for wider streets with a more constant and strong wind, although the manufacturing is notably more complicated.

The required energy for maintaining the lamp on for a media of 8 h/day during a year is of 876 Kw·h. To get that energy amount for a year, doing so an autonomous street lamp with the proper battery pack, is in both cases, and taking using the formulas and figures above, of:

Savonius type: $S = 4,3 \text{ m}^2$

Darrieus optimized type: $S = 1,9 \text{ m}^2$

Aesthetical requirements are also present. While a Savonius type can have beautiful shapes and can be decorated, using it as urban ornamentation, its size, at least to make a street light, seems too big for the case under study. But a light, thin Darrieus of 1,9 m², can be really tempting for its installation. If this is the case, the expected energy draw during strong winds is higher than the capacity of any battery to absorb that energy, so a network connection would be required.

New mixed concepts are being developed to improve low wind capabilities of Darrieus VAWT that will have the best of both designs, so no big investments and sizes will be required any more.

Guadua angustifolia Kunth as main material

As is known G.A.K. is an exceptional material suitable for all kind of applications.

In this paper we combine natural as cane or sticks application and technological as GAK composite for wind turbine blades or other highly mechanical demanded parts.

Guadua based components as main material for wind turbines.

When talking about long, slender structures, aeronautical structural design parameters can be used. Not by accident aluminium, and furthermore composite materials, are of main interest in aeronautical structures: Not only good mechanical properties are present, but they also have a low density while retaining high mechanical properties. In addition to the obvious weight savings, a less obvious factor usually is not taken into account when conceptualizing a new structure: For the same weight, a low-density material has always higher inertia modulus. Both characteristics, the weight related yield strength and the higher modulus are next analysed.

Bamboo is a low density material, the higher the density the higher its resistance.

Typical values of density and resistance are exposed in the next table:

Material Resistance Comparison

Material	Density	Flexural Bending		Parallel compression	
		Strength	Specific yield	Strength	Specific yield
		Kg/m ³	MPA	MPA/KG	MPA
GAK CANE	800	45	0,056	44	0,055
2024 ALUMINUM	2780	290	0,104	290	0.104
4130 STEEL	7850	841	0,107	841	0.107
GAK STICK	800	45	0,056	50	0.055
GAK STICK+CORTEX	800	80	0,100	60	0.187
GAK FIBERS	800	500	0,625		
GAK COMPOSITES A	1200	150	0,125		
GLASS FIBER AXIAL	2600	3445	1,325	1080	0.415

It can be seen that bamboo has not really much lower resistance. Besides, the construction of slender structures such as airfoils with metal is a difficult issue, as they must be empty inside due to their high density. Much better manufacturing could be conducted if a thick, solid airfoil is constructed. This can only be achieved by the use of low-density materials.

Higher modulus of inertia will be a factor to take into consideration when designing the airfoil, so main attention can be given to design of high thickness airfoils to exploit the bamboo's abilities as high inertia modulus material. In addition, when blades are manufactured in bamboo the outer skin can be manufactured in cortex GAK fibre, to reinforce the sandwich effect.

Finally, the high elasticity of bamboo makes it very adequate for absorbing constantly changing wind, as accelerations and response to the gust will be milder than in other cases. The structure would suffer deflections easily, but not approaching its yield limit, and gust would be naturally absorbed, improving the behaviour of the structure, the drive train endurance, and probably the noise emissions.

Natural Guadua cane as mast for lighting and wind turbines.

When talking about the mast, the natural fact of how bamboo forests behave with wind is an intuitive notion of that it will work as required. Particularly, when such mast is stripped of its branches and leaves, resistance to wind impact is still higher, and mast does not need to be treated or machined.

On the other hand, the cylindrical shape is symmetrical and a low wind resistance coefficient is present, although Von Karman street phenomena could appear and design features could be required depending of the mast design and wind conditions present. (figure 03)

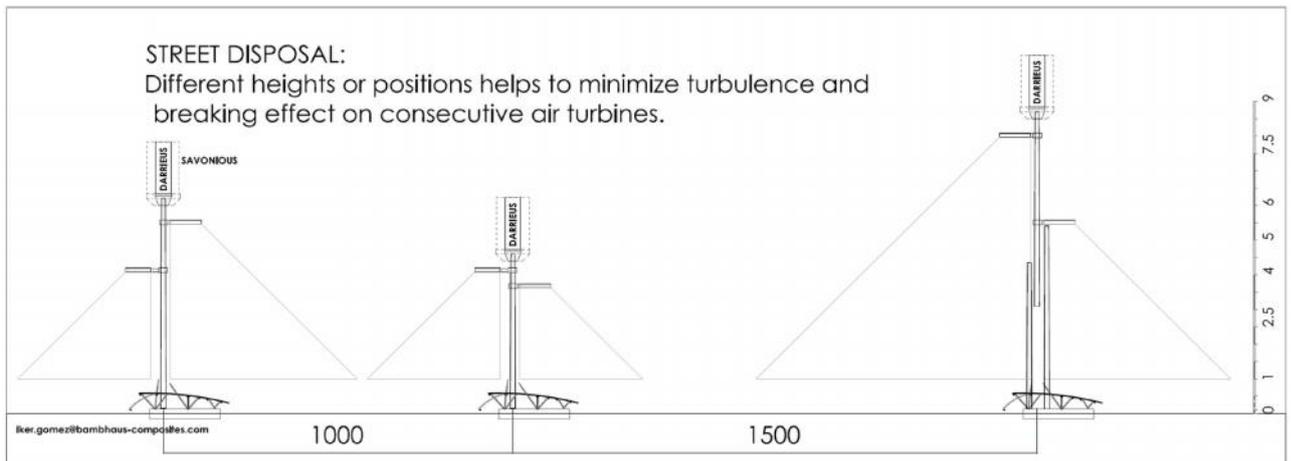


Figure 3. G.A.K. mast description and mechanical demand of the mast for a 250 km/h wind.

G.A.K. sticks made out of natural cane as comfort surfaces

Flexibility of G.A.K. Stick enables comfort while providing an excellent response to impact strength.

Guadua used for balancing CO₂ emissions and energy.

A 55% coefficient can be applied to translate GAK weight into CO₂ sequestered.

Procedures applied to GAK during its building process are considered too for measuring CO₂ balance.

Using GAK in its multiple choices seems the best choice to balance CO₂ emissions

Xilophagus and other wood predator protection treatment is mandatory for exporting G.A.K.

Immersion of the GAK in a non-toxic Borax solution is used.

When using GAK outdoors, as cane, UV protection of the mast is recommended to avoid cracking.

A non coloured water-based latur is used and 5 year protection is expected.

The remaining GAK parts will not be externally protected due to 2 main reasons: explore the wear resistance of GAK as sticks, and minimize the contact with synthetic materials although it would be harmless.

The values of carbon and energy consumption depend on the tasks involved in the process of the materials.

In order to simplify the task for approaching, components are assimilated beneath reasons of main materia.

Approach to embodied CO₂ and energy in the project made with G.A.K.

	Weight kg	Embodied CO ₂		Embodied Energy	
		kg CO ₂ /kg	total	MJ/kg	total
GAK. Cane	150	-0,55	-82,5	9	1350
GAK. Stick	500	-0,4	-200	10	5000
Concrete base:	500	0,13	65	1,3	650
Corrugated Steel	20	2	40	47,4	948
Steel and metals:	15	2	30	48,4	726
Electrics and Optics:	15	4	60	90	1350

windturbine G.A.K. based:	50	2	100	20	1000
		12,5 kg CO ₂		11.024 MJ	

Approach to embodied CO₂ and energy in the project made with Steel and Aluminium

Instead of trying to use GAK in every part of this project:

Mast is made of Steel and Wind turbine is made with Aluminium and polymer composites.

	Weight	Embodied CO ₂		Embodied Energy	
	kg	kg CO ₂ /kg	total	MJ/kg	total
Steel Cane	300	2	600	50	15000
GAK. Stick	500	-0,35	-175	10	5000
Concrete base:	1500	0,13	195	1,3	1950
Corrugated Steel	80	2	160	47,4	3792
Steel and metals:	0	2	0	48,4	0
Electrics and Optics:	15	4	60	90	1350
windturbine polymer composite:	40	8	320	20	800
Total:		1.160 kg CO ₂		27.892 MJ	

Conclusions

This project shows an example of how *Guadua Angustifolia Kunth* can be combined with Hi Technology equipment and design to make a self-functional urban lighted space.

Due to a good specific yield, GAK is a good alternative to metals like steel and aluminium. And should be studied in advance in order to provide an alternative to steel, aluminium and other synthetic materials used in the field of aeronautics or urban furniture.

There are different types of wind turbines; vertical axis are the most appropriate for changing direction winds. Due to a better efficiency on wind catching, Darrieus optimized type is better than Savonius type. Darrieus is lighter too.

Due to several conditions involved in the processing of materials CO₂ and energy embodied should be always take as an approach.

Although use of GAK, embodied CO₂ emissions are difficult to balance due to electric and lighting system.

In order to balance embodied CO₂ emissions, GAK, as happens with wood, should be used more.

Nevertheless if we would have done the mast with steel, the embodied CO₂ emissions would have been up a thousand kg more.

Acknowledgments:

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References

- Bleaney, A., Peskett, L., Mwayafu, D., 2010. REDD-plus after Copenhagen: what does it mean on the ground? COP 15 Briefing, January 2010.
- Boyd, E., 2009. Governing the Clean Development Mechanism: global rhetoric versus local realities in carbon sequestration projects. *Environment and Planning*. 41:2380 – 2395.
- Del Águila García, Alfonso. Industrialización de la edificación de viviendas. Tomo 2 “componentes” Madrid 2006
- green composites. 18th international conference on composite materials
- Ramirez F., A. Maldonado, J.F. Correal, M. Estrada. Bamboo-guadua angustifolia kunt fibers for
- Riaño, N.M., X. Londoño, Y. Lopez & J.H. Gómez. 2002. Plant growth and biomass distribution on Guadua angustifolia Kunth in relation to ageing in the Valle del Cauca - Colombia. *The Journal of the American Bamboo Society* 16(1): 43-51. Sociedad Americana del bamboo. Washington 2002
- Van Vuure, A.V. , L. Osorio, E. Trujillo, C. Fuentes, I. Verpoest. Long bamboo fibre composites. 18th international conference on composite materials, University of Bath, 2008. *Inventory of Carbon and Energy (ICE)*.

Study of the traditional joint of bamboo houses in earthquake areas by tilting tables

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Abstract

Most of the earthquake that occurred in Indonesia destroying non engineer buildings which had been built without calculated the strength and safety. The problem for these buildings is the error construction that was not appropriate with the rule of structure endure earthquake that appropriate with several variety of the materials.

The several earthquakes that found in the field establish that the house with bamboo construction had a bit damaged and it was not collapsed as the house that used a masonry wall whereas the connection system that used was very simply, that was used coco rope string and dowel/pin from bamboo or wood that is legacy of our great-grandparents. Because of that, Research Institute for Human Settlements carried out the research about the strength connection system to lateral force as a simulation with using tilting table. The lateral force works in the specimen get in the way correlate specimen mass, gravitation, and the declivity tilting table. This tool used because the modern test tool not available yet at that time. The specimen for full scale (6x6) m with used betung bamboo (ϕ 10 – 12 cm and string bamboo ϕ 8 cm) as the main structure while the connection system using coco rope string and dowel/pin from bamboo.

The test result indicates that after three times up and down to the declivity maximum 42° with interval slope 6° , the building was not collapsed and only had few damaged (in bamboo which has the age of young). According to the earthquake zones (SNI 03-1726-2002) concern about Tata Cara Perencanaan Ketahanan Gempa Untuk Bangunan Gedung then the specimen model can be apply in the earthquake areas.

This result study offers information for the practitioner, about the level of house vulnerability with bamboo structure and expect for the building planner to developing the bamboo potential for the economics building, easy to do but resistant on the risk of earthquake disaster.

Keywords

Tilting table, non-engineer building.

Introduction

Most of the earthquake that occurred in Indonesia destroying non-engineer buildings which had been built without calculated the strength and safety. Many other buildings can also be considered as non-engineered buildings such as masonry with/without reinforced concrete structures, wood/bamboo structures that were built without any engineering analysis since they were built by traditional method with ordinary masons/workers. The problem for these buildings is the failed construction that was not appropriate with the rule of structure endure earthquake that appropriate with several variety of the materials.

The several earthquakes that found in the field establish that, the house with bamboo construction had a bit damaged and it was not collapsed as the house that used a masonry wall whereas the connection system that used was very simply, that was used coco rope string and dowel/pin from bamboo or wood that is legacy of our great-grandparents.. Construction of elements made from bamboo are widely used because they are on account of the low cost, easy working, the aptitude of many users, the quick availability of bamboo and the traditional rural ways of construction appropriate to the economic situation of the lower income group. Bamboo like lumber, makes a light, flexible house that is much better than "modern" materials at surviving earthquakes.

Considering large number of non-engineered buildings, efforts should be made to make these buildings safer. Research Institute for Human Settlements carried out the research about the strength connection system to lateral force as a simulation with using tilting table. The lateral force works in the specimen get in the way correlate specimen mass, gravitation, and the declivity tilting table. This tool used because the modern test tool not available yet at that time. The specimen for full scale (6x6) m with used betung bamboo (ϕ 10 – 12 cm and string bamboo ϕ 8 cm) as the main structure while, the connection system using coco rope string and dowel/pin from bamboo.

The purpose is to know the characteristic and resistance of the bamboo house model to the lateral force using the tilting table.

A study of the traditional joints system of a building was given as an example of efforts in reducing vulnerability for non-engineered buildings, producing field manuals/guidelines and developing appropriate building technology to reduce structural damage because we are partly responsible for damaging the building in past earthquake

Why bamboo

Indonesia is one of the countries with the largest bamboo resources in the world. Nowhere, however is bamboo of greater importance than in building construction. As late as 1956 35% of all houses in Indonesia were constructed from bamboo and in further 35% bamboo was used in combination with timber. In 1980 in two largest cities of Indonesia Jakarta and Surabaya that 41.5% of dwellings of the low income groups were still being constructed from bamboo.

Bamboo can tolerate high values of deformations in the elastic range i.e. possesses high elasticity. Therefore bamboo houses when properly constructed are ductile i.e. being able to sway back and forth during an earthquake, without any damage to the bamboo poles. The construction materials for building a bamboo house should be readily available and accessible. The bamboo based house has a very low weight therefore foundation can be minimized.

Tilting table

The tilting table is one of the equipment to test the earthquake resistant building (static) which was installed in the Structure laboratory Research Institutes for Human Settlements. The slope is a forms of the simulation of the earthquake acceleration and this tilting table is suitable for frame construction

system then masonry. All of the equipment components made from steel plate and installed 3 m height from floor. The position of table supported reinforced concrete foundation.

The specification of tilting table are as follows;

- Size Wide = 700 cm, length = 800 cm and thickness of the table plate = 8.8 mm.
- Maximum the table slant = 42°

The lateral forces active when the tilting table moves (up and down) by two hydraulic jack with capacity 60 ton. The large of lateral forces same as building weight when the table was slope. All of the data test obtained from the transducers which connected to all of the joint between components than record to the computer

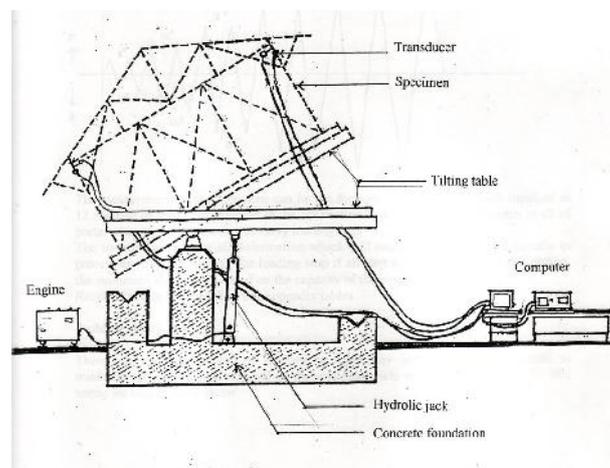


Figure 1 : Tilting table

Model of a bamboo house

4.1. Specification of house

- Size = (6 x 6) m with height of ceiling = 2.6 m and roofing cover by corrugated zink.
- Column and trusses included purlins use: Bamboo betung ϕ 12 cm and Bamboo tali ϕ 10 cm
- Joint connectors : coco rope and dowel/jig from bamboo.

4.2. Mechanical properties of bamboo

- Tensile strength varying results from 1000 – 4000 kg/cm²
- Compression strength varying result from 250 – 1000 kg/cm²
- Bending strength varying result from 700 – 3000 kg/cm²
- Modulus elasticity varying result from 100.000 – 300.000 kg/cm².

The model of bamboo house as follows ;

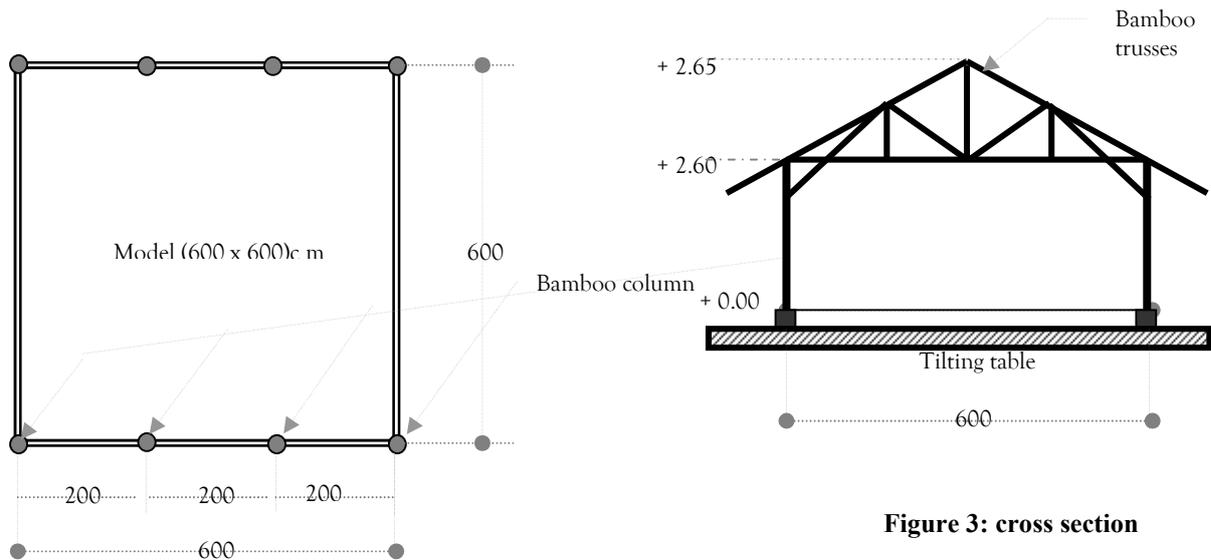


Figure 2 : Scheme of house

Figure 3: cross section

Methodology of testing

The procedure of testing is to move the table (up and down) with the interval 6 (six) degrees for each step (from 0 degree up till 42 degree) as mention below ;

- Step 1 = 0° - 6°
- Step 2 = 0° - 12°
- Step 3 = 0° - 18°
- Step 4 = 0° - 24°
- Step 5 = 0° - 30°
- Step 6 = 0° - 42°

The diagram of loading are determined as follows ;

The displacement (simpangan) of frame construction decided 1.75% (masonry is 1.5 %) from building height and data measurement can be get through 12 transducers which installed in 12 location of the frame construction and directly processed in computer..

Result of testing

Loading test was carried out in the cross section same as structure analysis calculation and in this direction the shear strength of building supported by four (4) portal trusses.

Same as some previously test for masonry houses the building safety determined = 3 to anticipate the difference quality works and building materials used (in laboratory and fields).

The result of testing based on data logger and computer calculation maximum response spectra = 0.67 G.

Using the Building Planning Resistant Code SNI 03-1726-2002 explains that this house model can be built on all of earthquake zone (hard and soft soil).

Table 1 ; The response spectra earthquake plan

Condition	Earthquake Zone					
	1	2	3	4	5	6
Hard	C = 0.33	C = 28	C = 0.24	C = 0.18	C = 0.12	C = 0.04
	Ok	Ok	Ok	Ok	Ok	Ok
Soft	C = 0.38	C = 0.36	C = 0.60	C = 0.30	C = 0.20	C = 0.08
	Ok	Ok	Ok	Ok	Ok	Ok

Note :

C = Coefficient of earthquake, calculate by gravitation

Ok = Building in the elastic condition

△ = Building in the inelastic condition

X = Building is collapse

The analysis using the relation of displacement and lateral forces shown as below tables ;

Table 2: The displacement of transducers

No of transducers	Displacement (mm)	No of transducers	Displacement (mm)
Tr - 1	5.61	Tr - 7	10.67
Tr - 2	36.32	Tr - 8	37.85
Tr - 3	37.55	Tr - 9	36.82
Tr - 4	37.44	Tr - 10	22.87
Tr - 5	34.51	Tr - 11	4.21
Tr - 6	(illegible)	Tr - 12	(illegible)

Not all behavior of the transducers clear to be read because some reasons as explain from the diagram of the relation between displacement and lateral forces below.

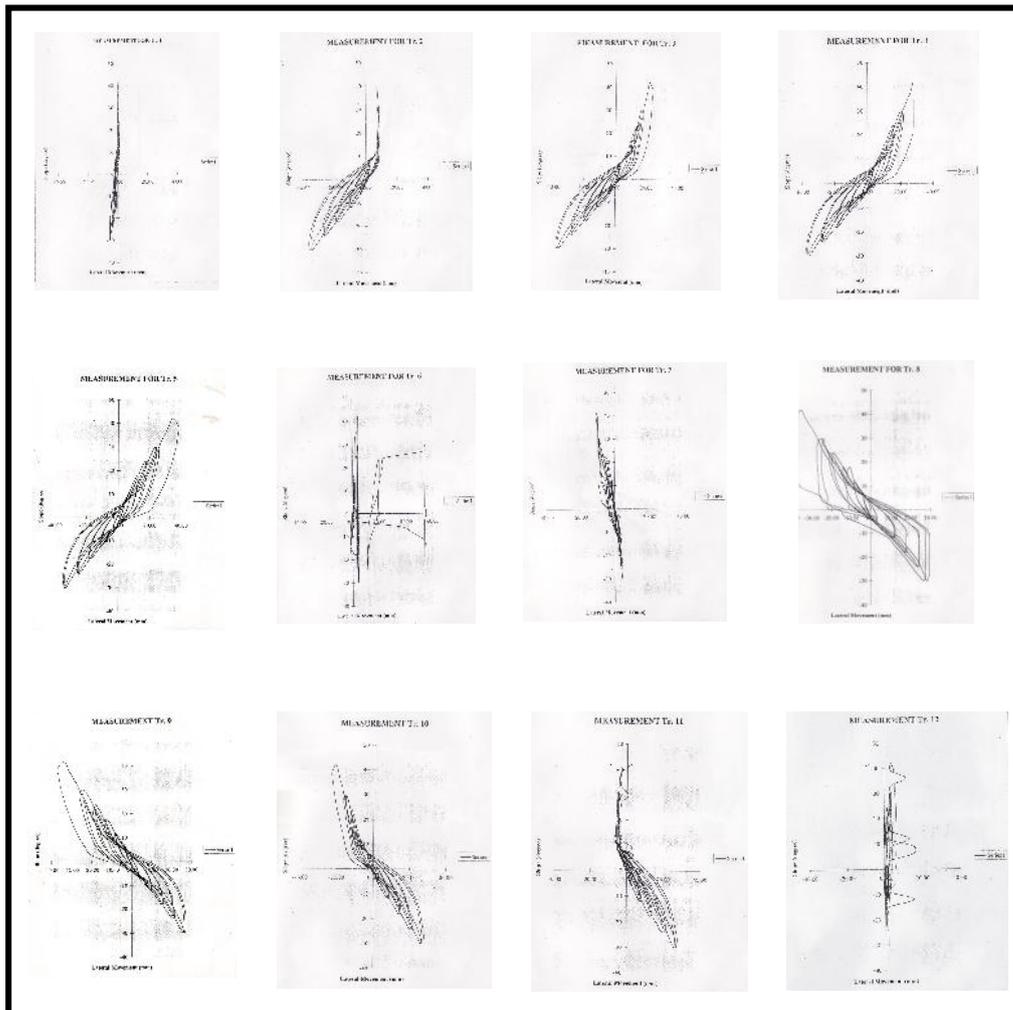


Figure 4 ; Diagram of the displacement and lateral forces

The above diagram shows that transducers no 6 (Tr-6) and no 12 (Tr-12) can not be read (illegible) because the position of transducers changed. Some of transducers can not be read i.g. Tr-2, Tr-7 and Tr-11 caused by condition of transducers or not in stable position.

Conclusion

- The connection use coco rope and bamboo dowel is a good system for materials with tubular shapes in particular bamboo,
- The structures design is available used in earthquake areas because the test result shows the displacement < 3.9 cm (1.5 % from the height of ceiling = 2.60 m) and in plain view the frame construction still in a good condition (not crack and collapse) up till the testing finish.
- Bamboo can tolerate high values of deformations in the elastic range i.e. possesses high elasticity. Therefore bamboo houses when properly constructed are ductile i.e. being able to sway back and forth during an earthquake, without any damage to the bamboo poles.
- The quality of bamboo must be more then 3 years old and the water contents < 10 % because very influence the construction strength.
- This system not so accurate like dynamic system because tilting table is only assumption use a simulation of the building gravitation forces to earthquake .

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References

- Directorate of Building Research, Buku Pedoman Perencanaan untuk Struktur Beton Bertulang dan Struktur Tembok Bertulang untuk Gedung 1992.
- Directorate of Building Research, *Indonesia Earthquake Study*, vol. 3, 1992
- Directorate of Building Research, *Technical paper No 12, Testing of Brickwork, 1989.*
- Krishnankutty, C. N.; Chundamannil, M.. *Journal of Bamboo & Rattan (VSP International Science Publishers)*, 2005, Vol. 4 Issue 4, p311-316, 6p; DOI: 10.1163/156915905775008381
- Murdiati and team, Laporan-laporan Penelitian Bangunan/Perumahan terhadap Gaya Gempa 2001.

Bamboo Design and Construction in the Philippines: the Cabiokid Experience

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Introduction

Long before, living spaces in the Philippines were built with biological materials coming from a variety of resources. People living in various eco-systems connected with their landscapes to build holistic and relevant habitats. There was a wide range of biological source materials coming from varied eco-systems like timber, palms, bamboo, and other grasses, which were collected as building and construction materials.

Then, living spaces in the Philippines were well connected with nature, not only because the house itself was built with biological materials but also because the surroundings, which provided for all the needs of the family living inside it, were well maintained and occupied vast tracks of land. Dwellings before would be situated in- or beside forests, on the flatlands, or near water bodies such as rivers and coastal areas. Such living spaces situated in different ecosystems opened varied and abundant possibilities for food, clothing, and other livelihood activities. All the dwellings of this era had a common denominator among them; they were primarily built with bamboo. In the vernacular, they are all called nipa hut or *bahay-kubo* (Fig. 1).



Fig. 1 - typical house design of a bahay-kubo which are on stilts with nipa roof

In the tropics such as the Philippines, dwellings are multifunctional in nature; they act as protection from the surrounding forces of nature, such as strong typhoons, rains, excessive heat and other animals. Dwellings also come in different designs based on the location and the weather of a specific area. The Philippines has the blessing of sufficient sun, water, wind and volcanic soil, added with various topographies like mountains, plains and coastal zones, which are all well distributed

throughout the archipelago. This fact led to the different design innovations of the local craftsman in creating their own living spaces (Fig. 2).



Fig. 2 – Cabiokid’s design of bamboo construction

Cabiokid’s challenge

Cabiokid is a permaculture experimental area, which serves as a fully functional farm and relevant techno park located in the central province of Nueva Ecija on Luzon Island. It is just 90 kilometers north from Manila and due to its location near the great central swamp area and flat in topography, agriculture remains the main source of livelihood in the area, with ricefields dotting the landscape (Fig. 3).

Cabiokid started experimenting with the benefits of bamboo since 2000. Bound by the principles of permaculture designing as its guiding framework, the organization aimed to fuse traditional and modern techniques to design and create functional and energy conscious living spaces out of biological and technological materials. As permaculture is primarily concerned with systems designing and natural sustainability, and with the farm located in the flat grasslands of Luzon, Cabiokid rekindled its relation with bamboo as a primary building material. Throughout its existence, Cabiokid provided simple ways and means on how to be reconnected again with nature by using biological materials in creating all of its facilities and living spaces. The discussions in this paper will attempt to zoom into the efforts of bamboo construction over a span of 11 years.



Fig. 3(left). Cabiokid's bird view and the surrounding landscape.

Goals of the Project

To showcase some experiences of Cabiokid Foundation with traditional designs and construction methods for building bamboo structures in its own development site and in the communities they are helping in other areas in Luzon island.

The paper will provide an explanation on how Cabiokid revived traditional construction methods in order to built creative and sustainable living spaces. Along, we will discuss the traditional techniques such as harvesting, weaving, and joining bamboo members and how we incorporated these techniques in today's era where cement and steel dominates construction methods. Furthermore we will show why it is relevant to construct primarily with biological materials rather than technological materials in a humid tropical country like the Philippines. Lastly we will also discuss the different species of bamboo, their characteristics and where they are best used in the construction process. All these efforts are situated in different sites in the Luzon island of the Philippines through the efforts of Cabiokid Foundation and various local organizations.

Our encounter with bamboo... species

Originally, the Philippines had relatively few bamboo species growing in its thickly forested areas and of these species most were climbing bamboos. These species were less useful for building and construction, but were often used for tying wooden beams together. Of the three species described below, "*Schizostachyum lumampao*", commonly known as *Buho*, is the only endemic species. Over the centuries other bamboos have been introduced in the country and at present there are 10 commercial species of bamboo growing in the Philippines, which may be appropriate for building structures. These species have been used by the people for over centuries to aid in the construction of their houses. Five of these bamboo species were cultivated by Cabiokid. These are widely used for

the construction of our buildings. In the process of experimenting we further minimized the species that we use into three in order to fully experiment on its potentials. Below is the list of the species that we selected for major construction projects:

Kawayan Tinik or Thorny Bamboo
“Bambusa blumea”

All over the Philippines, thorny bamboo is the most widely used variety of bamboo. It grows clumping in nature and is characterized by thorns surrounding the whole lower section of the plant. This species grows averaging from 15-30 meters. This species has different pole thicknesses from the bottom part to the tip making it usable for different kinds of construction (fig.4).

The bottom part is used for structural purposes such as foundations or structures as this is solid, thickly-walled and almost no hole can be seen when you cut it crosswise.

The middle part has the most functions in the house. It can serve also as the foundation, walling, and trusses. This is the most versatile part of the pole as the thickness can vary which will determine its use in the construction.

The tip is thinly walled and has the smallest diameter. In construction, they use this to build the rafters of the roof where the roofing material will be fixed. It is also used as the framing material for windows, doors, and other implements in the house.

Thorny bamboo grows well in all eco-systems, but does particularly well in areas with a long dry season. In wet areas this bamboo will often result in thinly walled poles. When this species is grown in dryer areas the poles are characterized to be slow growing but result into thickly-walled poles, which tend to be more favorable in construction. In average, this species can be harvested in 3 to 5 years after planting. Anything less or more than this time frame will result into weak or brittle poles.



Fig. 4 (left). Kawayan tinik clump

Fig. 5 (right). Buho clump

*Buho**“Schizostachyum lumampao”*

Buho bamboo has the characteristics of the tip of *Kawayang tinik* and has smaller diameter and thinly walled poles. This species grows fast and abundant in dry regions and can usually be harvested after 1 year from the start of the sprouting of the shoot (fig.5). Though it is thinly walled, the outer covering is harder and resists water compared to the other varieties. Its poles are relatively consistent in diameter from the bottom to tip and ranges from 4cm-7cm in diameter. Therefore, this is ideal for making long bamboo roof tiles and beaten bamboo walls. In many areas of the Philippines, the skin of this bamboo is used to make woven bamboo mats and handicraft items such as baskets and other household implements. The poles are not ideal to be used as structural members of bamboo structures due to its brittleness.

*Bayog**“Bambusa blumeana Luzonensis”*

Bayog shares the characteristics of the bottom part of *Kawayan tinik*. The pole's diameter is almost consistent from the bottom part to top. It is a thickly walled species of bamboo but does not grow as long as the thorny variety. The diameter of the poles can range from 6 – 8cm. This is usually favored for structural members of a bamboo building. The growth of this bamboo is mildly curving which makes it also favorable for building terraces balusters, window frames, chairs, and other furniture (fig.6).



Fig.6. Bayog clump

Cabiokid - experience with bamboo

In 2000, Cabiokid started developing its site in Nueva Ecija, a province in the central part of Luzon island in the Philippines. Cabiokid's goal is backed up by the principles coming from nature and it tried to make a workable system out of it by replicating patterns that we can see naturally. In Cabiokid, doing a variety of things with bamboo is one of the major focuses. Other activities in the area include organic farming, reforestation, wildlife rescue center, composting, and food processing.

90% of structures built in Cabiokid is made out of bamboo. Working with bamboo is one of the major activities because of its versatile potential, and thus the Cabiokid team started appreciating this material and since then many developments and improvements have happened, which will be tackled in the other parts of this paper.

In Cabiokid, there are two fields where we try to explore the potential of bamboo, those are: construction and handicrafts. Bamboo construction involves: house building, learning resource centers, small community centers, compost toilets, and water tanks. For handicrafts, we are experimenting with making furniture such as beds, chairs, small table items, and different designs of bikes. This paper will discuss our experiment and experiences on how we deal with bamboo to create different construction and handicraft objects (fig. 7 and 8).

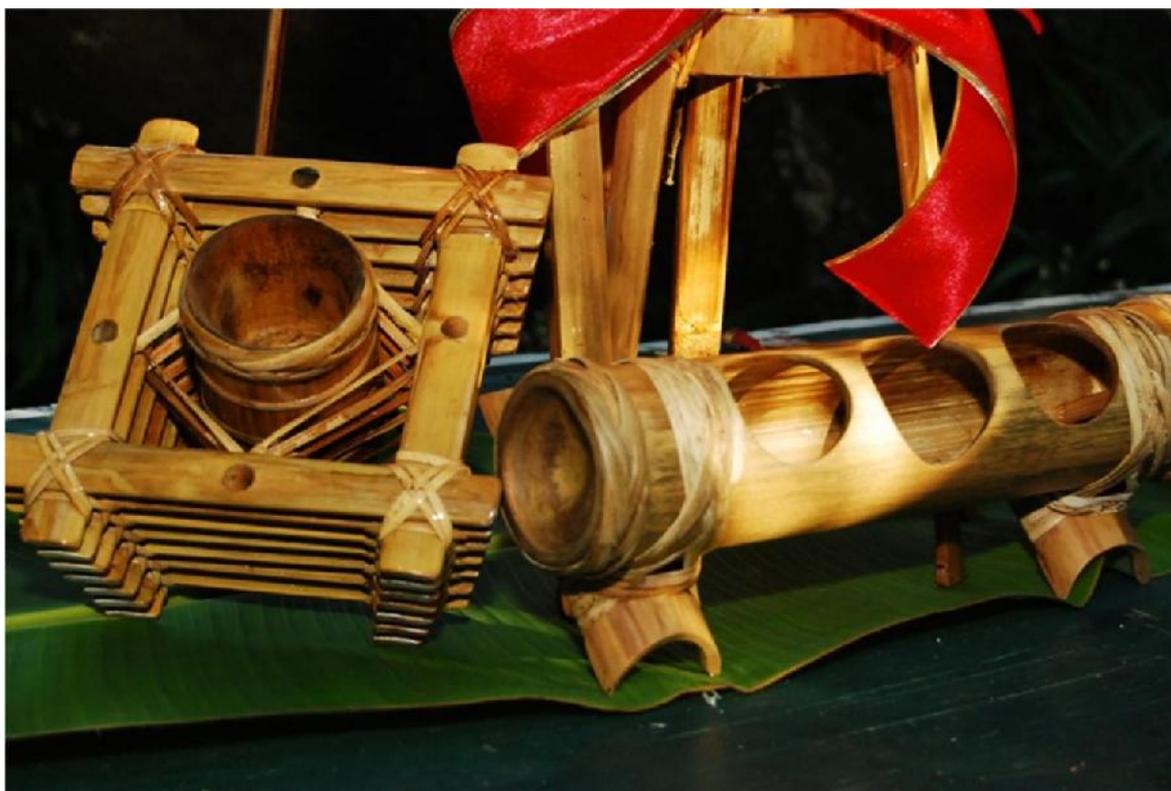
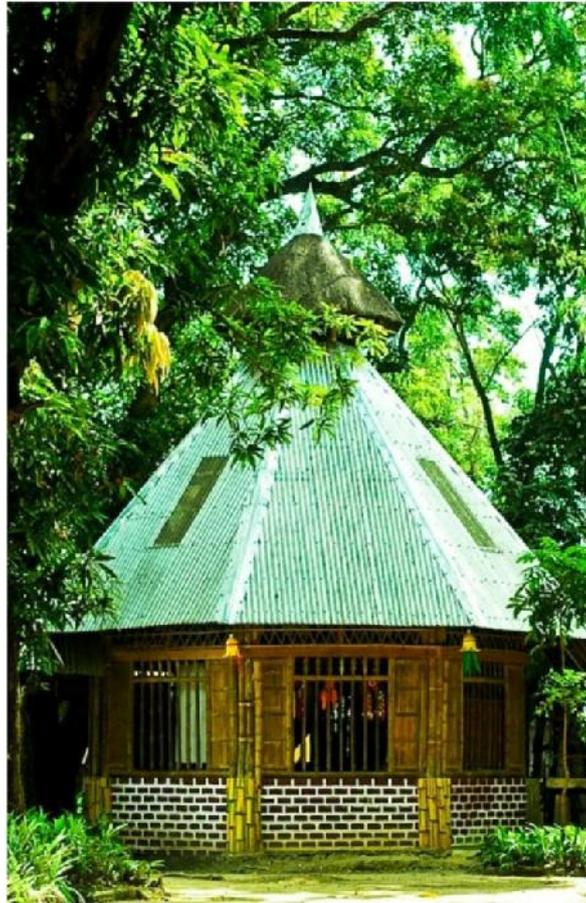


Fig.7 – some of the handicrafts made with bamboo

Cabiokid's first structure was built by tapping into the local people's knowledge with the bamboo. This was the first step towards a series of challenging experiments with the material. Some local craftsmen built our first bamboo building, now functions as the training center and sleeping area in Cabiokid. In this building, we realized that details of working with bamboo was almost forgotten; from connecting poles, selecting poles, and joining the members. They used metal nails to join bamboo members. Wooden planks were used as nailing beds for bamboo to create an even surface, and instead of tying the bamboo with natural materials such as rattan, tie wire and nylon wires were used. It was constructed in a six months timeframe, but later after three years, humidity started to develop rust with the metal nails leaving unwanted dark marks in the bamboos. Technical and biological materials were not carefully fused, which lead to some unwanted situations. Even with these, the building is still standing strong after seven years, with only some minor repairs on the grass roofing (fig. 9).



.8 – Ekopalas- a Learning Resource Center built with the help of Cabiokid in a nearby community. This serves as an alternative classroom for students



Fig.9 – Cabiokid training center – it's first bamboo building

Our Challenge

It was a kind of revelation to us that in a short span of time, a generation started losing the traditional knowledge which had been practiced for centuries when it comes to working with biological

materials. This became a challenge for our organization to look for existing skills and revive and utilize them for future bamboo construction.

Here are some the challenges and difficulties encountered at the first building in Cabiokid

- 1) Mixing cement and bamboo together
- 2) Using tie wire and nylon wire for tying joints
- 3) Using metal nails for bamboo
- 4) Putting some of the bamboo to close to the soil

Mixing bamboo and cement together without a binding agent results in to very low adhesion of bamboo with cement and vice versa. Since heat and rain in the tropics can be very extreme with humidity; these factors can cause the bamboo to expand or shrink thereby changing its diameter. This holds true during the wet season as natural materials tend to absorb water, which results into swelling and will revert back to its original size at the dry season.

Using tie wire and nylon wire for tying and joining bamboo members lead to the staining of the bamboo poles and hastening its decay as it is exposed to water and wind. On the other hand, nylon can somehow become brittle overtime especially if it is exposed to extreme sun light or fluctuations in temperature. The same is true for using metal nails. This will lead to the rusting of the bamboo pole or slats. The most important thing to avoid when using nails is that it has the capacity to split the bamboo component as it cuts through the vertical veins of the bamboo (fig 10 and 11).



Fig.10 – Connections made with tie wire and nylon showing stains on bamboo

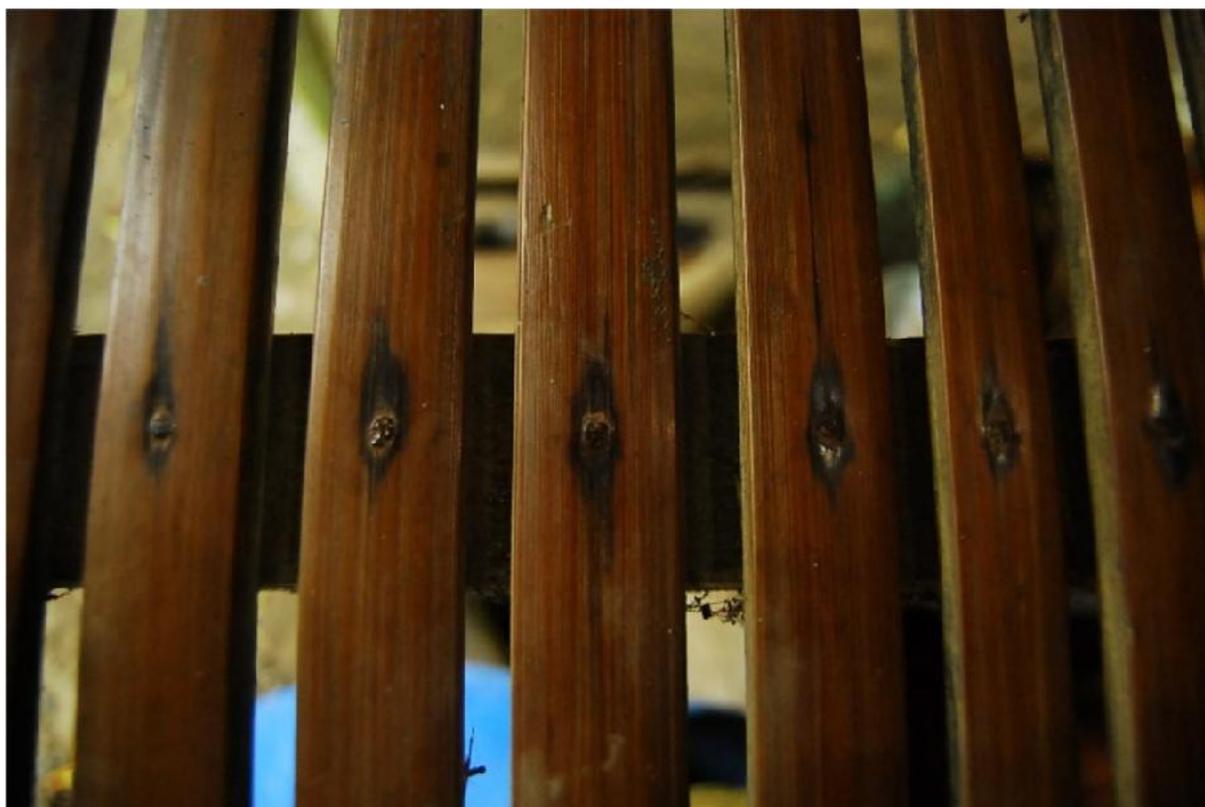


Fig.11 – Metal nail rust on bamboo showing dark spots

The building was an experiment for us. We wanted to prove that with proper treatment and handling of the materials, a longer lifespan of up to 25 years for the building can be achieved. This had been a major challenge for us: how will we build the next buildings without losing the properties and strength of the biological material as our primary building material? After all, our goal is not only to make buildings out of bamboo, but to make them grounded and connected to nature by tapping into all surrounding elements as much as possible. On the next building that we made, we tried to maximize all the natural energies surrounding the structure like the air, water, soil, and light.

Being connected with natural elements...

Natural elements possess some characteristics that make them all sustainable, productive and energy efficient at the same time. We tried to look at these principles, which could lead us to better understanding and designing of the next building that we would have to construct. With this, we mapped out design considerations to meet the goals we mentioned above.

Soil

A structure where people congregate and spend most of their energy must connect to the soil in a way that it can return excess resources coming from it. By making compost toilets, resource management facilities, a living space like a house can easily connect to the soil. Every structure put up in Cabiokid, follows such standards wherein human and kitchen waste can be turned into food for the surrounding flora of the house. The compost toilet is a facility with bamboo walls while the toilet itself is a cement casing reinforced with bamboo (fig. 12).

Water

Our bamboo structures in Cabiokid connect with the water by putting up rainwater harvesting tanks, which are being used for washing and bathing. Instead of using metal as reinforcement inside our

water tanks, we substituted the metal with bamboo. We built tanks of different sizes then we started sharing this to the other communities that we are assisting (fig. 13).



Fig.12 – Cabiokid compost toilets built with bamboo and cement



Fig. 13– Isabela craftsmen posing behind a construction of a rainwater collector with bamboo reinforcement

Sun

Another standard that we set when building bamboo structures is that it should trap sunlight for lighting and getting energy. We started with just inserting a polycarbonate sheet on our roof and later we ventured into putting solar panels to harness the free energy coming from the sun (fig. 14).



Fig. 14. Cabiokid's guest house utilizing polycarbonate sheets to allow daylight to illuminate the building

Air

In the tropics, humidity is one factor that must be avoided inside the dwellings. It leads to the proliferation of fungus especially with biological materials. In bamboo construction, instead of sealing and closing the walls which trap humidity, we tried using eaves and opening parts of the roof and bamboo slats for flooring to allow air to circulate freely throughout and inside the structure, therefore preventing moisture to accumulate and fungus to form (fig. 15).



Fig.15 – Cabiokid tent houses which are situated beside the forest to allow air ventilation

Revisiting traditional bamboo skills

We tried to improve on the first structure that we built inside the Cabiokid development site. We tapped the skills of people who are still adept and practicing the process of bamboo building. From different areas we collected existing knowledge based on their traditional skills of bamboo construction. These techniques started from choosing the tools, the selection and treatment of poles, improving simple tools, to finding different biological and technological materials that can blend with the characteristics of bamboo. We managed to meet people from the different communities that we are extending assistance to and who are highly skilled in basic bamboo skills. They were often the ones who taught us some of the forgotten skills related to bamboo construction.

Tools for working with bamboo

There are numerous tools used to work with bamboo. These tools minimize the processes in preparing and fitting the bamboo pieces together during construction. While it is still essential to use hand tools, machine tools are now deemed to be relevant as it cuts down the time in construction with bamboo. This part will discuss some of the tools that the local craftsmen are still using up to the modern machine tools that are also utilized.

Hand tools

Machete - Simple hand tools such as *machete*, *gulok*, *itak* or jungle knives are used to cut bamboo. They are sharp and heavy knives which are apt to cut the bamboo pieces with large diameters. This is the most versatile tool in bamboo construction as it can clean and skin the pole, it can splice and beat the bamboo (fig. 16).

Old scythe –scythes which doesn't have teeth anymore are used for skinning the bamboo. The blade is sharpened until its teeth disappear (fig. 17).

Chisel – the same function with woodworking, chisels are used to cut into corners of the bamboo members being joined. This is more favorable to use compared to *machete* when working on small

diameter of bamboo poles. Working with this tool is seen during the finishing phases of the house such as installation of bamboo flooring, balusters, doors and windows.



Fig. 16. Simple tools such as *gulok*, *itak* for working with bamboo



Fig.17 – Old scythe sharpened for skinning bamboo

Bamboo splitting jigs – these are multiple blades fixed in a single handle. Jigs can equally cut the bamboo pole to the desired number of slats. This is often used on bamboo with long lengths with thick walls. This divides the time of cutting the bamboo as compared to a machete (fig. 18).



Fig.18 – Bamboo splitting jigs with different number of blades



Fig.19 – Bamboo nail making jig

Saw – used the cut bamboo horizontally

Hammer/Mallet – accompanies the bamboo nail to be inserted to the pole. It is also used to adjust the bamboo pieces and make them fit in place

Bamboo nail jigs- these are specially manufactured metal jig with different sharp hole sizes. Bamboo sticks are pushed inside the hole. The jigs trim down the excess bamboo and what goes inside will turn out as uniformly sized nail. Variations are the same as the size of different drill bits (fig. 19).

Mechanical Tools

Drill – this may come hand-driven and motor-driven. Its primary use is to make the initial hole for the insertion of the nails. With the use of the whole saw, it can create holes for smaller bamboo poles to be inserted on larger diameter poles. Holes made by the drill can vary from 1/8” to 1.5”, while lengths can vary depending on how many members are being joined. Normally, the maximum length of a drill bit that is used in bamboo construction is 30cm (fig. 20).



Fig.20 – Drill with an extended drill bit for boring several bamboo poles

Grinder – before the period when there was grinder, to trim sharp bamboo edges manually, they use *gulok* or *machete*. Later on we used grinders but we improvised grinding discs which have different diameter and thickness. On this discs, different grades of sandpaper is attached to achieve the desired finish. Grinding capacity, finish, and quality can be achieved by attaching different grades of sandpaper (fig. 21).

With the grinder, the process and time consumed of trimming the bamboo poles/pieces together to make them fit is reduced. These are also used to achieve a smoother finish of the slats that will be

used for the flooring. It is also used to achieve a leveled surface of the spliced bamboo pieces. Excess of nails on the floor are also trimmed down with the grinder.



Fig.21 – Grinder with a customized disc with a 40grit sandpaper

Selecting and Harvesting

The locals have standards on when and how to harvest the bamboo. These are methods that need careful observation and understanding of the cycles of nature. Included in these traditional techniques include reading the full moon, harvesting on the season, and harvesting by tapping the pole itself. Careful and correct selection and harvesting is the key to achieving longer lifespan of poles. In the Philippines, poles should be 3 to 5 years of age before being harvested. However, some locals are observed to be tapping into the poles to know if the bamboo is matured. By knowing the sound of the poles, it lets you know if the bamboo is ready to be harvested. Another belief includes harvesting during the dry season as the level of the starch of bamboo is lower. On our experience, knowing the age of the shoot by marking it at the time of the first offset of the shoot is the best method. This allows you to harvest in any season knowing that the poles are 3 to 5 years of age.

Harvesting

Often times, harvesting of bamboo is always done through clear cutting. This method removes the whole bamboo clump regardless the age of the bamboo. However, doing this will increase the growth of new shoots but will take a longer period.

Another method is selective cutting of poles. This is more sustainable and efficient. This method practices the selection of matured poles. By selecting mature clumps, a space is given for the new shoots to grow. In observation of this method, shoots growing from this will result into straighter

poles which are ideal for construction. Selective cutting requires periodic maintenance of the clump to gain easy access and selection of poles from the whole clump.

Points to consider in harvesting:

- Pole maturity
- Handling of the pole
- Season / climate
- Clump maintenance

Preservation of bamboo

This is the most basic skill that we should take into practice when we want our bamboo structure to be sustainable and longer lasting at the same time. Treating bamboo nowadays can range from natural methods to using chemical to repel insects and other borers that can attack the pole.

In Cabiokid we built tanks for soaking the bamboo. Again, instead of using metals as reinforcement for the tank, we tried using bamboo and woven mats inside the cement plaster. Underneath is a septic tank to store the chemical solution for treating it (fig. 22 and 23).



Fig.22 – Bamboo reinforced tank building process. Workers are tying the mats before plastering it with cement



Fig.23 – On-going bamboo treatment in the tank

We used a mixture of borax and boric acid, which are commonly known chemicals to treat the bamboo at the beginning. This was due to lack of access of seawater and the cost-efficiency of getting seawater rather than buying chemicals. We soak the bamboo for two weeks in the solution then we put it in a shaded area to slowly dry it. Avoid direct sun drying as the heat can cause the poles to crack. This kind of bamboo treatment went on until the solution became lesser in strength in a span of 4 years. These chemicals are strong and should be handled carefully. Based on observation and experience, careless handling of treated poles or spillage will burn the leaves of the surrounding vegetation and could eventually kill the foliage. While, some species may still survive with the effect of the chemicals, it is still detrimental to the surrounding vegetation.

After this event, adding chemicals was never practiced and in the treatment process the aim to is to be organic by adding salt to mimic the seawater. Adding this element, produces the similar effect as compared to using borax and boric acid in treating the bamboo. After a year of practicing this process, we practiced treating the bamboo in a creek where irrigation water passes. This process is further economical compared to the two previous methods we have developed. (show pictures)

Skinning

In the Philippines, craftsmen building with bamboo will often remove the thin outer skin of the bamboo poles prior to using it for the construction. Skinning is primarily done for aesthetics of the structure to be built. However, skinning not only benefits the visual characteristics of bamboo but the process plays a vital role in releasing the excess moisture inside the bamboo pole. Removing the polished outer skin aids to draw excess water to go out of the bamboo. This process also allows easier penetration of binders that will be applied on the pole such as used oil and coal tar (fig. 24).



Fig.24 – Skinning process shown in the picture to remove the outer layer of the bamboo

Another possibility that skinning offers is that, this process exposes a mild sheen intrinsic in the skin of bamboo. For this instance, further application of different varnishes and top coats is minimized. This sheen tends to last longer in dry areas, while humidity can cause the area to develop mild fungus and darkening of the pole. Therefore, in humid tropical areas, proper attention and care of the pole after skinning should be done. Natural wax and used oil are some of the possible materials that could be applied to prevent fungus growth.

Using bamboo nails or pegs

The first building in Cabiokid has its bamboo flooring and various bamboo members stained with the rust coming from the nails. This rusting is caused by exposure to long wet and dry season and humidity. In order to prevent this, we started using bamboo pegs and nails to hold bamboo pieces together, especially in the flooring. The bamboo nails are uniformly done with a nail-making tool

An additional advantage of practicing this is it leads to maintained strength of the bamboo as no puncturing of the veins is done, therefore keeping the bamboo pole intact. Moreover, the bamboo nail, secured with wood adhesive provides better adhesion given that they are similar in properties as

opposed to using metal nails, which often splits the whole bamboo pole. Bamboo pegs or nails vary in size depending on the need and application. Normally, it matches the diameter of drill bits available in the market. The drill bit creates the hole for the nail to enter and secure the bamboo pieces together. Drilling the bamboo pole has very minimal effect to the veins of the bamboo thereby maintaining its strength (fig. 25).



Fig.25 – Different nail sizes for different applications

In practice, nail making is the only process that doesn't require removal of the outer skin of the bamboo. Keeping the outer shell will hold the nerves of the bamboo nails. Bamboo nails are sourced from the bottom part of the bamboo since it is the only solid member of the pole.

Structural methods

Building a bamboo structure is backed with life span and strength of the structure in mind. This starts from harvesting the bamboo properly until joining the bamboo members itself. It is possible to build rigid residential structures out of bamboo up to three storeys. This is very specific in the case of the bamboo growing in the Philippines which is only limited to an average of 25 meters in length. We recommend limiting the structures to this height as building additional height poses constraints on the connections of the poles. More height also requires rigid joining and securing of the poles which would eventually lead to consumption of more bamboo and materials and also more effort in replacing the bamboo poles in case of decay.

Mixing bamboo and cement

It has always been a rule that never put biological materials attached to the soil as this will increase the decay of the material which will come in contact with humidity, insects, and other harmful elements. This fact holds true with bamboo. In order to prevent this, coal tar or used oil is brushed to the bamboo pole before inserting this in a 30x30cm cement foundation. This is the minimal size of cement foundation that is utilized in our construction. We elevate the bamboo and encapsulate it with minimal cement to prevent moisture and insects from entering it. In other structures we built, we also experimented with tarred poles and inserted it directly to the soil with a salt bed, 3" thick to prevent termites, ants, and other insects in entering the poles (fig. 26).



Fig. 26. Bamboo brushed with coal tar encapsulated in cement

Jointing Techniques

Bamboo poles are often joined in angles, inserted, or placed on top of each other.

Bamboo pole is normally joined in 30-90 degree angle with another pole. In most cases, joining is secured by a bamboo nail then wrapped with rattan to further strengthen it (fig. 27).



Fig.27 – Roof showing application of angle jointing of bamboo

Bamboo inserted to another member is usually done on bamboo finishes such as making balusters and bamboo walls (fig. 28).



Fig.28 – Smaller bamboo member inserted to a bigger pole to form a wall

Bamboo placed on top of each other is mainly seen in structural pole members and roofing. Support for flooring joists are also made in this manner (fig. 29).



Fig.29 – Butt jointing where a bamboo horizontally sits on a post. Butts are made close to the joint

It is important to consider that in jointing; poles should be cut and joined closest to the node, approximately 2-4cm before it. The nodes are support forces of the bamboo pole to prevent continuous splitting. In case that this is not possible, the pole that should be connected shall be filled with a smaller diameter of bamboo inside it that will act as the node of the pole. This also decreases the chance of the material to break or split.

Design Checklist for jointing:

- Select poles of almost the same diameter
- Secure the joints with a tied rattan
- Cut the poles to be connected close to the joint

With our subsequent structures, nylon wires were totally eliminated due to its brittleness, and more so, the waste that it generates is never recycled. We recommend using rattan for tying the bamboo to further add strength with the joining initially made by nailing together bamboo poles. Joining the poles together with the use of rattan to tie and secure the joined bamboo pieces together in place creates a tighter bond between the joined bamboos. This process also adds to the visual characteristics of the building (fig. 30).

Tying the bamboo members with rattan is an added protection for the joined bamboo made by bamboo nails. Nails can only provide a unidirectional form of holding the bamboo while further securing it with rattan maintains and further supports the position in which the members are joined. In

cases of the building which was only done with nails, there are noticeable movements to the bamboo members which could increase in due time and damage the structure.



Fig.30 – Example of a rattan tying of bamboo

Tying with rattan is an art itself already. This practice is an age old technique and has different variations which are dictated by the width and length of the rattan, the numbers of members to be joined, and the imagination of the craftsman. Rattan achieves the best result when it is soaked in water for two days prior to use. Soaked rattan are flexible and is easier to work with. Another advantage when tying with soaked rattan is, the moment the tying is finished, as it dries, it tightens itself, making a more secured support for the bamboo. If this is carefully and imaginatively done, it is able to make and impressive view of the then sharp angled connections made by joining bamboo pieces with nails.

Splitting , beating, and weaving bamboo

In a traditional *bahay-kubo* in Philippines, large bamboo poles are split to make slats of different width and thickness. Variations in width and thickness have different applications in the structure. For example, slats with 4-6cm in width are used as rafters for the roof while slats with 2.5-3.5cm width are used for walling and the floor of the building. The same function comes with beaten bamboo but more often, it is used as sidings for the wall. Splitting them in different widths and thickness will determine its function in the structure.

Splitting

Splitting is one of the oldest and well-known techniques used to process bamboo poles. Poles are then cut vertically following the direction of the veins of the bamboo. Numerous splits can be achieved depending on the size and diameter of the pole as well as its function for the structure. We observed from the locals that the middle part of the bamboo; which has average thickness and diameter is the

most commonly used part for splitting. 2 to 6 split bamboos can be made out of a single pole. The length of the pole will be determined by where it is to be used in the structure. Moreover, split bamboos with the thickness ranging from .35-1cm are flexible. This allows more applications on the house. For example, a curving roof that needs rafters will use split bamboos with this thickness.

There are improvised tools to splice the bamboo easily rather than slicing one by one from the pole. Simple splitting tools with fixed number of blades can equally slice the pole. This process also breaks the nodes of the bamboo. Doing this cuts the production time of achieving evenly cut splits by as much as 80%. Uniformly width bamboo splits are achieved with the use of these tools (fig. 31).



Fig.31 – Different sizes of split bamboo

Below is a table of comparison of the splices, their width, and their designated use

Number of splits from a pole	Width	Function
2	10cm – 11cm	Foundations for small structures such as chicken cages, floor joists
3	7.5 cm – 8.5 cm	Floor joists
4	4cm – 6 cm	Roof rafters
6	2.5 – 3.6 cm	Bamboo flooring and walling

Beating

Beaten bamboo or *tadtad*, are achieved by softly hammering the bamboo pole with a *gulok/machete*. Bamboo achieved here is similar to a mat but are fitted together tightly. This material is used primarily for the walling of the structure in replacement of the woven bamboo mats or *sawali* (fig. 32).



Fig. 32 (left). Beaten bamboo fixed on a wall.

Fig. 33 (right). Woven bamboo mats fixed on a wall

Woven bamboo

Woven bamboo or *sawali* are a tightly woven bamboo strip that creates a mat. This can span from 2 to 8 meters and can cover large area. *Sawali* is commonly used as a walling material. Air circulation is not lessened with this material (fig. 33).

Roof

Roof is a defining factor in construction and design. Different designs can lead to varied visual value of the structure as well as aesthetics. Building the roof with bamboo offers many possibilities. Due to the flexibility of material, different roof designs can be achieved. However, it is still important not to compromise air ventilation and rigidity of the structure.

The roof that we make should always be connected to a water tank, as much as possible. We build steep roofs in order to catch rainwater faster and to prevent water stocking on roofs with natural materials. Traditionally, roofing materials can also be obtained from bamboo with the *buho* variety. Other natural materials also exist such as *nipa, cogon, and anahaw*. However, water that passes through these materials tend to color the water and turn it murky brown, which makes it not ideal for taking a bath and washing. With the advent of technological materials, galvanized iron sheets, corrugated plastic sheets, and various polycarbonate sheets, we increased the propensity to catch clear water. Water passing on to these are suitable for consumption inside the house; for washing and for bathing. This is further improved by putting a mesh on top of the gutter where the water is passing to prevent larger sediments from entering your water tank.

Design Checklist to improve water catchment:

- Make higher angles of roof, minimum is 45 degrees

- Use technological materials such as: corrugated plastic sheets, galvanized iron sheets, and polycarbonate for channeling water
- Putting wire mesh on the gutter
- Checking the gutter regularly for rust

Designing with organic shapes and natural materials

We deviated already from the traditional four-sided structures of building with bamboo. Bamboo offers flexibility in construction in aspects such as; the difference in diameter determines its functions, poles are not straight and some bends could be observed which can lead to different applications of bamboo.

One aspect we tried to put into practice is looking at modern ways of building posts. In most of our buildings, poles of 3 to 5 members are tied together to form bigger posts. Later innovations include the extent of exploring with different angles of posts but still maintaining the strength that is must provide to the building. Moreover, in the structures we built, some roofs are planes while some possess conical shapes resembling objects we can see from different living species.

Currently we are trying to construct non-rectangular nor square structures. Domes and circular structures are created to allow more sides, bends, and edges. We found out that domes, circles and lobed shaped roofs are resistant to strong winds. Such roof structures function as a web that has complex and stable connections that distributes forces created by rain and water on it. (fig. 34).



Fig. 34. Cabiokid geodesic dome roof – example of flexibility of bamboo on roofs

We tried to practice high angles of roof which makes the channeling of water faster thus preventing other sediments from stocking in the roof, which can then cause the rotting of bamboo and the roofing material. Low-angled roof showed faster degradation of the natural roofing material due to water penetration which leads to fungus then decay of the material.

Below are some of our designs inspired by the abundance of materials coming from nature as well as shapes that we can see in the surrounding.

1. Community based, Learning Resource Center, TUMANA – Tiwi, Albay

This building was built to serve as a Learning Resource Center in the middle of a 10-hectare permaculture site situated in the Southern Luzon province of Albay. The province is widely known as the entry point of the typhoons coming from the Pacific to the Philippines, and these are characterized

by winds that can reach up to 280 kph. Withstanding typhoons and earthquakes from volcanic activities are the major design challenges for this building.

Tiwi, Albay is blessed with stones coming from a nearby active volcano, clay soils and with bamboo and *anahaw* (a palm roofing material). Blending of various local and biological materials for the building was exercised. It was built with volcanic stones for the first floor while bamboo on the second floor and the *anahaw* for the roof. Fired clay tiles have been used to cover the ground floor.

The building is facing the Pacific Ocean and is designed to channel strong winds from the typhoon. It is pointed in front sloping downwards, analogous to a bird's beak. The roof is designed to minimize the catchment of strong winds. It is lobed at the far end, imitating slopes of airplane wings to swiftly channel strong winds from the typhoon away from the building (fig. 35).



Fig. 35 – TUMANA Project Learning Resource Center

Connecting with natural elements:

Soil

- Utilizing natural materials abundant in the environment such as volcanic stones, bamboo, and *anahaw*
- Building compost toilets
- Building on-site materials recovery facility (MRF) out of bamboo
- clay floor tiles

Water

- Trapping excess rainwater by building rainwater tanks
- Trapping into the free flowing water with dams and planted bamboo around it

Sun

- Used polycarbonate sheets on some parts of the roof for additional daylight source

Air

- Used large windows made of bamboo
- Eaves in the roof for exhaust
- Bamboo slats for the flooring to allow air circulation

Innovations in bamboo design:

- Joining three to five bamboo members together to create posts
- Imitating reinforced concrete bars by using bamboo brushed with coal tar which is then inserted to cement posts
- Developed different patterns for roof trusses to achieve circular, dome, lobed shaped roofs
- Use of metal pegs and screws to hold extending posts together
- Utilized rattan for securing bamboo connections after joining them with the pegs or nails
- Used bamboo pegs and nails for joining bamboo members for floors, posts, rafters

2. Cabiokid's social "Tent" Houses

Triangle shapes are widely found in nature where they symbolize and exert stability. We experimented with this shape to build some of the facilities inside Cabiokid. This is our example of a structure which is cost-effective, built of light materials, easily built. This was also built after a strong typhoon hit the Philippines in 2009. This is an example of a house which serves as a structure which can be easily built after a calamity and can house a family with five members.

Our first building experiment with this kind of structure was in 2009. The structure is 3 meters wide and 4.5 meters in length. It is situated beside Cabiokid's forest to maximize the passage of air inside the structure. It is elevated supported by cement posts where the building is resting on. We took advantage of the canopy of the forest where there is less light and heat, this lead us to putting more skylight roof sheets similar to polycarbonate sheets but of lesser strength. The sheet is translucent allowing light to penetrate softer the interior of the structure. At both ends, we still used *cogon*, a common grass used for roofing on most of the bamboo houses.

At a later stage we built another triangle house near the previous one, and improving it, we placed the two structures beside one another. It created an inverted triangular space in between, which we intended to become a rainwater harvesting tank. The tank is made of high density polyethylene plastic

but is supported by bamboo frames. This is advantageous to the both structure as it provides a cooler temperature inside the two rooms that comes from the water tank (fig. 36).



Fig.36 – Cabiokid’s social tent houses. The middle part functions as a rainwater collector

Connecting with natural elements:

Soil

- Dedicated small space for the building, but more land available for an edible garden

Water

- Steep roofing out of polycarbonate plastic to channel water to the water tank
- Rainwater harvesting out of corrugated plastic sheets

Sun

- Use of corrugated plastic roofing sheets to enhance soft ambient light to illuminate the interior of the building

Air

- The buildings are located beside the forest to benefit from air circulating inside the forest

Innovations with bamboo designs:

- Elevated the bamboo by placing it on a cement post
- Used salt beside cement posts to prevent termites from eating the bamboo
- High angled roof structure for better air circulation
- Fused *cogon* with corrugated plastic sheets for roofing
- Experimented on different patterns of tying rattans

3. Cabiokid's Geodesic Dome building

This is the most recent attempt of a building that we made. It is similar to techniques used in building geodesic domes but instead of using metal pipes we utilized bamboo poles in replacement of metal pipes. This is our first time to totally deviate from the traditional building methods that are normally seen in Philippines; four sided walls, hip roofs, and similar methods.

In joining the bamboo pieces together, we used metal pipes that act as fixtures to fit the bamboo poles together. It is secured by a tie wire attached to the metal pipes. Each metal pipe fixture holds 3 to 5 bamboo pieces together. The connections are then strengthened by wrapping it with rattan, which holds the bamboo poles attached to the metals.

The building took 3 months to complete. Careful detailing of metal pipe fixtures and wrapping it with rattan ate most of the work hours, but it is already proven to give enough strength and beauty to the building. The whole building is covered with *sawali*, a type of woven bamboo mat and *cogon* (fig. 37).



Fig. 37. Cabiokid's geodesic dome

Connecting with natural elements:

Air

- Create eaves for exhaust of hot air inside the structure
- Large triangular windows following the polygons created by the dome

Sun:

- Used polycarbonate sheets for some parts of the roof to enhance ambient light inside the structure

Innovation with bamboo designs:

- Bending bamboo pieces to follow a circular shape inside and outside the structure
- High angled roof for easier rainwater discharge
- Used bamboo mats for the walling
- Using metals pipes as fixtures to connect bamboo pieces together
- Improved tying techniques in supporting metal pipe connections of the dome

Rain Water collectors

As mentioned earlier in this paper, Cabiokid also utilizes bamboo as a replacement for reinforced concrete bars. In the communities that are being assisted by Cabiokid, we build rain water collectors with the use of bamboo and wire mesh, which is then serving as the concrete reinforcement. It took around 2 months to build a circular tank of 3 meters in diameter. Now, the tank serves as the major water source for the local livelihood cooperatives. The stored rainwater is being used for tree seedlings, but also to provide for the daily water needs of the community (fig. 38).



Fig.38 – Finished Isabela water tank

Bamboo Handicrafts

Bamboo methods mentioned earlier in this paper such as jointing, using bamboo nails, and tying are not only limited to construction. These methods were also utilized in our attempt to produce smaller crafts.

Cabiokid E-trike

Tricycle is one of the most common forms of transportation in the Philippines. The e-trike or electric tricycle is an attempt to show the potentials of bamboo in the aspect of transportation. This is a prototype to show an alternative way of transport using electricity stored in batteries in the country's

popular mode of transport. It is small and fast which can easily used for short travels. While electric vehicles exist already for decades, there have been no attempts yet to produce vehicles for mass transportation out of bamboo (fig. 39).



Fig.39 – Cabiokid e-trike

In this prototype, similar methods of joining bamboo members were used to create various parts of the e-trike. Bamboo parts in this prototype were heat treated to strengthen the pole. This process crystallizes the sugar in the pole and binds the veins of the bamboo together. However, the nodes of the bamboo should be broken to allow the heat to be quickly released out of the bamboo. If this is not done, possibility of the pole to crack is maximized. Heat treated bamboo is rigid compared to non-heat treated ones. It also gives an appealing caramelized color to the bamboo. In this prototype, metals for the chassis are still present and some mechanical parts in making the bike's frame.

The prototype works in two ways, it can be pedaled or it can be driven by the motor attached to the batteries. In instances wherein the battery loses power to drive the motor, pedaling can be done to continuously run the vehicle.

It still uses moving parts of a tricycle seen and gasoline-powered tricycle such as the wheels, the handlebars, fork, and pedals.

Similar to the production of bamboo bikes in joining the poles for the bike's frame, we used epoxy saturated *abaca*, a fiber endemic to the Philippines, to wrap and secure the joints. On the other hand, the carriage utilized traditional bamboo techniques such as using spliced bamboo for its flooring and fixing it with bamboo nails. Angled joints are still wrapped with *rattan* to fix the bamboo members

Innovations with bamboo designs:

- Used *abaca* for the bike frame
- Saturated *abaca* with epoxy
- Heat treating the bamboo

Bamboo bikes

After building the e-trike prototype, we delved into building single transportation mode of vehicles which are the bamboo bikes. Though bamboo bikes are around for more than a decade already, it is difficult to get first-hand information on knowing the processes involved in manufacturing it. We tried to build our own prototype similar to the method we used in making the e-trike.

The frame of the bike is 95% percent bamboo with metals inserted on some parts such as the dropout, which is the end of the frame, the steering tube, the seat tube, and the bottom bracket for the pedals. Mechanical parts such as wheels, cranks, and forks are still made of alloy or steel.

In the frame building process, the bamboos are soaked in running water to remove the starch for 7 days, and then heat treated to cook the excess starch, and then air dried for another week before skinning it. The bamboo connections in the frame are fixed by epoxy saturated *abaca* fiber. For finishing, the dried epoxy-fiber connection is then sanded down to achieve a smooth finish of the bamboo and the epoxy-fiber connections. (fig. 40).



Fig. 40. Cabiokid bamboo bike

We discovered that heat treating the bamboo also contributes to the finishing of the bamboo. As it is treated, the "caramelization" done by the heat turns the thin outer layer of the bamboo lustrous, giving a polished satin-like finish on its surface. This process reduces the possibility of applying additional finishing lacquers for the bamboo.

We built four prototypes of bikes with different designs already. Bringing it to an in-depth analysis, bamboo poles with 3.8-4.8cm in diameter are the ones ideal for building the bike frames. Our frames built with less than this diameter shows more flexing leading to cracking. This range of pole size is given to minimize the flexing of the frame caused by high torque from pedaling, turning, standing while pedaling, and crashes from the bike.

Building bike frames are rider specific. Meaning, no standard diameter of bamboo poles and its thickness can suit each and every rider. Rider's weight, height, handling capacities are notable considerations in making the bike frame.

On the other hand, bamboo, as numerous researches will say, has the properties found with carbon fiber, steel, and aluminum. However, on building bikes and with first-hand experience, it has numerous good qualities. One of this is that, Bamboo bike frames offers a smooth ride compared to other bike frame materials.

Our future challenge for this attempt is to make it ecological. While bamboo is one of the most ecological materials available, Epoxy resins, on the other hand, are harmful and wasteful at the same time. Wasteful and harmful properties include unavoidable vapor inhalation, its resin and chemical hardeners themselves, the sanding process, and its disposal. We are in the process of sourcing local resins that can have the same effect of as the epoxies found in the market. Future challenges will also include innovation in bike designs, its function, and its possibility for making livelihood out of it with the communities.

Innovations with bamboo designs:

- Used *abaca* for the bike frame
- Saturated *abaca* with epoxy
- Heat treating the bamboo
- Tying techniques of fiber for the bamboo

Improvements and Conclusion

Cabiokid continuously challenges every new bamboo construction that is presented. We try to improve and research not only in buildings but also in developing the handicraft aspect of bamboo. In doing so, we are now in a process of pooling interested bamboo craftsmen to build more ecological structures in the country. The organization is aiming to harness or enhance local skills, which can then be gradually shared to other communities in order to return some of the forgotten knowledge and the abundant connections with nature. What matters most is how and in what way communities will appreciate and embrace such technologies.

In buildings, we are still in the process of looking possibilities on constructing and designing buildings. We haven't explored most of the possibilities yet with working with different natural as well as technological materials without losing the characteristics and functions of both materials. This is true for example when working with metals and bamboo. We are still on the side of using mostly

natural materials and less on technological materials. As we observed, technological materials do not go back to the soil and are non-renewable resources. For example in bamboo bikes, a prime challenge for us is to look for natural resins to bind the *abaca* fiber and the bamboo together. The same is true with our bamboo treatment, where we are in search of plants that can do the same effect as the chemical can do. These are some of the notable challenges that we are in the process of looking for possible ecological solutions.

References:

- Bahay-kubo picture <http://direkaleckx01.blogspot.com/2011/06/bahay-kubo-nosebleed-version.html>
- Dacanay, Julian et. Al., *Balai Vernacular: images of the Filipino's private space*, 1992, Cultural Center of the Philipines, Manila.
- Farrelly, David, *The book of bamboo*, 1984. Sierra Club books. California, USA.
- Van Lengen, Johan, *The Barefoot Architect*, 2008. Humid Tropics structures and humidity. Shelter Publications, California, USA.

The Hyperbolic Bamboo Bridge- Chiang Mai, Thailand.

Mark Emery

Abstract

This presentation is a continuation to the life's work of bamboo bridge master builder "Jorg Stamm". By adding to his "7 concepts for bamboo bridges"(2009) with an **8th** new structural concept for building a bamboo Bridge:

1. The Beam
2. The Arch
3. The Suspension Bridge
4. Cable Stade Bridge and Cantilever
5. A Bridge with form active surfaces
6. The Truss
7. Space frames
8. **The Hyperbolic Bamboo Bridge.** (Figure. 1)

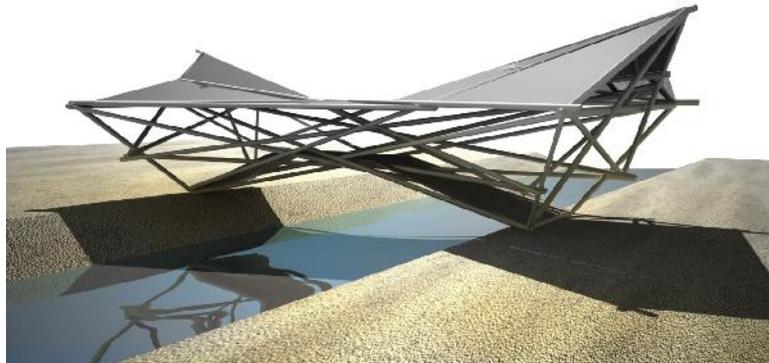


Figure 1

The *Hyperbolic Bridge* was born out of this inherent need to increase the durability of the bamboo structure by employing 'Protection by design Principles' for UV and water. (Jansen 2005) This has resulted in many roof types for bamboo bridges – the lightweight canvas roofs of the Cúcuta bridge, The heavy tile roves of the Santafe Bridge and the palm thatched feature roof horns of the Soneva Kiri bridge. (Figures- 2, 3, & 4),



Figure 2



Figure 3



Figure 4

The *Hyperbolic Bridge* plays with our preconceived notions of space (ie Wall, floor, roof) as the floor beams become handrails; and handrails becomes the roof joist, and roof joist becomes the floor beams.(Figure. 5)



Figure 5

The structural composition of the Chiang Mai *Hyperbolic Bridge* consists of two outward leaning trusses tied together with an inner and outer ring from which the long bamboo poles twist to create an inner void where the pedestrian may pass.

(Figure. 6)



Figure 6

With the completion of the first *Hyperbolic Bridge* we have learnt how to manipulate and tension each cable like a piano string to stiffen the deck and bring security for the user. (Figure. 7, 8) The pure simplicity of this concept has some challenges; for installation, treatment, transportation and specialized joinery for long spans – These topics will be analyzed in detail within this paper, with a focus on the construction methods and bamboo species that we are pioneering in Thailand.



Figure 7



Figure 8

On the shoulders of our forefathers, we raise the standards and compete with modern materials making bamboo bridges a commercially viable alternative.

References

- Emery, M.J. 2009. A tale of two Bridges, 8th World Bamboo Congress, Bangkok, Thailand.
- Hidalgo, L. O. 2003. Bamboo - The Gift of the gods. ISBN 958-33-4298-x, Bogotá, 2003.
- Janssen, J.A. 2000. Designing and Building with Bamboo. Inbar Techn. Rep. No 20. Beijing.
- Janssen, J.A. 2005. International standards for bamboo as a structural material. Structural Engineering International, January.
- Liese, W. 1998. The Anatomy of Bamboo Culms. Inbar, ISBN 81-86247 -26-2, Beijing.
- Liese, W, 2003. Bamboo Preservation Compendium. Inbar, ISBN 81-901808-0-0, Beijing.
- Stamm, J. 2004. "Analysis of techniques and Work Methods of traditional European Carpentry applied during 10 years in Covered Bamboo Bridges," 7th World Bamboo Congress, New Dehli, India.



Figure 7



Figure 8

On the shoulders of our forefathers, we raise the standards and compete with modern materials making bamboo bridges a commercially viable alternative.

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- Emery, M.J. 2009. A tale of two Bridges, 8th World Bamboo Congress, Bangkok, Thailand.
- Hidalgo, L. O. 2003. Bamboo - The Gift of the gods. ISBN 958-33-4298-x, Bogotá, 2003.
- Janssen, J.A. 2000. Designing and Building with Bamboo. Inbar Techn. Rep. No 20. Beijing.
- Janssen, J.A. 2005. International standards for bamboo as a structural material. Structural Engineering International, January.
- Liese, W. 1998. The Anatomy of Bamboo Culms. Inbar, ISBN 81-86247 -26-2, Beijing.
- Liese, W, 2003. Bamboo Preservation Compendium. Inbar, ISBN 81-901808-0-0, Beijing.
- Stamm, J. 2004. "Analysis of techniques and Work Methods of traditional European Carpentry applied during 10 years in Covered Bamboo Bridges," 7th World Bamboo Congress, New Dehli, India.

- Stamm, J, 2009. Seven Concepts for Bamboo Bridges. 8th World Bamboo Congress, Bangkok, Thailand.
- Stamm, J. 2008. Following the Natural Advantage of the Giant Grass, Popayán Conferencia de Bambu, December.
- Vélez, S. 2000 Grow Your own House, ZERI-VITRA.
- Villegas, M, 1996. “Bambusa guadua”, Villegas editores,

Machinery Processing Issues & Solutions For Bamboos

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Manufacturers and designers of machinery and tooling for Bamboo and Wood

Abstract

With an experience of nearly 35 years in machinery and tooling in the field of wood working, bamboo working, plastic working, insulation processing and special purpose machinery and tooling. Our firm has developed and learnt lot of specific design solutions to meet machinery and processing needs of our clients. This includes providing them with better machinery suited to there specific bamboo. We provide machinery specific to bamboo in use and do not use a standardized or a same approach for all bamboos.

Machinery being developed is basically for the usage of bamboo specific species, machinery selection based on the bamboo parameters like wall thickness, outer diameter, inter nodal distance, amount of moisture at the time of processing and similar factors. We have thus made some improvements so that the processing solutions required to achieve optimal solutions in this field like external knot cleaning and skin removing machines that can also be used for bent bamboos, slicing machines that can slice even the most hard or dry bamboos, planning machines for one and all solutions, splitting machine for heavy duty and better finish splitting using hydraulic power.

We have developed many manual tooling and machinery from pedal operated machinery to high accuracy slicing machines that can be operated using manual or electrical power as and when required. Newer processes have been established to provide better and more feasible usage of bamboo. For specific purposes like resort making, treatment systems, stick making units, strip board units, mat board units and similar areas we have established a lot of special and specific machinery for artisans, mass production houses and hobbyists.

Our approach is to provide specific customer oriented solution not go with trends and processes previously established but to develop and design systems as per bamboo and product specific requirements.

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Introduction

Bamboo is the main source of livelihood for rural people especially those living on the fringes. Galloping rise in human and livestock population has led to an increase in demand, which is much above carrying capacity of the forests. This has led to deforestation and consequent scarcity of bamboos in the area.

Natural resources contribute significantly to economic development and improvement in the quality of life. They provide food, fodder, fuel, fibre, water etc. and support the agricultural production system. Over the course of this century, the forests have undergone severe degradation due to various reasons viz., biotic pressure, commercial exploitation, illicit felling and lopping, mismanagement and their common property nature. This all contributed in degradation of the forest as well as semi forest areas.

"The Poor Man's Timber" is one of the most ubiquitous gifts of nature to mankind. Its versatile properties and scope make it a great partner of the nature as well as human beings. There is need to propagate, preserve and conserve bamboo for ecological security and to cater to the need of the future generations.

Bamboo, a tall grass with about 1250 species in 75 genera, is one of the most important and versatile group of plants known to humankind. Technically and taxonomic ally bamboo is a grass. But with majestic looks of a tree, this tall grass is one of the most important and versatile groups of plants known to humans. Bamboo plants occupy a special place in lives of people, especially in Asia. Probably, bamboo is the most beneficial plant known to humans. Its unique properties make it a versatile plant in both rural and urban areas. Little wonder, it is often called as "the poor man's timber" in India, and "Friend of People" in China.

Bamboo is amongst the most ancient grasses surviving today. It made its appearance on earth some 200 million years ago when dinosaurs still ruled this planet. Bamboo is one of the most ubiquitous plant species found in the tropical, subtropical and temperate regions of all continents - except Europe and West Asia - from lowlands to the foothills of the Himalayas. It grows on a wide range of soils: from perennially poor to perpetually rich and tolerates soil moisture conditions from drought to drowning. Its remarkable survival capabilities match closely with those of the millions of the poor dependent on the plant for sustenance.

Bamboo is one of the fastest growing plants and is a quick renewable resource. It produces maximum biomass per unit area per unit time compared with other forest plants.

About us

We, at Garnet Tools, Garnet Machines, Garnet Engineering and Garnet Machines and Tools India Pvt. Ltd., are into manufacturing of machinery and tooling for Bamboo, Wood, Copper, Aluminium, insulation, Plastic and other ferrous and non-ferrous applications. We have been in this field for the last 35 years and have specialised in providing turn key solutions as per raw material and customer specific requirements.

We have nearly 150 types of standard machinery and 1500 types of standard tooling.

We develop special purpose machines and tooling as per customer requirement and have been able to achieve repeated orders from throughout the world in this field. We are an ISO 9001:2008 certified company including design and development clause which enables us to develop, design, prototype, test, manufacture and supply machines and tooling that are thoroughly reliable, highly productive, efficient and have a low maintenance cost.

We also train, develop skill and impart technical knowledge for development of projects for processing of bamboo, wood, pressboard, insulation and other similar related materials.

About Bamboo Species

Bamboo is a group of woody perennial grasses in the true grass family Poaceae, which is a large family with over 10,000 species. In the tribe Bambuseae also known as Bamboo, there are 91 genera and over 1,000 species. The size of bamboo varies from small annuals to giant timber bamboo. Bamboo was only added to the world between 30 and 40 million years ago, after the demise of the dinosaurs. Bamboo is the fastest growing woody plant in the world.

In bamboo, the inter-nodal regions of the stem are hollow and the vascular bundles in the cross section are scattered throughout the stem instead of in a cylindrical arrangement. The dicotyledonous woody xylem is also absent. The absence of secondary growth wood causes the stems of monocots, even of palms and large bamboos, to be columnar rather than tapering. Bamboos are some of the fastest growing plants in the world, as some species have been recorded as growing up to 100 cm (39 in) within a 24 hour period due to a unique rhizome-dependent system. (Chisholm, Hugh, ed)

Bamboo – Technical aspects and processing parameters

Bamboo and technical aspects for processing

- Grows 3 times as fast and can be harvested 4 times as often as Eucalyptus.
- Yields 6 times more cellulose than fast growing trees
- The grains of Bamboo are along its length and thus have high elasticity
- Found extensively in natural forests and is suitable for afforestation of degraded lands
- 2.5 billion people worldwide use Bamboo
- Is a very strong plant with a very high Modulus of Elasticity: 9000- 10000 N/mm²
- Indian Bamboo sector generates about 432 million work days annually

(Data from national Mission on Bamboo Applications)

Bamboo is unique in that it is strong in both tension and compression. While tensile strength remains the same throughout the age of the bamboo plant, compressive strength increases as it gets older. There is some controversy in determining proper testing protocols, as it is important to test bamboo which is at least three years old, and that the test should occur on a piece of bamboo with an entire inter node and two intact nodes. Some testing research has not used these criteria, and thus the results are not as useful.

To utilize bamboo to its best capabilities, several conditions are important to consider. One consideration is that bamboo grown on slopes is stronger than bamboo grown in valleys, and that bamboos that grow in poor dry soils are usually more solid than those grown in rich soils. Bamboo will shrink diametrically, so Oscar does not recommend tied connections. Bamboo takes at least four months to dry, and should not be kiln dried, as the moisture inside leaves mostly through the ends. There are certain limitations of the use of bamboo in construction. The starchy interior is attractive to insects. In addition, because bamboo has a slick waterproof coating, it cannot be painted. However, this coating allows bamboo to be used as water pipes.

As bamboo is extremely flexible from 6-12 months of age, it can be used to create a number of curving forms. In India, curving roofs called Chocals were developed, and bamboo domes have been built in New Guinea. A parisian architect named Friedman built some beautiful ringed buildings in India, but they were unfortunately destroyed by insects within a few years, as they were not treated. Indeed, the type of bamboo construction used can greatly affect the longevity of buildings. Ref. Oscar Hidalgo

Bamboo when looking at the processing there are some issues that are important:

- Wall thickness
- Outer diameter
- Inter-nodal distance
- And bendiness or straightness

But there are some more important factors also in processing

- Moisture content
- Type of grain structure
- Hardness of skin
- Number of branches and thickness of bamboo knots near branches

Bamboo can be used to manufacture a variety of products (Figure 1).



Figure 1. Bamboo Product range. Source: Garnet Tools presentation, 2011-12

Initial processing issues and differentiation from Moso

When we saw the processing methods used around the world regarding the bamboo product manufacturing. We saw a vast difference in the approach as taken by the Moso bamboo or similar related processing and the processing required for other types of bamboos.

If bamboos are straight they can be machined easily like a cylinder but as they are not always straight. In most species found in India, Thailand, South East Asian, African and South American and similar regions there are lot of bent in the bamboo this can be due to

- Improper plantation
- Protruding nearby branch nodes(these cause slicing of bamboo to be improper)
- Highly dense plantation with bamboos not planted correctly.
- Windy conditions and not thick or firm soil

Moso is similar to machine grown bamboo straight, less hard grains and low machining inaccuracies. Moso bamboos can be used to make a variety of products like furniture, boards, strips, incense sticks and other similar species of bamboos.

When machining other bamboo species the results are much different than expected, the machines have to be designed and suited according to the end product and finish required. Other bamboos have nearly indifferent results and thus the pre-established processing methods and solutions for Moso and there methods are irrelevant to our present and other different bamboo species.

Bamboo is useful for different things at different ages:

<30 days it is good for eating

6-9 months for baskets

2-3 years for bamboo boards or laminations

3-6 years for construction

>6 years bamboo gradually loses strength up to 12 years old

Bamboo for construction is best cut right after new shoots have started to grow, as the plant will have given all its starch to the new culm. It is important to cut bamboo just above the node at the base.

The age of the culm is very important to know in order to select culms with the greatest strength for bamboo construction. One-year-old bamboo is an emerald color with the sheaths just beginning to fall off. Bamboo 2-3 years old has white spots on the culm, indicating the beginning of lichens. At 5-6 years these lichens can be clearly seen. Branches also tell the age of a bamboo plant. Every year each culm of bamboo loses its branches which are replaced with new branches. Old bamboo is attacked by insects from the interior of the plant, which can be difficult to detect. (Retrieved from Oscar Hidalgo, Notes by Cassandra Adams, <http://www.networkearth.org/naturalbuilding/bamboo.html>)

Lookout for a change and different approach to processing

When we encountered the machinery issues and the problems associated with bamboo processing like not having the strip, slices, sticks of even sizes due to change in structure and bamboo species we had to look out for a solution to solve the issue. Method for search of solutions:

- Changes found from the hand operated tools and learning from the artisans
- Designing on the ideas of artisans
- Procedural usage and satisfaction from artisans
- Making them to change to machinery from had usages
- Using worker skill ad repetitive feedback and working close to them

These have been the key to learn and develop the machinery ad tooling as per requirements.

Effects of bamboo species on the processing method selection and machinery selection

Bamboo species play a defining role in designing the machines and tooling that can be used to manufacture the product as per requirement. A few examples of change in processing is mentioned below:

Solid bamboos

Solid bamboos can be used to do a lot of purposes. Even solid bamboos can be used to provide great products and product simplification if processed using the right amount and kind of machines. Converting solid bamboos into straight ones can be used to make slices, strips, sticks etc.

Dendrocalamus giganteus

Dendrocalamus giganteus with its huge structure and dimensions can be used to process a lot of products with a very high productivity and can be of use to make a number of highly cost effective and productive products from bamboo due to higher weight to length ratio. This bamboo can found highly productive usage not only in construction but also in mat making, stick making and similar applications.

Ochalandra Travancorica

Ochalandra Travancorica is very thin with diameter between 0.8 inch to 1.5 inches, wall thickness between 3-6mm and inter-nodal distance between 0.8 to 1.3 meters. This bamboo has inter knot distance of nearly one meter and has a shiny exterior giving it an excellent finish and looks on the surface. This bamboo is used to make handicraft and bamboo products with artistic value. When machining this bamboo and processing it. We do not used the standard procedure of machines we have designed a specific process of machining to achieve knot free and high quality thin slices used by the industrious workers to make bamboo mats and slices. Mats are made using fine slices bamboo and mostly the mats used in India are made by weavers. They are feed with machine made fine slices. After splitting of this bamboo we directly slice it into thick slices and then convert them into fine and clean slices. There is no need of knot processing, planing or strip making operations.

Development of machinery to suit bamboo

On processing bamboo of various types we found that when using naturally grown and a variety of species the bamboo specifications do tend to vary a lot so the processing methods and the usage and the optimal application would also vary. On our trials at our works and client locations we found that cleaning of external knot and the exterior skin and even the heavy internal knots after splitting was a issue. Bamboo near its knots having branches has high level of unevenness and requires at least a proper cleaning of branch node parts. These branch nodes does not allow for proper splitting as well as the slicing of bamboo as these regions are highly woody in nature and so the shearing along the grains becomes much more difficult. Some common issues at the bamboo regions and solutions are mentioned below:

External knot

We first initiated with removal of knots as they cause bamboo unevenness and the bamboo strips or splits are not straight. So the first issue in processing of bamboo is removing external knots. External knots in a bamboo can be highly protruding and thick. In Indian Bamboos the external knot is very dense and does not have to allow for proper machining until unless they are cleaned. Sometimes near the branch nodes the external knots are very thick and the bamboo bent is a major issues. Bent bamboos cannot be machined using a chisel and rotating the bamboos.

This is achieved using bamboo being rotated with our hand and pushed towards a rotating high speed Carbide tipped cutter. Our Bamboo External Knot Removing cum Skin Finishing Machine is designed to clean the external knot as well as the outer skin of bamboo. Removing of the outer skin and knot is very crucial for further operations on the bamboo. Their cleaning assures longer life of cutters and chisels. The machine uses specially designed Carbide Tipped External Knot Removing Cutter. The cutter is dynamically balanced and is suitable for all sizes of bamboo, i.e. up to 200mm and even higher on special requests. The machine is unique in itself, as for the first time it has been that easy to clean the outer skin and knot of the bamboo. The operator has just to rotate the bamboo and move it along the axis, and the skin and knot are cleaned by the cutter, smoothly (Figure 2).



Figure 2. External Knot Cleaned Picture. Source: Garnet Tools Factory trial pictures, 2003-04

Internal knot cleaning

As similar to external knot processing, internal knots in bamboos are very hard to pierce or use it for making strips, slices and similar products. Thus we designed a very heavy duty internal knot cleaning system. Our Bamboo Internal Knot cum Skin Removing Machine is designed to clean the internal knot as well as the outer skin of bamboo. The machine uses hardened chisels for the operation. Irrespective of the final product to be made, the machine is a necessary part of any bamboo processing plant. There are two chisels, one for removing the internal knot and one for removing the outer skin of the bamboo split. In the process of cleaning the upper and the lower skin, the output attains a flat surface on both the sides. This helps in further processing of the splits (Figure 3).



Figure 3. Internal Knot Cleaned Strip. Source: Garnet Tools Factory trial pictures, 2003-04

Splitting of bamboo

Heavier and harder bamboos that can be not be split easily due to denser knots and thicker wall thickness for example the bambusa gigantus and similar species like asper and these require heavier machinery to be processed require better machinery in terms of design. We made some economical but better machinery specification wise changes like:

Chain splitter replaced with a hydraulic splitter(Figure 4)

Hydraulic splitter machine with power pack and control panel with this the process is slower but the strength of bamboo can be easily overcome. This process is much more convenient and systematic in terms of processing as it gives more options and a bamboo can be split to even 32 parts also depending upon the bamboo and the grill used



Figure 4. Hydraulic Splitting Machine. Source: Garnet Tools Factory trial pictures, 2003-04

Hydraulic Splitter features:

- Foot Switch for free hands and holding
- Hydraulic power makes splitting better
- Accuracy is higher with better splitting and evenness of pieces
- Extremely safe and precise operation with minimum chances of operator hazards

Process or product specific solutions

Multi splitting systems and grill designs

We have established a capacity to design grills that can split a bamboo into two splits to 32 part splits and can be designed accordingly and suited to the end product requirement. We developed these products for our customers so that they can achieve machining outputs and productivity. The blades are designed using hardened steel highly durable and maintenance and easy to re-sharpen blades (Figure 5).

Also we have designed parallel splitting grills that can be used to get parallel splits that could only be achieved previously using two parallel placed cutters which could cause a lot of material loss. This

could be used in hydraulic splitting systems for better accuracy and even can be worked on chain splitting machines as well.



Figure 5. Grills along with Parallel Splitting Grill (left side bottom). Source: Garnet Tools Factory trial pictures, 2004-05

Four side planning

Four side machining of harder bamboos is much more difficult. Removal of knots, cleaning of skin and the deviation near the knots are a major cause of concern for most operations.

First cleaning of internal knots, external knots, skin cleaning, rough planning and the finish final planning would be done later. The machine required for the same are very sturdy and have a stronger gearbox.

Multi product and utility product solution

Our Universal Woodworking Cum Bamboo Application Machine is capable of doing a number of operations just by changing cutters and fixtures. Tools like circular saws, slotting saws, knot removing cutters, parallel splitting saws, planning blades, designing cutters, three side planning cutter set, drills of various sizes, polishing plate etc. can be used on the same machine. These tools can do jobs like, cutting, angle cutting, grooving, slotting, removing external knot and internal knot, skin finishing, parallel splitting, planning and thickness planning, designing, three side planning, drilling etc. Hence, the machine is ‘universal’ in itself, i.e. capable of doing nearly all the operations which a small workshop of bamboo and wood requires (Figure 6).



Figure 6. Universal Woodworking Cum Bamboo Application Machine. Source: Garnet Tools Factory trial pictures, 2005-06

Stick and slicing issues

For making sticks we need to process slices then into strips as the wall thickness found is much higher we first make slices then process them into sticks this gives higher productivity and better results. Slicing on harder bamboos becomes difficult as the bamboo with external knots and internal knots become denser and thicker. The machinery process is a shearing of the strip into required thickness as per slicing requirements. The process is not always completed in one go. You are required to make thin slices using specialised machinery and fine slicing can only be done using highly accurate slicing and roller feeding system.

Overview of Incense Sticks Market in India

The incense stick market in India is valued at \$400 million with an annual compounded growth rate at 20%. Further value addition occurs when incense paste is hand-rolled, mostly by women, on to the sticks to make “raw” incense sticks, which are then perfumed to obtain finished incense sticks. About 1,000 billion incense-sticks are produced annually, and bamboo sticks account for only 7-8% of the total cost of incense sticks. This puts the current value of bamboo in incense sticks at \$30 million. Annually 0.67 million tons of bamboo is consumed by the incense stick industry. Incense stick production is a home-based industry engaging about 500,000 people, mostly women. Though both domestic and export markets are growing, only 10% of the total cost of finished incense sticks is realized by the producers because of marketing and financial constraints. Transportation, marketing and advertising often claim 60-70% of the total cost, causing both producers and retailers to be at the

mercy of agents and wholesalers. This is beginning to drive people out of the industry, especially in South India. Note that agarbatties use inputs that are outputs from other enterprises – bamboo sticks, charcoal and jiggat (tree bark powder) (INBAR website).

The incense stick market faces a sudden upsurge in demand during the festive season. Demand from both domestic and international sectors peaks up during festivals like Dassera and Diwali. Some of the popular fragrances have captured the imagination of consumers both in India and abroad.

Today, the incense industry has not only become a major revenue earner for the Government it has also become a prominent source of employment for women in rural areas. Presently, the incense industry in India is a cottage industry and the market is largely unorganized. Only twenty percent of the market is occupied by organized players while the rest is controlled by small operators. The government needs to pay urgent attention to this industry if it wants the industry to retain its leadership position in the industry.

The incense stick industry has also promoted social entrepreneurship in the rural and semi urban areas as more and more people have acquired the confidence to start their own businesses. Some reputed Indian corporate have taken the lead in promoting this trend by training rural women in bamboo cutting, incense rolling, raw incense sorting, perfuming and packaging of incense sticks. The entry of corporate has helped in mechanization at each step of the manufacturing process. Earlier rural women used to cut bamboo with an axe leading to injuries. This process has been done away with the introduction of bamboo cutting machines.

Production Process:

The process of Incense Stick making:

1. Cutting of Bamboo in Required Shape
2. Splitting of Bamboo if the wall thickness is low
3. Slicing of Bamboo
4. Square Stick Making
5. Polishing of Dry Sticks
6. Preparing raw material for coating i.e. wood dust, coal powder and solution
7. Coating of incense Sticks
8. Drying and Heating of Sticks
9. Perfuming of Sticks
10. Packaging and forwarding

Most operations on the bamboo are done manually in India and the process is now changing we are now providing manual, non-electrical and electrical cum manual systems, machines and tooling.

Manual tools and machinery

Manual machines form a key to rural upliftment and society welfare in developing countries. Providing machines that do not require electrical power or can be used in house holds can be a value added enterprise and thus we have developed a variety of had tools and machinery that can help a simple labourer to ear nearly two times the daily wages earned from a much environmentally and health

Manual machines include machines for cutting bamboo, splitting bamboo, skin cleaning, tooling and machine for making sticks, slices and handicraft products. These machines are simple to operate and are more economical and the financial aids required can be fulfilled very easily. These machines have been boon to artisans to help them increase productivity and to enrich themselves and their lifestyle still keeping their culture intact (Figure 7).



Figure

tool

Tool

11

Newer developments in products and technological advancements in processing

Rounding of bamboos

Rounding of bamboos or making cylindrical straight bamboo poles is a major issue when we think of making furniture, handicraft and related construction material. Previously rounding of bamboos as required is done using manual tools and required a lot of time and labour efforts. One person used to make bamboo straight and bent free, second person used to clean the knots, third person used to remove the skin of the bamboo, fourth person was required for finishing of bamboo pole and another person for sanding if required. This is a tedious and long time taking operation requiring lot of training, human skill and hard work. One standard bamboo chair or bed requires nearly 10-20 bamboo round poles to be sanded and then polished and fitted in the machine to achieve the required quality and price that could be fetched in the market (Figures 8).

Rounding of bamboo can be achieved by cleaning knots, removing skin from the bamboo, making a round from the bamboo skin removed pole and if removed sanding can be done for giving the finish as required for the particular application. Now to go into mass production or at least make the product

marketable, reachable to metros and urban community its important to use replaceable, repeatable and highly durable parts in place of skill prepared parts with less chances of repeatability and durability.



Figure 8. Rounding of Bamboo.

Source: <http://www.amazuluinc.com/images/solid-bamboo-finished-solid-bamboo-001.jpg>

Flattening machine

This machine is used to flatten bamboo after cleaning of internal and external knots and branch nodes. This machine can process the bamboo without any additional wastage. The process is carried out by sequential rollers with decreasing curvature to flatten the bamboo splits into a flat section. The cracks developed on the bamboo strips looks artistic and the bamboo can be used for natural decoration, as front face of boards and as a filler material for matboard or other board making industries(Figure 9).

Joinery methods

Joinery in bamboo has always been a much talked about and also still a vastly unexplored region. We have developed a few solutions that we provide to our specific and customers in the field of constructions, furnitures and board industry. The kind of joinery defines the yield and productivity from the bamboo. Joinery in furniture can enable an able manufacture to ship more, produce in mass, manufacture products. Joinery should be such that it should compliment the products aesthetic value and add to its durability and longevity. Normally we observe that the joints and its looks are the most bothered about issues related to a product and its quality. Similarly to make a strip from a bamboo and to make its joint with similar strips or perpendicular joints of making much more stable and boards with different joinery systems that can increase the yield from a bamboo by nearly 20% is the need of the hour. We have indigenously developed a few of them and looking to develop them using industrial ad technological partners.



**Figure 9. Flattening Machine with flattened bamboo pieces.
for manuals, 2011-12**

Source: Garnet Tools photo gallery

Way ahead and future in Bamboo

Low Cost Bamboo Floor Board Making

Bamboo flooring boards have always been considered a high value and high investment products. We can but provide much lower costing but equally efficient system for processing of bamboo into strips and then pressing them into boards and finishing them into fine finished boards. The processing machines designed depends upon the type of bamboo and the end product finish required.

The machinery thus provided at the time of turn key solution projects can be varied and tested on the customer provided or similar bamboo species and thus the process is indigenous and has a lot more flexibility and even hobbyist can manufacture bamboo floor boards in small quantities using tools and machinery provided by our concern.

Furniture, Resort Making And Bamboo Construction Machinery

To manufacture a bamboo bed, chair, house, hut, bridge and similar constructions you need to treat, cut, shape, make strips, round bamboos, drill and similar operations are required. But have people calculated the time thy can save to reduce the amount of your labour and skill involvement. Creating designs and following them makes products repeatable and maintaining a proper design tolerances when at the time of commissioning of bamboo product resources. Highly planned systems work and perform better.

Strand Woven and Fibre Based Joining

The bamboo fibers when pressed at high pressure and using appropriate temperature, primary processing and settling of bamboo fibers result in a very strong and highly durable bamboo beam or board. These boards or strips or furnitures or any bamboo product made from them would have high strength. These boards and sheets can be used in most places and the usage of bamboo is also very high. Thus we can utilize a lot of bamboo that would have been wasted in making strips and similar processed to make boards. For this process we only need to crush and segregate the strip slices to get a strong bonding and glue penetration. The pressing system and other machinery are much different from the primary processing methods but the machinery after pressing or board or block making is very similar to any board or beam processing machinery as used in wood or bamboo sheets. But the machinery and its load bearing capacity should be very high in order to accommodate the high density structure. This is the future in the processing of bamboo.

Conclusion

“Bamboo resources when used at optimal levels can be a boon to any economy.” – Mr. Abhay Banthia, Garnet India Group. Bamboo with its variety of products and applications can be used in furnitures, handicraft, constructions, incense, fiber, boards, charcoal, food and many more. Each Bamboo can be used to higher extent if we understand and use it using the marketable and most productive ways. We can increase the yield per bamboo or per kilogram by 10-30% depending upon the product and the bamboo species being in use. Also of proper machinery, tooling and bamboo specie to manufacture a product that is marketable, processing issues are solved and have a high yield would be a proper mix for any industry to flourish and develop into a profit making as well as much more environmentally friendly system as well.

References

- Chisholm, Hugh, ed (1911). "Bamboo". *Encyclopædia Britannica* (11th ed.). Cambridge University Press.
Retrieved from Oscar Hidalgo, Notes by Cassandra Adams, <http://www.networkearth.org/naturalbuilding/bamboo.html>
<http://www.inbar.int/Board.asp?BoardID=225> inbar website about incense stick Home > Our programmes > [Livelihood and Economic Development](#) > [Innovative Products](#) > Incense sticks
Adams, C. <http://www.networkearth.org/naturalbuilding/bamboo.html>

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Keywords

Architecture , construction techniques



Figure 1.

Introduction

In the lush green surroundings of a former fruit orchard, where Thailand's highest mountains meet the flat rice fields, Panyaden School contracted 24H to design its environmentally friendly school buildings.

Panyaden is a private bilingual school with a Buddhist approach. The students will be the ambassadors to introduce green living into the lives of their communities.



Figure 2.

Concept

The school consists of an informal arrangement of pavilions (salas), organized along pathways inspired by the shape of the tropical antler horn fern.



Figure 3.



Figure 4.

There are two main types of buildings.

First, the classroom pavilion type has load-bearing walls from rammed earth, dividing the building into 3 classrooms. The curved contours of the waving bamboo roof structure mirror the mountains at the horizon.



Figure 5.

Second, the sala pavilion type is used for common functions such as the assembly hall and the canteen. Columns consist of bamboo bundles reaching up to the bamboo canopy from their stone foundations, giving a feeling of walking through a majestic bamboo forest.



Figure 6.

Design

The design adopts all bioclimatic aspects to suits its humid tropical environment. The roof cantilevers up to 3m acting like a big umbrella providing shade and protection from the heavy rains. The open(able) facades provide natural cross ventilation.

The entire school has been built from local earth and local bamboo. Organic vegetables and rice are grown in the school's garden. Environmentally friendly waste water treatment and food waste recycling producing organic fertilizers and biogas for cooking, round up the picture of an environmentally friendly school with a negligible carbon footprint.



Figure 7.



Figure 8.

Bamboo

A variety of local bamboos has been used, utilizing the specific qualities of each of them. *Pai Tong* (*Dendrocalamus asper*) created the main structure, *Pai Sisuk* was split and woven into the curvy roofstructure mesh and was applied in a flattened form for ceiling panels and roof shingles. *Pai Liang* (*Bambusa multiplex*) was used in parts of the roofstructure.



(image 9)



Figure 10.

Bamboo Umbrella Housing

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Facing the task to improve the quality of life in combination with an environmental protection, initiative thinking arose to develop a sustainable housing production that considers nature's demands - such as the use of local and renewable resources like bamboo - and simple technologies that can be processed by the local population to enhance local developments by local production chains.

In this context the present project is implemented using bamboo as the dominant construction material and exposing its architectural and structural possibilities.

The project was commissioned by the National Training Service for the Construction Industry (SENCICO) from the Ministry of Housing, Construction and Sanitation of the Republic of Peru, through the Research Management and Standardization, responsible for promoting research, development studies, testing, training and standardization of new technologies and procedures, designed to buildings and housing in particular.

As part of the research and promotion of local materials, the institution has been working with bamboo as a construction material for over 10 years in different places and with concern on different investigation results. The present case is located in the northern Peru.

The design and construction of the present housing prototype generally aims at the spreading of bamboo constructions in Peru. At a local level, the project promotes the proper use of bamboo as a construction material by demonstrating and exposing its architectural possibilities in that specific area with properly managed bamboo forests plantations.

The architectural concept comes from the shape an umbrella. The basic components are the "umbrella roof", made of four main trusses, borns in four central's columns, and four secondary trusses, generating an octagon and a circular roof; and bamboo poles as "vertical blinds", covering all sides, leaving openings for doors and windows, that harmonize with the architecture of the prototype and the relationship of interior space with the surrounding space.

Area: 58.45 m²

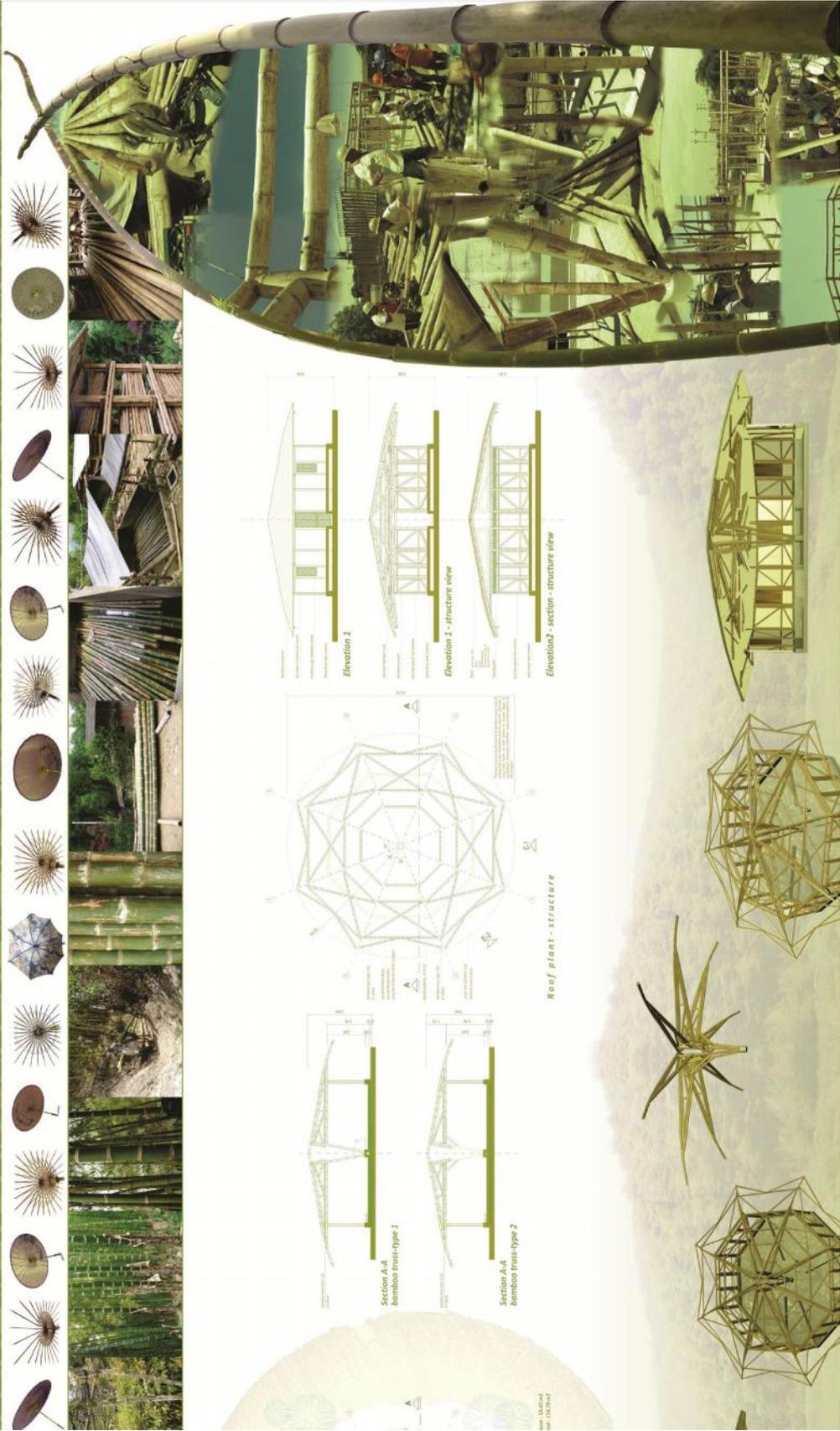
Location: Chiclayo, Peru.

Bamboo Forest: Florida, Perú.

Specie: *Guadua angustifolia* Kunth

Bamboo Umbrella Housing

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Session 2. Development and Country Reports

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Current uses and options for commercialization of bamboo Resources in Ethiopia: Entrepreneurs' and Technology Perspective

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Abstract

Bamboo based entrepreneurship will have tremendous opportunity for augmenting rural development efforts in Ethiopia. Given a huge resource base, distributed in wider geographic areas, fast growth characteristics, numerous product and service function, bamboo can top many of the species list for integrated rural development in Ethiopia if the production technology is upgraded. The highland bamboo has features useful for construction and furniture production. The lowland bamboo with solid culm and long fibers is most preferred for charcoal and paper making. Both of them have diversity of local uses. However, this species has been neglected and has not given sufficient attention for its commercialization. This paper presents an exploratory of innovative commercialization attempts from technology and entrepreneurs' perspective based on survey of actors. Bamboo production in Ethiopia is still a cottage industry runs as a family business. The technology they use is indigenous technology with some modification based on design manuals, internet sources and person to person experience sharing. Small scale wood and bamboo processor firms claim that they can produce quality products that can fulfill many of the design requirements of the consumers if they are supported by institutional actors in market creation. There is also limited awareness of the potential of the resource from the private sector and the quality of bamboo from consumers' perspective. Thus the major gap rests on promotion of the product to engage the private sector in the processing, marketing and consumption of the resource as well as promotion of the product for broader marketability. Side by side, further development of existing and new innovations and diffusion of new innovations can accelerate bamboo utilization culture and thereby rural households' income. By doing so, the huge environmental benefits of bamboo can also concurrently be tapped.

Keywords

bamboo commercialization, technology, Innovation, entrepreneurs, Ethiopia

Introduction

Bamboo is a versatile resource with a multiplicity of uses ranging from the traditional to state-of-the-art applications in modern technology. The abundant availability, fast growth, good mechanical property, low cost, and its excellent environmental service roles makes the species at a higher priority for commercialization to enhance its product and service functions. Despite the potential which existed the government and to some extent the private sectors, the product still considered by the majority of the population as inferior product and used for low quality demanding utilities.

Ethiopia has over 1million hectare of bamboo resource which can sustainably produce 3million meter cube of dry weight annually (Embaye, 2003). Despite its large resource base, immense economic and environmental potential, its contribution thus far is insignificant. Its use has been limited to traditional uses like fences and low quality furniture. The underlining reason for this is low level of product commercialization. Commercialization attempt is underway in the country. However, it is not based on a sound knowledge base that can be used to accelerate the rate and enhance consumer and producer acceptance of the commercialization approach. Little research attention has been given so far.

The resource is mainly in natural forest stand and has been largely intact from human action due to its relatively inaccessible location. Recently however, the resource has been under threat by two major forces. The first is natural death of the stand followed by clearing the dried stand and cultivating with cereal crops, preventing regeneration potential of the bamboo resource. The second factor is deforestation for private investment and small scale farming.

Commercialization of the resource considered as an option to prevent the depletion of the resource while providing income for rural and other dependent households who otherwise depend on destructive landuses. The generation of benefit through commercialization, however, among other things, depends on the innovative production and processing technology as well as well-connected market system. Thus, the present research explores the role of entrepreneurs and innovative technology in commercializing bamboo resources of Ethiopia.

Method

This paper is based on data collected from Addis Ababa, Hawassa and Bahir-Dar cities of Ethiopia. Ethiopia is located in East Africa. The country has diverse altitudinal range which leads the country to have diversity of fauna and flora including bamboo species.

To generate the required data and answer the research questions, a qualitative survey of several actors in the bamboo processing and trade has been interviewed. Interview of 16 medium and large agricultural entrepreneurs to understand their decision behavior to engage in bamboo based business; 10 cottage industry to understand existing processing and innovation condition, 35 urban residents to understand the awareness level and willingness to buy bamboo products and three bamboo experts were also interviewed to get their insight about technology, research and development and market role in bamboo commercialization. Focus group discussions were also conducted to understand market knowledge and response of consumers. A review of secondary data has been also made. Personal experience and private communication with experts and peoples engaged in the sector has also been used as source of data for this paper. Data has been analyzed qualitatively and logically depending on the data type.

Result and discussion

Current uses and marketing of bamboo

Bamboo resources of Ethiopia distributed in many parts of the country. Every region is inhabited by different cultural group. Consequently, the use of bamboo varies in relation to their cultural requirements. In general the following uses of bamboo have been documented based on the various data sources.

Bamboo for construction material: in all bamboo growing areas of Ethiopia, though the design and parts of the house which uses bamboo varies, uses bamboo in all or some parts of the house. In some regions, all parts of the bamboo use to construct house without any other materials included. The leaves used for thatching, the twigs used for tying and the culms used as complete raw material for building houses. Recently high standard tourist resorts and recreational places prefer bamboo to wood. This could be attributed to the fact that tourists are generally foreigners and elite Ethiopians who have better awareness of bamboo products. Another most common use of bamboo is fencing of crops, homestead and cattle yards. Construction of bridges and water ways is also observed in some places.

Furniture; from rural to urban areas, one can find various bamboo furniture products with a diverse design and quality level. In rural areas bed, small chairs and table are constructed from bamboo culms with little modification and aesthetic addition to the raw bamboo. In urban areas, the design somewhat improved, culms may be molded into different structures and various finishing and aesthetic activities made. As expected, products in the urban center are more expensive than rural areas. Producers and consumers are different persons in the urban case while the rural area producers and users of produces are often the same person.

Household utensils: bamboo is used for storage of crops (granary), water container, various agricultural implements, walking sticks, beehives, baskets etc. . Bamboo is also used as support structure for some fruit and vegetable crops which have weak stems.

Hand crafts: bamboo handcraft availability in craft shops of many tourists has surged recently. Crafts symbolize ancient Ethiopian buildings, traditional cultures, musical instruments, orthodox culture, military and other ways of life. With relative political stability and increase in tourist number the demand for handcraft including bamboo based products has increased significantly.

While many of the traditional bamboo produces designed and constructed by the users, other products like basket handcraft, furniture and raw material for construction of those products marketed locally, in small regional markets and the capital city with an increasing price and market connectedness.

Generally there is a consensus by all the informants that there is a relative increase in the bamboo market in the last ten years. So do the number of bamboo processors and traders. For instance, the price of highland bamboo culm has increased by three fold and the price for furniture more than double in the same period. The aesthetic preference for bamboo houses especially in recreational centers has growing rapidly. However, the innovativeness of actors in all the value chain is still poorly developed. Thus value addition through innovative design, organizational competency and product quality and diversity can lead to a significant transformation in the market system and structure.

Commercialization challenges

For the last six years, Ethiopian economy is growing in more than 10%. There is also huge increase in investment by local and foreign companies in various sectors of the economy. Business Motivation and assertiveness has grown fast. Several business sectors have shown accelerated growth. Agricultural investments such as flower industry, apiculture, horticulture, oil and seed has been growing both in volume of production and process technologies with national and international actors as players. An equally assertive business opportunity has existed for bamboo resource. However, interest for the product has been less encouraging. Interview with medium and small enterprise shows

that only three of the interviewed entrepreneurs are aware of the resource potential for large scale international trade. The rest do not think and have no idea about the business opportunity in the bamboo sector. However, all of the interviewed entrepreneurs know bamboo as a resource used by rural households for low quality subsistence use and if it has any potential for trade only for local market. The former group cited several reasons for their pessimism in the bamboo business in Ethiopia. The first reason is that, the plybamboo, bamboo lumber and furniture production could have high wastage which curtail its profitability coupled with the long distance of bamboo raw materials from the centers of market. They also mention that the commercially useful bamboo resource is scattered in several regions of the country and decision on where to establish bamboo processing factors is a challenge. If it is constructed in Addis Ababa, the capital, transportation of culms will be costly. Equally challenging is locating processing industrial plant near the resources base. However, the resources are distributed in different places and overexploitation may happen unless large land for plantation bamboo made available. Thus, entrepreneurs may be deterred from entering the bamboo business or it may take some time to enter to the processing business. One of the interviewees is already in feasibility study and dealing with government for leasing land for plantation.

More than two-third of the respondents have mentioned availability of market as a problem even in the future. But five of the respondents think that what matter is production quality. And if they produce quality product and if existing policy condition in the country support them in establishing the business and market search, they believe that they can create market locally and attempt to enter in the international market. Two of the respondents believe that in international market they will face stiff competition from Asian entrepreneurs and creating local market at first and building from here could help them survive as a business. However, they assert that they can still produce low technology products such as bamboo charcoal where technological disparity has no major impact. They also think that they can find an unexploited market segment even in the international market. One respondent also mentioned that accessing western market may be easier for us than do the Chinese entrepreneurs. Thus there is divergent response from the entrepreneurs as to the feasibility of bamboo processing and trade in Ethiopia. The government can stimulate them to inter to the business with several incentive mechanisms including searching and facilitating market opportunities.

Technology

Recently two sources of technology are applied for bamboo innovation development in Ethiopia. The government led innovation approach inclined to foreign technologies. It has been trying to import processing technology, bamboo propagation and even organization system of research and development by establishing Chinese type ministry (Ministry of science and technology). However, success is far less significant. The first reason is the technology is not owned by the local stakeholders. Secondly the technology is not properly selected and adoption activities were poorly done. Third development was preceded to research. There are several research institutes and large numbers of personnel were engaged in the sector in china while in Ethiopia, there are few institutes who consider bamboo as one program in their research undertaking. The government and NGOs has given several trainings to improve the skill of processors. However, trainers little benefit from the training as the training is not demand driven.

The second source is traditional technologies that gradually developed by local artisans and grow from rural simple processing to relatively improved cottage industry in urban sector with the use of imported machines and tools to improve quality of products. This technology generally seems successful, and at least, existing production and consumption depend on this technology. In addition to machines some artisans also use design standards of international bamboo processors by looking in the internet and buying design manuals.

The middle ground may produce better result. However intensive demand driven training should precede development effort. In order to select the most appropriate technology, product and extension methods, local research and development has no substitute. Thus, the government has to establish strong bamboo research and development institute that has clear mandates with an interdisciplinary team of researchers. Government support that is capable of integrating R&D, Market, communication among entrepreneurs and among all major actors in the production to consumption chain can yield better result. Cross functional communication and knowledge sharing are the key factors for developing the technology and accelerate commercialization in Ethiopia.

Bamboo innovations and learning interactions

Bamboo processors state that they have introduced several design innovation, through personal creativity, copying from other firms and looking at design books from international bamboo furniture producers. About 60 percent of SME has introduced one or more machine for better design and aesthetics. Most of them are satisfied with the market condition; however, their customers are foreigners and informed local residents. In order for bamboo to access broader consumer, strong company cooperation for innovation assisted by sustained promotional work by the state and the private sector are found essential.

The cooperation among entrepreneurs is meager. Under this condition, promising technological innovation development will be less likely. Cluster based firm developments in many countries has become successful and Ethiopian bamboo processors can also benefit by interaction and experience sharing. Clustering is also started in better forms in other sectors of Ethiopia while the situation for bamboo is not yet encouraging.

Conclusion

The fact that bamboo products has never had a place for high quality uses and the traditionally held believe that it is a poor-man's timber largely curtailed efforts for its commercialization. Existing uses are limited for fencing, low quality constructions. Yet there are some cottage industries in the cities utilizing bamboo culms to construct furniture and souvenirs for tourists. Interestingly products produced by the cottage industry are bought by foreigners and elite Ethiopians.

The production technology is also very low and even many of the processors have no appropriate processing Equipment. They argue that limited market is behind the under development of their production system. However, true it may be, in reality they do not also have the proper training and information for better quality product. The government and the private sector need to work hand in hand to improve entrepreneurs' capability and accelerate innovation to transform the technology and product quality.

Another issue acclaimed crucial for bamboo innovation and commercialization is to organize small entrepreneurs in some form of joint action or clustering based on geographic and sectorial homogeneity. This will create a strong competitive advantage as they will get new experience from each other (Porter, 1985) to improve their production process and production equipment. Until now, there are no bamboo based clusters, but there are couples of associations and they can be upgraded into clusters. Jote(2009) argues that since there is no viable bamboo SMEs cluster, we need to start from scratch and develop into strong clusters through appropriate cluster modeling strategy.

Communication among peers is found very limited as a result of competition for existing small number of customers and fear of bankruptcy. However, experiences in other sector and countries shows that, clustering can improve their technological capability and competitiveness if properly designed and implemented. (Juma, 2011) recommend that government can nurture a quick flow of

investment, ideas, and even personnel from the public sector to private firms. As government-funded initiatives deliver proof of concept, governments should make way for private enterprise.

References

- Embaye, K. 2003. Ecological Aspects and Resource Management of Bamboo Forest in Ethiopia. Phd dissertation. Swedish university of agricultural sciences, Uppsala, Sweden.
- Jote, N. 2009. Cluster Approach for Enhancing the Productivity and Competitiveness of Micro and Small Enterprises /MSEs/: Case Study on Bamboo Micro and Small Enterprises. M.Sc Thesis, Addis Ababa University, Ethiopia.
- Juma, C. 2011. The new harvest : agricultural innovation in Africa . Oxford University Press, Inc., UK
- Porter, M.E. 1985. Competitive Advantage: Creating and Sustaining Superior Performance. New York: The Free Press.

Bamboo – Guadua in Colombia: Administration and Environmental Sustainability

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Abstract

Bamboo is considered one of the principal species of bamboo like the biggest use in importance in America and in Colombia; its distribution in all the Colombian Andean zone and it's managing and use. Given in the last fifty years (50), since the intuitionalized, it has allowed that this species of bamboo be considered the most close to sustainable concept thanks to its removability and to the great offer to the social environmental services and cultural that aport to the urban communities and rural. The bamboo - guadua in Colombia, is regulated, administered and handled by the Environmental Authorities, that in I associate with the usuary communities have united efforts to cause its promotion and sustainability, looking for in addition to position it like the species that in the future next is base, for the mitigation of the Climatic Change.

Keywords

Guadua, Environmental Services, Environmental Authorities, Sustainability, Climatic Change

Abbreviations:

C.R.Q (Regional Independent Corporation of the Quindío).

Bamboo in Colombia

The bamboo *Guadua*, *Guadua angustifolia* Kunth original from America it is distributed from Central America to South of Ecuador and Peru; Colombia has approximately 45.000 hectares localized in the Central Andean region, where its use and its sustainable manage it's the product of the importance and the role that it represents the communities, therefore, the experience and knowledge of the Environmental Authorities in the Country, that for more than forty years (40) have regulated, encouraging and the most important is that they have putting together all the efforts around to this specie, and in this way, they have consolidated processes such as the national chain of bamboo, the guadueros federation, The Colombian Bamboo society and lately the National Red of Bamboo – *guadua*, which is constructed around the International Red of Bamboo and Rattan (INBAR).

Administrative Diagram

The bamboo rodals, denominated “bamboos” are used for their owners, under the addressing of the environmental authorities in the country, who for its experience have developed a technological package that allows the sustainability of the resource, through the rational use, this is considered, the principal activity of the forestry and it obeys to a “Managing Plan for Using Sustainable of the Forestall Bamboo”, that includes previous forestry labors such asunhooking, cutting, planning the pick outs, primary transformation processes and managing processes post harvesting. In the same way is the “Environmental Authority”, who followed the performance and control to the activities described above, this has allowed to increase the level of “Sustainable Managing of the Bamboos” from the part of the user Communities of this resource in Colombia, who, besides in partnership with Governmental Institutions, offer processes such as the Unification of the Norms, the Voluntary Forestall Certification (The *Guadua* the only bamboo in the world with standards for the certification), the conformation of the forestall nucleus, Productive Alliances and the development of the clusters such as Construction and Bioengineering that they look to improve the competitively and marketing of the products derived from this kind of jungle.

Environmental sustainability and *Guadua*

The *Guadua* in Colombia over other species for its particular capacity for: soil protection, hydrologic regulation, for being a niche of flora and fauna, for its rate of removability that guarantee the highest capacity of set up the CO₂ and for being an strategic specie for restoring the degrade rated ecosystems, (widest range of the distribution and the adaptability).

In Colombia actually the *Guadua* is overview like the principal native species that must be employ for affronting situations like the ones generated for “Weather Changes phenomenon” (droughts and winters) that are alliterating the dynamic of the micro basins where are localizing our populations.

The *Guadua* in Colombia, it is thought and used for performing in a world level. Mitigation processes, such as an environmental forestall restoration in areas around the rivers and currents in middle zones and in low micro basins; in the same way and as contribution to reduce the Risk and Mitigation of the impacts, the *guadua* is employed as a principal material in the construction of the bioengineering labors and/or biomechanics, reducing consumes and cost that affects the environment; finally, is very important to mention the foment to set up the forestall plantations of *Guadua* that pretend to increase the capacity to stick the CO₂ in the national context and in strategic zones or highest vulnerability in front of the Global Heating phenomenon.

Bamboo shoot as a resource for health food, food security and income generation in North-East India

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Abstract

The North East region of India is a rich resource of biodiversity in general and bamboos in particular. It harbours about 43% of the total bamboo wealth of India and plays an important role in the life of the people especially in the rural areas. Though bamboo shoot is not a popular food commodity in the Indian sub-continent, it is a delicacy in the north-eastern states being used in fresh and fermented forms. Bamboo shoots have high content of proteins, carbohydrates, minerals, fiber and vitamins and are even richer in nutrient components than some of the commonly used vegetables. They are endowed with health enhancing properties due to the presence of phenols, phytosterols and fiber. However, this highly nutritious vegetable is being neglected and replaced by other food items and its usage in local households is gradually diminishing. Very few people are aware of the nutritive value of the shoots and with the passage of time, it is becoming neglected as other indigenous plants like finger millet, buckwheat, taro, amaranth etc. and considered as food of the tribals and poor people. Concerted efforts need to be taken up to utilize this natural resource not only to meet the increasing demand of food and food security in the region but also to encounter malnutrition widely prevalent in the country and provide income generation for the local people. Despite the enormous production of bamboo shoots in the region, processing and packaging of the shoots is in its infancy with only a few units being operational. Taking into account the increasing demand of bamboo shoots worldwide, and the enormous economic potential, development programs need to be framed for utilizing the vast resource to generate employment opportunities for the weaker sections of the society and help in their social and economic up-liftment.

Keywords

Bamboo shoot, North-East India, income generation.

Introduction

The North Eastern region of India comprising eight states namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim, is one of the richest reservoirs of genetic variability with its own unique biodiversity, habitats and ecosystems, which together make it a diversity rich resource. The north-eastern area falls under the Indo-Burmese region which is the 6th among the 25 mega diversity hotspots of the world. It is also one of the 8 hottest biodiversity hotspots with more than 7,000 endemic plants (Myers *et al.* 2000). This region is considered as the “Bamboo Paradise of India” and is a treasure house of bamboo diversity harboring 43% share of the total bamboo wealth of India. About 66% of bamboo resources of the country and 28% of the total bamboo area of the country are found in this region (Madhab 2003). With respect to species diversity also, it is North-East region of India which has more than 54 species out of 128 reported from India (Nathani 2008, Nimachow *et al.* 2010). As far as bamboo species are concerned, the richest North-eastern State having species diversity is Manipur with 53 species, next is Arunachal Pradesh, with 50 species. The whole region is very rich in bamboo species diversity as well as stock. In India, bamboo is spread over 1,00,000 sq km of forest area (12.8 %) of the total forest area. From time immemorial, people in the region are using bamboo for various purposes right from bridges over mighty rivers like Siang to sitting mats, rain coats, carry bags, writing pens, house utensils, cradle, walking stick for old man and finally bier to carry the dead body. Bamboo is also food for big animals like elephant as well as for humans. Young juvenile shoots, the new culms that just emerge from the ground, are delicious and nutritive food for the people of the region and constitute a range of traditional delicacies. There are a number of tribes in the region and each tribe has its own way of using young juvenile shoots as food either, fresh, fermented or dried.

North-East Region of India and bamboo in the region

The North-East region of India has 8 per cent (2,62,179 sq km) of the total area and has around 4 per cent (4,55,87,982) of the total population of the country (Table 1). The population density in the region ranges from a minimum of 16 persons per sq km in Arunachal Pradesh to 397 persons per sq km in Assam (Table 1). The people of the region are of different tribes or ethnic groups which numbers more than 135. Majority of the population in the region still directly or indirectly depend on agriculture and natural resources like bamboo, pineapple, ginger, cardamom, coal, lime, etc for earning their livelihood.

Bamboo is one such natural resource which is abundantly available in the region. Bamboos are intricately associated with the day-to-day life of people of North-East because of its multipurpose economic uses. The uses of bamboos in day-to-day requirements range from household construction to utensils to food and medicine. The bamboo stock in the region is around 42.27 million, which is more than 60 per cent of the total bamboo stock of the country (Table 1). States like Mizoram have more than 40 per cent of their total geographic area covered with bamboo only. There are states like Manipur and Meghalaya have 21.0 and 32.0 per cent of their total forest area, respectively, covered with bamboo (Table 1). Bamboo is spread on 9.2 per cent (24,110 sq km) of the total geographic area of the region, maximum (9,210 sq km) being in Mizoram state. The region is also rich in bamboo diversity with more than 54 species. Manipur and Arunachal Pradesh are the richest states with bamboo diversity with 53 and 42 species, respectively followed by Meghalaya and Assam with 35 and 29 species, respectively. Sikkim is also very rich in bamboo diversity with just 7,096 sq km of total geographic area has 26 species of bamboo (Table 1).

Bamboo shoot as a Health Food in the region

In addition to its multifarious uses, the young bamboo shoots are also used as food in the region. Soft juvenile bamboo shoots of all most all species are used as food by all tribes and ethnic groups of the region. However, there are some species like *Bambusa balcooa*, *B. bambos*, *B. tulda*, *Dendrocalamus giganteus*, *D. hamiltonii*, *Melocanna baccifera*, *Chimonobambusa callosa*, *D. hookerii*, *D. giganteus*, *D. sikkimensis*, etc. which are preferred. The young shoots are either used fresh or fermented and dried for later use. In terms of yield, it is *Dendrocalamus* species (*D. hamiltonii*, *D. sikkimensis*, and *D. giganteus*) which are harvested maximum for shoots. Every tribe or ethnic groups of the region have their own methods of fermentation and use of bamboo shoots as food (Table 2). The Khasi people in Meghalaya mainly ferment shoots in plastic or glass bottles filled with water, whereas Meiteis of Manipur ferment bamboo shoots in black clay pots or in bamboo baskets. Generally bamboo shoots, fresh or fermented are used for making pickles, curry like Soibum (by Meiteis) or prepared with pork (by Khasis). However, these cuisines are traditional followed for generations and may not be palatable to all. Hence, new cuisines should be developed taking into account the changing food habits of the modern world. There is a need to develop new food items from bamboo shoots to popularize it as an important health food as well as a solution for food security in the region. In developed countries, bamboo shoots are an ingredient of food items such as cookies, dairy and meat products, beverages, ketchups etc. and because these are popular food items, they are readily consumed by people.

Though bamboo shoots have been a popular food commodity for generations in the region, with changing trends in the food habit, it has become a neglected food item. Younger generations are not aware of the nutritional value of the shoots. It is generally despised as tribal or village food and do not find any place in the plates of city people. Very few people know that worldwide bamboo is listed third out of six most healthy foods. Also people in the region are not aware that phytosterols present in bamboo shoots have the cholesterol lowering activity and it is termed as appetizer due to presence of high cellulosic content in it (Nirmala *et al.* 2011). The fiber content in the bamboo shoots which ranges from 3-5 g/100 of fresh weight helps in lowering cholesterol in blood and bamboo shoots are known to protect neurons from oxidative stress and has anti-fatigue activity (Akao *et al.* 2004). Bamboo shoots have many amino acids and minerals like potassium, phosphorus, sodium, magnesium, calcium, etc which are absent in many common vegetables that people take in the region (Table 3). Bamboo shoots have many medicinal benefits from preventing cardiovascular diseases, cancer and weight loss to improve digestion. Due to high potassium content in shoots, bamboo is considered as heart protective vegetable.

Table 1. North-East States of India: population, geographical area, forest area and bamboo stock in the region.

North-East Indian States	Geographic Area (sq km)	Population (2011 census)	Population Density	Forest Area(sq km)	Per cent Forest Area of Total Geographic Area	Bamboo Area (sq km)	Per cent Bamboo area of Total Forest Area	Per cent Bamboo Area of Total Geographic Area	Total Bamboo Growing Stock (000,000 tonnes)
Arunachal Pradesh	83,743	13,82,611	16	51,540	61.5	4,596	8.52	5.5	1,616
Assam	78,438	3,11,69,272	397	26,832	34.21	1,813	6.56	2.3	9,844
Manipur	22,327	27,21,756	103	17,418	75.01	3,692	21.81	16.5	11,470
Meghalaya	22,429	29,64,007	132	9,496	42.3	3,102	32.67	13.8	4,407
Mizoram	21,081	10,91,014	51	16,717	75.6	9,210	57.80	43.7	10,890
Nagaland	16,579	19,80,602	119	9,222	55.6	758	8.8	4.6	3,657
Sikkim	7,096	6,07,688	85	5,841	82.3	-	-	-	-
Tripura	10,486	36,71,032	350	6,294	60.0	939	14.92	9.0	860
TOTAL	2,62,179	4,55,87,982	1253	1,43,360	486.52	24,110.0	151.08	95.4	4,27,44

Table 2. Fermented and other food products of bamboo shoots in different tribes of North-East India

States	Tribes	Bamboo Shoot Product	Procedure of Fermentation
Arunachal Pradesh	Apatani	Hikhu	Chopped pieces of bamboo wrapped in banana leaves and kept for 6-8 days
		Hiring	Sliced shoots kept in bamboo cylinders and covered with leaves, 7 days
		Hithyi	Sliced bamboo shoots sun dried and stored in bamboo basket
	Adi	Ekung	Sliced bamboo shoots kept in a basket and covered with ekkam leaves, 5-6 days
		Edung	Fermentation in bamboo cylinder
Manipur	Meitei	Soibum	Sliced shoots fermented in a clay pot or in bamboo basket
Meghalaya	Khasi	Lung Siej	Fermentation in plastic/glass bottles
Nagaland	Aao	Eishie	Fermentation in plastic/glass bottles
Mizoram	Mizo	Mautuai	Fermentation in plastic/glass bottles

Table 3. Comparison of macro-nutrients and mineral elements in bamboo species and some other common vegetables.

Bamboo species and common vegetables		Macronutrients (mg/100g)								Mineral elements (mg/100g)						
Common names	Scientific names	Proteins	Amino Acids	Carbo hydrates	Starch	Fat	Fiber	Vit C	Vit E	Ca	P	K	Fe	Mn	Mg	Na
Lam Saneibi	<i>Bambusa bambos</i>	3.57	3.98	5.42	0.25	0.50	1.90	1.90	0.61	0.36	30.12	576	2.99	0.47	5.38	10.06
Saneibi	<i>Bambusa tulda</i>	3.69	3.65	6.92	0.59	0.48	3.97	1.92	0.61	4.06	19.31	408	3.19	0.7	8.68	12.96
Wanap	<i>Dendrocalamus hamiltonii</i>	3.72	3.18	5.50	0.47	0.41	3.90	2.45	0.71	3.0	28.12	416	2.69	0.16	6.09	9.32
Amaranth	<i>Amaranth gangeticus</i>	4.0	1.3	6.1	--	0.5	1.0	1.0	43.3	397	247	31	1.8	0.36	55	20
Cauliflower	<i>Brassica oleracea var. botrytis</i>	5.9	0.4	7.6	--	0.4	2.0	2.5	46.4	33	57	303	1.23	0.2	15	30
Cabbage	<i>Brassica oleracea var. capitata</i>	1.8	0.3	5.6	--	0.1	1.0	2.6	32.2	47	23	246	0.6	0.18	18	18
Carrot	<i>Daucus carota</i>	0.9	0.2	10.6	--	0.2	1.2	1.2	3.0	80	530	108	1.03	0.16	18	35.6
Radish	<i>Raphnus sativus</i>	0.7	0.1	3.4	--	0.1	0.6	1.6	15.0	35	20	393	1	0.22	10	39
Spinach	<i>Spinacea oleracea</i>	2.0	0.3	2.9	--	0.7	0.2	0.6	28.1	99	49	558	2.7	0.9	79	79
Potato	<i>Solanum tuberosum</i>	1.6	0.2	22.6	15.4	0.1	0.4	0.4	19.7	12	58	421	0.8	0.2	23	11

Ladies finger	<i>Abelmoschus esculantus</i>	1.9	0.3	6.4	--	0.2	1.2	1.2	13.0	66	56	103	0.35	0.19	11	6.9
Tibda	<i>Citrullus vulgaris</i>	1.4	0.2	3.4	--	0.2	1.2	1.0	--	--	--	24	0.9	--	--	35
Cucumber	<i>Cucumis sativus</i>	0.6	0.1	2.5	0.1	0.1	0.4	0.7	3.2	14	25	136	0.9	0.14	12	2
Pumpkin	<i>Cucurbita maxima</i>	1.4	0.2	6.5	--	0.4	1.1	0.7	9.0	21	44	340	0.8	0.1	12	5.6
Bottle gourd	<i>Lagenaria siceraria</i>	0.6	0.4	3.4	--	0.1	0.6	0.6	12.0	26	13	150	0.7	0.1	11	2
Tori	<i>Luffa acutangula</i>	1.2	0.1	3.4	--	0.1	3.3	0.5	5.5	18	26	160	0.46	0.07	14	2.9
French beans	<i>Phaseolus vulgaris</i>	18.8	0.3	20.1	--	2.0	1.8	4.6	--	186	304	1316	3.4	1.2	188	18
Brinjal	<i>Solanum melongena</i>	1.4	0.2	4.0	--	0.3	1.3	1.3	12.0	18	47	200	0.9	0.13	10	3

Bamboo shoots and food security in the region

It is estimated that 1.2 billion people in the world do not have enough food to meet their daily requirements and a further 2 billion people are deficient in one or more micronutrients especially in the developing countries (Kotecha 2008). In India, the situation is worst as according to World Bank Report of 2005 the prevalence of underweight children in India is amongst the highest in the world and is double that of sub Saharan Africa. North east is abundant in nutritious crops like buckwheat, flax, taro, local rice, bamboo shoots and many wild pulses which are being neglected. Many of the rural households use traditional underutilized crops to meet their needs but these crops are being displaced due to pressure from imported species, demography and changing food habits. In addition, loss of agrobiodiversity and farmers dependence on a few highly selective crops resulting in narrow food baskets, have also caused food and nutrition insecurity and poverty to rural and urban communities. Bamboo shoot, like many other local crops, is a neglected crop in the region due to lack of a coherent strategy for evaluation and development. This commodity resource, if properly utilized, can help meet the increasing demand for food and nutrition, energy, medicine and industrial needs. Bamboo grows naturally or is cultivated in homesteads and farms, and is one of the underestimated natural resources in the national and international scenario. Consumption of bamboo shoots as food is now mainly restricted to rural areas particularly among the poor people. Urban people do not prefer it due to reasons like lack of easy availability in the vegetable market, difficult to clean and get the soft edible part from the harvested shoots, short shelf-life, unpleasant smell due to presence of cyanogenic glucoside and homogentisic acid (HGA). Moreover, people in cities prefer ready to cook packs which is at present totally lacking for bamboo shoots of the region.

Bamboo shoot is such a food commodity in the region which is available at least for five to six months (June to October) in the region and further it can be preserved by simple method of fermentation and storage for more than one year. At present bamboo shoots grow naturally without any ecological disturbance on all type of soil and climatic conditions. Shoots are harvested from jungle, peeled of sheaths and sold in the local market in very low price than other vegetables. All common vegetables available in the market are one or two times costlier than bamboo shoots (Table 4). Bamboo shoot is the solution for food security as well as for nutritive food for the region.

Table 4. Market cost of bamboo shoots and other vegetables in Shillong (one of the important cities of NE India and capital of Meghalaya state)

Bamboo species and common vegetables		Price of 1kg in Indian Rupees (Oct., 2011)
Common names	Scientific names	
Bamboo shoots (fresh)	<i>Dendrocalamus hamiltonii</i>	20
Bamboo shoots (fermented)	<i>Dendrocalamus hamiltonii</i>	25
Cauliflower	<i>Brassica oleracea</i> var. <i>botrytis</i>	40
Cabbage	<i>Brassica oleracea</i> var. <i>capitata</i>	40
Carrot	<i>Daucus carota</i>	40
Radish	<i>Raphanus sativus</i>	20
Potato	<i>Solanum tuberosum</i>	20
Cucumber	<i>Cucumis sativus</i>	20
Tori	<i>Luffa acutangula</i>	30
French beans	<i>Phaseolus vulgaris</i>	80
Brinjal	<i>Solanum melongena</i>	30
Pea	<i>Pisum sativum</i>	80
Onion	<i>Allium sativa</i>	25
Tomato	<i>Lycopersicon esculentum</i>	30
Turnip	<i>Brassica rapa</i>	20
Beetroot	<i>Beta vulgaris</i>	70
Ladies finger	<i>Abelmoschus esculentus</i>	40

Table 5. Bamboo shoot harvest in North-East region of India.

States of North-East India	Number of species (Number of main edible species)	Annual Shoot Harvest (tonnes)	Annual Income From Bamboo Shoots (in Million Rupees)
Arunachal Pradesh	42 (5)	1979	11.95
Assam	29	----	----
Manipur	53 (8)	2188	8.56
Meghalaya	35	442	1.97
Mizoram	25	433	1.31
Nagaland	22 (5)	442	2.56
Sikkim	26	----	----
Tripura	10-15 (6)	201	1.61
		5685	27.96

Bamboo shoot for income generation and community development in the region

At present, over two million tonnes of bamboo shoots are consumed in the world annually (Yang *et al.* 2008). In the international market, China earns 6500 million Indian rupees annually from export of edible shoots, with USA importing around 44,000 tonnes accounting for 14.5% of the total world import. USA imports 30,000 tonnes of canned bamboo shoots from Taiwan, Thailand and China (Daphne 1996). However, though India is the second largest resource of bamboos after China, it has not been able to figure in the international market as steps have not been taken to exploit the immense potential of this valuable resource. At present, bamboo shoot is mainly a vegetable of rural people and marketed at local level only with average harvest of only 5685 tonnes (Table 5). Except three small units, there are no big factory/industry in the region based on bamboo shoots as present in China, Thailand and Taiwan. The bamboo shoot processing units are located at Dimapur, Nagaland (900 tonnes/year) and Jorhat, (200 tonnes/year) and and Bongaigown, Assam (300 tones/year) and one in Aizwal, Mizoram (Nimachow *et al.* 2010). Whereas in China there are around 700 factories involved in canning and export of bamboo shoots to the tune of 2,50,000 tonnes annually. Similarly in Prachinburi Province of Thailand there are 25 bamboo shoot canning industries and Thailand earns around US \$ 31 million from bamboo shoot harvest (Thamincha 1988). Statistics show that about 26.2, 435 and 426.8 tonnes of bamboo shoots are harvested annually in the Northeastern states of India like Sikkim, Meghalaya and Mizoram respectively (Bhatt *et al.* 2003, 2005). India's size of domestic economy is currently estimated at 200 million rupees. The market potential of bamboo is estimated at 450 million Indian rupees which will increase to 26,000 million rupees by 2015, thus enabling five million families crossing the poverty line (Farooquee *et al.* 2007)

Bamboo shoots are becoming a popular food item globally mainly due to its nutritive value and health enhancing properties. There is a growing demand for processed and packaged bamboo shoots in the national and international market as the shelf life of freshly harvested shoots is only 2-3 days. In India,

there is hardly any organized bamboo shoot processing and marketing industry to even cater to the needs of the domestic market mainly the restaurants and high-end hotels serving oriental dishes. North-East region of India with a total bamboo cover of 24,110 sq km and standing stock of 42.74 million tones has great opportunity to develop bamboo shoot canning and processing industry in the region to meet the growing demands both at home and outside the country. Presently, the world market for bamboo shoots is of around US \$ 10 billion which will be doubled in 2015. Bamboo shoot being reported as functional and nutritive food is becoming very popular within India as well as in other parts of the world. Taking into account the high potential of income generation from bamboo shoots, a scientific and systematic developmental program for utilizing the vast resource needs to be taken up to uplift the socio-economic status of the underprivileged weaker section of society and generate significant employment opportunities for the people of the North-East.

References

- Akao, Y.; Seki, N.; Nakagawa, Y.; Yi, H.; Matusumoto, K.; Ito, Y.; Ito, K.; Funaoka, M.; Maruyama, W.; Naoi, M.; Nozawa, Y. 2004. A highly bioactive lignophenol derivative from bamboo lignin exhibit a potent activity to suppress apoptosis induced by oxidative stress in human neuroblastoma SH-SY5Y cells. *Bio and Med Chem*, 12, 4791-4801.
- Bhatt, B.P.; Singha, L.B.; Sachan, M.S.; Singh, K. 2005. Commercial edible bamboo species of the North-Eastern Himalayan region, India. Part II: fermented, roasted, and boiled bamboo shoots sales. *J. of Bamboo and Rattan*, 4(1), 13-31.
- Bhatt, B.P.; Singha, L.B.; Singh, K.; Sachan, M.S. 2003. Some commercial edible bamboo species of North-East India: Production, Indigenous uses, Cost- benefit and Management Strategies, *J. Am. Bamboo Soc.*, 17, 4-20.
- Daphne, L. 1996. Bamboo shoots: delicious to eat, easy to sell. *Washington Tith*, Autumn. 7-9.
- Farooque, N.A.; Dollo, M.; Kala, C.P. 2007. Traditional wisdom of Apatani Community in the management and sharing of natural resources in North Eastern India. In: Mishra KK (ed) *Traditional knowledge in contemporary societies: challenges and opportunities*. Pratibha Prakashan, Delhi. pp 110-126.
- Kotecha, P.V. 2008. Micronutrient malnutrition in India. Let us say “no” to it now. *Journal Community Medicine*, 33, 9-10.
- Lobovikov, M. 2003. Bamboo and rattan products and trade. *J Bamboo Rattan* 2(4), 397-406.
- Madhab, J. 2003. The Green Gold: Under Exploited Wealth of the North-East India. *Dialogue*, 5 (2), 45-52.
- Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; da Fonseca, G.A.B.; Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858.
- Naithani, B.B. 2008. Diversity of Indian bamboos with special reference to North-East India. *The Indian Forester*, 134(6), 765-788.
- Nimachow, G.; Rawat, J. S.; Dai, Y. 2010. Prospects of bamboo shoot processing in North-East India. *Curr. Sci*, 98, 288.
- Nirmala, C.; Bisht, M.S.; Sheena, H. 2011. Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews Food Science Food Safety*, 10, 153-165.
- Thammincha, S. 1988. Some aspects of bamboo production and marketing. In: *Bamboo: Current Research*, (eds. Rao, I.V.R., Gnanaharan, R. and Sastry, C.B.), pp. 320-326. Proceedings of the International Bamboo Workshop, Cochin, 14-18, November, 1988, KFRI and IDRI, Canada.
- Yang, Q.; Duan Z.; Wang, Z.; He, K.; Sun, Q.; Peng, Z. 2008. Bamboo resources, utilization and ex-situ conservation in Xishuangbanna, South-eastern China. *J Forest Resource*, 19, 79-83.

Bamboo shoots as a functional food

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Abstract

With increasing health consciousness globally, consumer demands for functional food have increased significantly in the last decade. Functional foods are foods that influence specific functions in the body and thereby offer benefits for health, well-being or performance beyond their regular nutritional value. Though health benefits of oats, flax, soybeans and many other vegetables are well known, the concept of bamboo shoots as a health food was unknown until recently. Bamboo shoots, a popular ingredient of South Asian cuisines, are available in fresh, frozen, fermented and canned forms. Juvenile bamboo shoots in addition to being rich in nutrient components have bioactive compounds such as phenolics, phytosterols and dietary fibres that have health enhancing properties and reduce the risk of developing certain chronic diseases. Phenolic acids have mild anti-inflammatory properties and are potent antioxidants. The shoots are a good source of phytosterols that have cholesterol lowering activity as they inhibit the absorption of dietary cholesterol in the intestines. Dietary fibre is associated with a number of health benefits including reducing the time of release of ingested food in colon, reduced exposure to carcinogens and bowel protection. Bamboo shoots being highly nutritious, rich in bioactive compounds, free from residual toxicity as they grow without the application of fertilizers and cheap compared to normal vegetables can be used as a functional food.

Keywords

Bamboo shoots, nutrient components, bioactive compounds.

Introduction

The importance of bamboo as an eco-friendly raw material capable of meeting multifarious needs of the people at large is gaining global acceptance. From its all-pervading dominance in the agrarian economies of the East during the middle ages and the subsequent oblivion in the 19th and 20th centuries, bamboo is once again emerging as a much sought after material in the hi-tech world of the 21st century (Sastry 2008). From a raw material known as the "*poor man's timber*" bamboo is currently being elevated to the status of "the timber of the 21st century". So varied is bamboo's application that one finds its utilization on a massive scale today in environment protection, as a nutrient food, high-value construction material and also in about 1,500 other listed applications. Though extensively used for industrial purposes, the use of young bamboo shoots as food is only confined to some countries, where they constitute a part of daily food ingredient. A lesser known fact is the health benefits of bamboo shoots (Nirmala et al. 2011). Health and "healing foods have a long history in many cultures, where food and medicine are considered to be equally important in preventing and curing diseases. It is mainly the advances in understanding the relationship between nutrition and health that resulted in the development of the concept of functional foods which in simple terms can be defined as foods that may provide health benefits beyond basic nutrition. Functional foods, also known as nutraceuticals or medical foods are driving food markets around the world and are expected to be one of the emerging trends for the food industry in the new millennium. Consumption of functional foods provides a practical and new approach to achieve optimal health status by promoting the state of well-being and possibly reducing the risk of disease. In the last two decades, consumer demands in the field of food production have changed considerably as they have become more conscious and believe that foods contribute directly to their health. Today, foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition related diseases and improve physical and mental well-being of the consumers (Menrad 2003). The increasing demand on such foods can be explained by the increasing cost of health care, the steady increase in life expectancy and the desire of older people for improved quality of their later years (Roberfroid 2000; Kotilainen 2006). Bamboo shoots have been regarded as a traditional Chinese medicine for more than two hundred years when it was proclaimed that they were beneficial to human health. This has been authenticated by modern research wherein, their medical benefits have been revealed. Consumption of bamboo shoots is mainly concentrated in Southeast Asia, mainly China, Japan, Thailand and Taiwan where they are a popular ingredient in the local cuisine. China has the largest bamboo industry producing approximately 1.3 million metric tons of fresh bamboo. Worldwide, more than 2 million tons of bamboo shoots are consumed annually of which about 1.3 million tons are produced in China alone (Xuhe 2003; Kleinhenz et al 2000). They are loved mainly for their crisp and crunchy quality and rich aroma. Available in canned, frozen and fermented forms throughout the year, bamboo shoots are available fresh only in the months of March and April as it is a vegetable of the spring. Though canned and preserved shoots dominate the international market, due to increased consumer consciousness for health leading to demand for non-processed food, fresh shoots are preferred as they are more nutritious and have far better flavour and texture. They are mostly used in making appetizing soups, delicious snacks, hot curries, spicy stir-fries, attractive salads, pickles, aromatic fried rice, spring rolls, and other stewed and fried dishes. The shoots are not only used as vegetables but are also processed and preserved in many forms such as dried, fermented, salted, pickled, water soaked, and canned. The present paper elucidates the nutritional value of bamboo shoots, its health benefits and potential of being used as a functional food.

Nutritive value and health beneficial properties of bamboo shoots

The criterion for healthy nutrition, in so far as food intake is concerned, constantly evolves with an understanding of food-health relationship. Recently, the research and exploration of bamboo as a food commodity has witness tremendous activity. Due to this, major advances have been made in fresh shoot production and processing and analysis of nutrient component of edible shoots. In addition to having high nutritive value, the shoots contain bioactive compounds that provide us health benefits.

Nutrient components

Based on nutritional analyses, bamboo shoots are good source of food energy and have been projected as a new health food in the Indian sub-continent (Nirmala et al. 2011). In addition to being delicious, bamboo shoots are rich in some nutrient components, mainly proteins, carbohydrates, and minerals but have a low fat content. Bamboo shoots also contain phytosterols and a high amount of fiber that have cholesterol-lowering and anticarcinogenic activity and therefore could be called nutraceuticals or natural medicines. The shoots are free from residual toxicity as they grow without the application of hazardous fertilizers or pesticides. The nutritive values of ten bamboo species have been analysed. The fresh shoots have a high content of carbohydrates, proteins, starch, amino acids and fiber but are low in fat (Table 1). Carbohydrate content ranges from 4.9g to 6.17g/100g fresh weight of which the maximum is found in *Bambusa tulda*. *Dendrocalamus hamiltonii* which is a commercially popular species, has the highest protein content of 3.71g/100g fresh weight. Shoots of *B. bambos*, *B. tulda* and *D. hamiltonii* have good flavor, are tender and very tasty and most popular being liked by all consumers. Major mineral elements present in the shoots include calcium, phosphorous, magnesium, potassium, sodium and zinc (Table 2). Comparison of bamboo shoots with vegetables has revealed that some species have higher nutrient content than the commonly used vegetables (Nirmala et al. 2011). Dietary fiber content of *B. tulda* and *D. hamiltonii* is higher than that present in *Luffa acutangula*, *Solanum tuberosum* and *Cucumis sativus*. The potassium content in both the bamboo species is also higher than cauliflower, cabbage, carrot and radish. Nutrient composition of bamboo shoot varies among species. Such changes also depend on the age of shoot and the harvesting time.

Bamboo shoots are not only delicious and nutritious but have certain health benefits. They are diathermic, detoxificant and diuretic. The tender shoots are low in calories, one cup of half-inch long slices containing a mere 14 calories, half a gram of fat and 2.5g of fibre. Fibre helps to keep cholesterol level in check and plays an important role in preventing colon cancer. Lignans, an important component of fibre, are reported to have anticancer, antibacterial, antifungal and antiviral activity. Bamboo shoots are labelled as a heart protective vegetable because of its high content of potassium, that helps to maintain normal blood pressure and a steady heart beat. High content of up to 40% cellulose increases the movement of the intestines, helping digestion and prevents the accumulation of sebaceous matter. It also prevents constipation, colon cancer and decreases body fat. To eat fresh bamboo slices for several days is good for fever and phlegm (Shi and Yang 1992). Phenolic acids present in the tender shoots have mild anti-inflammatory properties and are potent antioxidants. Antioxidants prevent cancer and the blood vessel injury that can start atherosclerosis. Another very important constituent of bamboo shoots are the phytosterols which act as nutraceuticals. Bamboo shoots, both fresh and fermented, are a good source of phytosterols which are the precursor of many pharmaceutically active steroids found in plants

Table 1. Macronutrients (g/100 g fresh weight), vitamin C, vitamin E (mg/100 g fresh weight), moisture, dietary fiber and ash content in the freshly emerged juvenile shoots of various species.

Name of species	<i>B. bambos</i>	<i>B. kingiana</i>	<i>B. nutans</i>	<i>B. tulda</i>	<i>B. vulgaris</i>	<i>D. asper</i>	<i>D. brandisii</i>	<i>D. giganteus</i>	<i>D. hamiltonii</i>	<i>D.membr anaceus</i>
Proteins	3.57 ±0.03	3.57 ±0.08	2.84 ±0.12	3.69 ±0.03	3.64 ±0.03	3.59 ±0.06	2.31 ±0.12	3.11 ±0.17	3.72 ±0.12	3.38 ±0.10
Carbhy -drates	5.42 ±0.02	5.45 ±0.12	5.47 ±0.05	6.92 ±0.04	6.51 ±0.05	4.90 ±0.11	4.90 ±0.10	5.10 ±0.04	5.50 ±0.08	5.40 ±0.03
Starch	0.254 ± 0.042	0.337 ±0.030	0.398 ±0.02	0.591 ±0.12	0.273 ±0.052	0.359 ±0.08	0.490 ±0.042	0.506 ±0.061	0.469 ±0.031	0.229 ±0.040
Fat	0.50 ±0.02	0.35 ±0.03	0.40 ±0.02	0.48 ±0.07	0.50 ±0.01	0.40 ±0.06	0.24 ±0.10	0.39 ±0.03	0.41 ±0.02	0.43 ±0.05
Vitamin C	1.90 ±0.08	2.10 ±0.12	1.19 ±0.10	1.42 ±0.06	4.80 ±0.11	3.20 ±0.06	1.59 ±0.10	3.28 ±0.02	2.45 ±0.08	1.58 ±0.06
Vitamin E	0.61 ±0.05	0.50 ±0.10	0.47 ±0.06	0.61 ±0.14	0.52 ±0.10	0.91 ±0.13	0.42 ±0.10	0.69 ±0.03	0.71 ±0.03	0.65 ±0.10
Moistur e	89.83 ±0.08	90.00 ±1.02	92.00 ±0.23	83.60 ±1.26	90.60 ±0.82	89.40 ±0.98	89.80 ±0.15	90.70 ±0.12	92.51 ±0.51	89.30 ±1.34
Dietary Fiber	3.54 ±0.02	4.490 ±0.06	2.28 ±0.01	3.97 ±0.02	4.24 ±0.01	3.54 ±0.07	4.03 ±0.09	2.65 ±0.03	3.90 ±0.03	2.91 ±0.06

Table 2. Mineral element contents in the juvenile shoots of various species (mg/100 g fresh weight).

Species	<i>B. bambos</i>	<i>B. kingiana</i>	<i>B. nutans</i>	<i>B. tulda</i>	<i>B. vulgaris</i>	<i>D. asper</i>	<i>D. brandsii</i>	<i>D. giganteus</i>	<i>D. hamiltonii</i>	<i>D. membranaceus</i>
Minerals										
Ca	0.358 ± 0.020	0.566 ± 0.003	3.390 ± 0.001	4.064 ± 0.002	1.356 ± 0.002	5.506 ± 0.001	4.905 ± 0.001	6.802 ± 0.001	3.004 ± 0.021	2.722 ± 0.001
Cu	0.28 ± 0.015	0.50 ± 0.010	0.36 ± 0.006	0.44 ± 0.012	0.38 ± 0.017	0.32 ± 0.020	0.24 ± 0.023	0.56 ± 0.006	0.29 ± 0.010	0.16 ± 0.020
Fe	2.990 ± 0.002	2.398 ± 0.001	2.453 ± 0.004	3.185 ± 0.003	2.234 ± 0.002	3.366 ± 0.020	3.348 ± 0.002	2.433 ± 0.002	2.693 ± 0.003	3.317 ± 0.004
Mg	5.380 ± 0.020	6.170 ± 0.001	8.10 ± 0.020	8.676 ± 0.003	7.30 ± 0.006	10.14 ± 0.006	9.82 ± 0.012	10.09 ± 0.21	6.912 ± 0.001	5.834 ± 0.003
Mn	0.472 ± 0.012	0.456 ± 0.003	0.454 ± 0.002	0.696 ± 0.003	0.298 ± 0.003	0.414 ± 0.002	0.320 ± 0.001	0.342 ± 0.002	0.160 ± 0.003	0.878 ± 0.001
K	576 ± 1.154	336 ± 0.577	384 ± 1.155	408 ± 2.309	248 ± 1.155	464 ± 0.576	290 ± 0.577	288 ± 1.155	416 ± 0.577	232 ± 1.154
P	30.12 ± 0.030	25.09 ± 0.006	24.00 ± 0.006	19.31 ± 0.017	20.90 ± 0.012	40.95 ± 0.006	22.09 ± 0.030	15.90 ± 0.006	28.12 ± 0.021	26.95 ± 0.030
Se	0.0003 ± 0.0001	0.0002 ± 0.0001	0.0001 ± 0.0001	0.0004 ± 0.0001	0.0004 ± 0.0001	0.0010 ± 0.0001	0.0003 ± 0.0001	0.0005 ± 0.0001	0.0008 ± 0.0001	0.0004 ± 0.0001
Na	10.06 ± 0.006	15.60 ± 0.006	12.26 ± 0.030	12.96 ± 0.006	9.76 ± 0.010	10.14 ± 0.004	8.00 ± 0.010	8.22 ± 0.012	9.32 ± 0.020	10.10 ± 0.020
Zn	0.574 ± 0.001	1.018 ± 0.002	0.694 ± 0.002	0.716 ± 0.003	0.908 ± 0.003	0.852 ± 0.012	0.950 ± 0.001	1.086 ± 0.002	0.696 ± 0.001	0.722 ± 0.001

Bioactive compounds

Bioactive compounds are essential and nonessential compounds (e.g., vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human health (Beisalski et al. 2009). These substances are present as natural constituents in foods at low levels and provide health benefits beyond the basic nutritional value of the product. There is increasing scientific evidence indicating that consumption of certain foods rich in bioactive compounds may lead to reduced risk of developing certain chronic diseases like cardiovascular diseases, cancer and other age related conditions as they can influence metabolism and interact with disease precursors and developing mechanisms. Bioactive compounds found in food include flavonoids, phenolic acids, lignans, saponins, stilbenes, carotenoids and plant sterols. Bamboo shoots contain lignans, a component of fiber, phenolic acids and phytosterols.

Phenolics

Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. They are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways, are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens (Randhir et al. 2004). They are a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic hydrocarbon group. These compounds are one of the most widely occurring groups of phytochemicals, and are of considerable physiological and morphological importance in plants exhibiting a wide range of physiological properties such as anti-allergic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia et al. 1997, Manach et al. 2005, Middleton et al. 2000). The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years.

Table 3. Phenolic content in fresh and processed shoots of some bamboos.

Bamboo species	Fresh	After 15 min boiling	After 30 min boiling	After 60 min boiling
<i>Dendrocalamus asper</i>	554.0	92.0	85.0	80.0
<i>D.giganteus</i>	346.0	240.0	180.5	116.4
<i>D.membranaceus</i>	482.8	317.6	193.2	118.7
<i>D.hamiltonii</i>	508.0	160.0	87.3	82.0
<i>Bambusa balcooa</i>	189.6	60.9	58.9	54.1
<i>B. tulda</i>	450.0	221.9	120.4	114.5

Phenolic acids have mild anti-inflammatory properties and are potent antioxidants. Antioxidants help prevent cancer and the blood vessel injury that can start atherosclerosis. Most of the research studies have focussed on the functional activities of bamboo leaves and stems and there are only few reports on the functional properties of bamboo shoots. Park and Jhon (2010) identified eight phenolic acids viz. protocatechuic acid, p-hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid, syringic acid, p-coumaric acid and ferulic acid from powdered bamboo shoots of two species, *Phyllostachys pubescens* and *P. nigra*. Phenolic content in *Bambusa tulda*, *B. balcooa*, *Dendrocalamus asper*, *D. giganteus*, *D. hamiltonii*, and *D. membranaceus* was determined in both fresh and processed shoots (Table. 3). Though *D.asper* had the maximum phenolic content in the fresh shoots, it reduced drastically after processing. In *B. tulda*, *D giganteus* and *D membranaceus*, the phenolic content decreased to a lesser extent and would thus be more suitable for canning purposes than *D. asper*.

Phytosterols

Phytosterols exist as naturally occurring plant sterols that are present in the nonsaponifiable fraction of plant oils. They are plant components that have a chemical structure similar to cholesterol except for the addition of an extra methyl or ethyl group. These compounds possess a double bond at carbon-5, which can be saturated by enzymatic hydrogenation in plants or during food processing to form plant stanols. Most commonly found phytosterols are β -sitosterol, campesterol, stigmasterol, saturated phytosterol such as sitostanol and campestanol. Rich sources of phytosterols include grain legumes such as sesame, chickpea, lentils, peas, wheat, corn, millet, rye and barley. Since bamboo shoot is not a common food, the presence of phytosterols in the juvenile shoots has not been highlighted until recently. Phytosterol consumption reduces intestinal cholesterol absorption, leading to decreased blood LDL-cholesterol levels and lowered cardiovascular disease risk. In addition to their cholesterol-lowering actions, there are evidences that suggest that phytosterols possess anti-cancer effects against cancer of the lung, stomach, ovary and estrogen-dependent human breast cancer (Mendilaharsu et al. 1998; De Stefani et al. 2000; Ju et al. 2003; McCann et al. 2004; Bradford and Awad 2007; Woyengo et al. 2009). Predominant phytosterols in bamboo shoots are β -sitosterol, campesterol and stigmasterol (He and Lachance 1998). The total sterol content in bamboo shoot ranges from 112.4 to 279 mg/100g dry weight in four species *Phyllostachys pubescens*, *P. praecox*, *Pleioblastus amarus* and *Dendrocalamus latiflorus*). Fermentation of the fresh shoots led to increase in the phytosterol content probably due to anaerobic digestion of microorganisms that caused degradation of organic matter resulting to enrichment of phytosterols. Bacteria leading to the fermentation of the shoots have been isolated from *Schizostachyum capitatum* (Jeyram et al. 2010)

Table 4. Dietary fiber (NDF) and its components in the freshly emerged juvenile (jv) and 10 days old emerged (old) shoots of the five species (g/100g fresh weight).

Sl No.	Names of species	NDF		ADF		Lignin		Hemi-cellulose		Cellulose	
		jv	old	jv	old	jv	old	jv	old	jv	old
1.	<i>B.bambos</i>	3.535 ±0.015	9.640 ±0.105	2.810 ±0.010	7.560 ±0.092	1.460 ±0.030	4.802 ±0.041	0.725 ±0.005	2.080 ±0.013	1.350 ±0.001	2.758 ±0.051
2.	<i>B.tulda</i>	3.970 ±0.0020	10.690 ±0.102	3.360 ±0.031	8.206 ±0.089	2.300 ±0.010	5.542 ±0.056	0.609 ±0.989	2.484 ±0.013	1.060 ±0.021	2.754 ±0.033
3.	<i>D.asper</i>	3.540 ±0.065	10.850 ±0.023	3.000 ±0.014	8.270 ±0.010	1.260 ±0.010	5.680 ±0.008	0.475 ±0.054	2.580 ±0.013	1.740 ±0.004	2.690 ±0.002
4.	<i>D.giganteus</i>	2.645 ±0.025	13.840 ±0.041	2.150 ±0.009	9.520 ±0.021	0.560 ±0.010	6.320 ±0.014	0.495 ±0.016	4.320 ±0.020	1.589 ±0.999	3.200 ±0.007
5.	<i>D.hamiltonii</i>	3.900 ±0.030	8.200 ±0.020	3.230 ±0.026	6.105 ±0.012	2.170 ±0.017	3.890 ±0.009	0.670 ±0.004	2.095 ±0.008	1.060 ±0.009	2.095 ±0.003

Dietary fibres

Dietary fiber consists of remnants of edible plant cells, polysaccharides, lignin and associated substances resistant to digestion by the alimentary enzymes of humans. It has been one of the most enduring dietary interests worldwide and has been a topic of immense medical research and is recommended as part of the treatment and prevention of a number of diseases. The benefits of dietary fiber are mostly related with gastrointestinal function as it reduces the time of release of ingested food in colon. Living on a diet rich in fibre can help reduce cholesterol, regulates blood sugar, cancers and obesity, checks constipation, colitis and colon cancer and even haemorrhoids. Dietary fiber consists of two main components, soluble fiber that is readily fermented in the colon into gases and physiologically active byproducts, and insoluble fiber that is metabolically inert, absorbing water as it moves through the digestive system, easing defecation.

Bamboo shoots are a rich source of dietary fiber, the amount ranging from 2.23-4.20 g/100g fresh weight of shoot (Nirmala et al. 2009) which increases with age (Table 4). The fiber in bamboo can be classified as Nutrient Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). The former is the indigestible component consisting of hemicelluloses, cellulose and lignin and the later, primarily representing cellulose and lignin. The dietary fiber content varies even within the same species and the way the shoots are preserved. Generally, the content increases with age and processing like fermentation and canning. Dietary fiber content in fresh, fermented and canned shoots of *D. giganteus* was estimated (Nirmala et al. 2008). Whereas, the fermented shoots have a lesser amount of ADF, the canned as well as the fresh shoots have nearly equal amount of ADF. Lignin content in both the fresh and canned shoots was less than the fermented shoots. The fermented shoots have higher amounts of cellulose than the fresh shoots while the canned shoots have lower amount of cellulose than both the fresh and fermented shoot.

Conclusion

A large number of scientific evidence supports the observation that functional foods containing physiologically-active compounds, either from plant or animal sources, may enhance health. There is a new global awareness of the concept of functional foods beyond simple nutrition both in developed and developing countries as people have become more health conscious. Health benefits provided by cruciferous vegetables, citrus fruits, soybean, garlic, oats, flax seeds, tomato, fish etc. are well known. However, until recently, the health benefits of bamboo shoots were not known, as they are not consumed worldwide being concentrated mostly in the in the South Asian countries. Despite the fact that bamboo shoots are more nutritious and cheaper than some commonly used vegetables and have health enhancing properties, it still remains as a neglected plant. It is estimated that 1.2 billion people in the world do not have enough food to meet their daily requirements and a further 2 billion people are deficient in one or more micro-nutrients mostly in the developing countries. Bamboo with significant food and/or industrial potential remains underutilized through lack of a coherent strategy for their evaluation and development. If properly utilized, this enormous untapped commodity resource can help to meet the increasing demand for food and nutrition mainly in the rural areas.

References

- Benavente-Garcia, O.; Castillo, J.; Marin, F.R.; Ortuno, A.; Del Rio, J.A. 1997. Uses and properties of citrus flavonoids. *Journal of Agriculture and Food Chemistry*, 45, 4505-4515.
- Biesalski, H.K.; Dragsted, .LO.; Elmadfa, I.; Grossklaus, R.; Muller, M.; Schrenk, D.; Walter, P.; Weber, P. 2009. Bioactive compounds: Definition and assessment of activity. *Nutrition*, 25(11-12), 1202-1205.
- Bradford, P.G.; Awad, A.B. 2007. Phytosterols as anticancer compounds. *Molecular and Nutritional Food Research*, 51, 161-170.
- De Stefani, E.; Boffetta, P.; Roncho, A.L.; Brennan, P.; Deneo-Pellegrini, H.; Carzoglio, J.C. 2000. Plant sterols and risk of stomach cancer: A case control study in Uruguay. *Nutrition and Cancer*, 37, 140-144.
- He, Y.H.; Lachance, P.A. 1998. Effects of dietary bamboo shoot on fecal neutral sterols and bile acid excretion in the rat. *FASEB J*, 12(4), 210.
- Jeyaram, K.; Romi, W.; Singh, A.; Devi, A.R.; Devi, S.R. 2010. Bacterial species associated with traditional starter cultures used for fermented bamboo shoot production in Manipur state of India. *International Journal of Food Microbiology*, 143,1-8.
- Ju, Y.H.; Clausen, L.M.; Allred, K.F.; Almada, A.L.; Helferich, W.G. 2004. β -sitosterol, β -sitosterol glucoside and a mixture of β -sitosterol and β -sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells in-vitro and ovariectomized athymic mice. *Journal of Nutrition*, 134, 1145-1151.
- Kleinhenz, V.; Gosbee, M.; Elsmore, S.; Lyall, T.W.; Blackburn, K.; Harrower, K.; Midmore, D.J. 2000. Storage methods for extending the self-life of fresh bamboo shoots [*Bambusa oldhamii* (Munro)]. *Postharvest Biology and Technology*, 19, 253-264.
- Kotilainen, L.; Rajalahti, R.; Ragasa, C.; Pehu, E. 2006. Health enhancing foods: Oportunities for strengthening the sector in developing countries. *Agriculture and Rural Development Discussion Paper* 30.
- Manach, C.; Mazur, A.; Scalbert, A. 2005. Polyphenols and prevention of cardiovascular diseases. *Current Opinions in Lipidology*, 16, 77-84.
- McCann, S.E.; Freudenheim, J.L.; Marshall, J.R.; Graham, S. 2003. Risk of human ovarian cancer is related to dietary uptake of selected nutrients, phytochemicals and food groups. *Journal of Nutrition*, 133, 1937-1942.
- Mendilaharsu, M.; Stefani, ED.; Deneo-Pellegrini, H.; Carzoglio, J.; Roncho, A. 1998. Phytosterols and risk of lung cancer: A case-control study in Uruguay. *Lung Cancer* 21(1):37-45.
- Menrad, K. 2003. Market and marketing of functional foods in Europe. *Journal of Food Engineering*, 56, 181-188.
- Middleton, E.; Kandaswami, C.; Theoharides, T.C. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacological Reviews*, 52, 673-751.
- Nirmala C, David, E, Sheena H. 2009. Bamboo shoots: A rich source of dietary fibres. In *Deitary fibres, Fruit and Vegetable Consumption and Health* (eds. Klein, F, Moller G). pp 15-30. Nova Science Publisher, USA.
- Nirmala, C.; Bisht, M.S.; Sheena, H. 2011. Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. *Comprehensive Reviews in Food Science and Food Safety* ,10, 153-169.
- Nirmala, C.; Sharma, M.L.; David, E. 2008. A comparative study of nutrient components of freshly emerged, fermented and canned bamboo shoots of *Dendrocalamus giganteus* Munro. *Journal of American Bamboo Society*, 2, 33-39.
- Park, E.J.; Jhon, D. 2010. The antioxidant, angiotensin converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. *Food Science and Technology*, 43, 655-659.
- Randhir, R.; Lin, Y.T.; Shetty, K. 2004. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. *Asia Pacific Journal of Clinical Nutrition*, 13, 295-307.
- Roberfroid, M.B. 2000. Concepts and strategy of functional food science: The European perspective. *American Journal Clinical Nutrition*, 71, S1660-S1664.

- Sastry, C.B. 2008. A 2020 vision for bamboos in India: Opportunities and challenges. Proceedings of International Conference “Improvement of Bamboo Productivity and Marketing for Sustainable Livelihood. 15-17th April. New Delhi, India. pp. 6-15.
- Shi, Q.T.; Yang, K.S. 1992. Study on relationship between nutrients in bamboo shoots and human health. Proceedings of the International Symposium on Industrial Use of Bamboo. International Tropical Timber Organization and Chinese Academy, Beijing, China. Bamboo and its Use; p. 338-346
- Woyengo, T.A.; Ramprasatha, V.R.; Jones, P.J.H. 2009. Anticancer effects of phytosterols. *European Journal of Clinical Nutrition*, 63, 813-820.
- Xuhe, C. 2003. Promotion of bamboo for poverty alleviation and economic development. Proceedings of the International Workshop on Bamboo Industrial Utilization. October 2003, Hubei Provincial Government and Xianning Municipal and INBAR.

Production to Consumption Study on Bamboo in Burundi

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International Network for Bamboo and Rattan

Abstract

Burundi has joined the International Network for Bamboo and Rattan as a member country in 2010. INBAR is exploring with the Burundi Government the options for a close cooperation to develop the bamboo and rattan sectors in Burundi (mostly focus on bamboo in Burundi). This study aims to examine the status of the bamboo sector, the socio-economic relevance and environment and identify constraints, opportunities, and areas for potential intervention. This has been done in close cooperation and coordination with the Burundi focal point of INBAR and other stakeholders in Burundi. The study and the recommendations such as method management improvement and improved utilization of bamboo will provide a focus for developing the bamboo sector in Burundi to improve the livelihoods, generate employment and income through relevant interventions.

This study summarizes the environmental, social and economical findings from the data that were collected in the various ministries, institutions as well as through local surveys. In addition; we have analyzed the various supply chains to understand the bamboo stakeholders' interactions and the impact of bamboo on the local communities.

Keywords

bamboo, development, resources, poverty, markets, livelihood

Acronyms and abbreviations

ABO	Association Burundaise de la protection des oiseaux
AFDB	African Development Bank
AFD	Agence Française de Développement
AIDS	Acquired immune deficiency syndrome
CDM	Clean Development Mechanism
CFA	Centre de Formation d'Apprentis
CIA	Central Intelligence Agency
CIE	Centre d'information environnementale
CNUCED	Conférence des Nations unies sur le commerce et le développement.
CO2	Carbon dioxide
DFID	Department For International Development
DRC	Democratic Republic of Congo
EUA	European Union Allowance
FAO	Food Agriculture Organization
FBU	Franc Burundais
GDP	Gross Domestic Product
GPS	Global Positioning System
GW	Gigawatt
GWh	Gigawatt heure
HDI	Human Development Indicator
HIPC	Initiative for Heavily Indebted Poor Countries
HIV	Human immunodeficiency virus
IDA	International Development Agency
IGEBU	Institut Géographique du Burundi
IMF	International Monetary Fund
INBAR	International Network for Bamboo and Rattan
INECN	Institut National pour l'Environnement et la Conservation de la Nature
ISABU	Institut des Sciences Agronomiques du Burundi
ISTEEBU	Institut de Statistique et d'Etudes Economiques du Burundi
KTOE	Kilo Ton of oil equivalent
MCI	Minister of Commerce and Industry
MEM	Minister of Energy and Mines
MEEATU	Ministre de l'Eau, de l'Environnement, de l'Aménagement du Territoire et de l'Urbanisme
MINIPLAN	Ministère du Plan
NGO	Non-Governmental Organization
ONUDI	Organisation des Nations unies pour le développement industriel
PCS	Production to Consumption Study
Pers	Person
PNUD	Programme des Nations Unies pour le Développement
REGIDESO	Régie de Production et de Distribution d'Eau et d'Electricite
PAS	Structural Adjustment Plan
Y	Year
VAT	Value Added Tax
WB	World Bank

Introduction

Bamboo grows in every country in Africa from the tropical forests down to Cape Town in South Africa. Bamboo is used widely in Africa from commonplace necessities like cattle pens to fuelwood and charcoal to more exotic uses like bamboo wine/beer. Bamboo has traditionally been used for construction, handicrafts, to support tea and banana plantations, and to make sundry household articles. The mountain gorilla populations of Africa include bamboo in their diet, just as pandas and elephants do. Bamboo can also be one of the main sources of fodder for ruminants, rabbits and fish.

Africa's deforestation rate is four times the world's average. Over the past 20 years, illegal logging, charcoal production and agricultural fires have had disastrous impact on the environment leading to soil quality deterioration, depleted underground water table and modification of the microclimate. As a result, local communities cannot rely on traditional biomass resources any longer to ensure a regular source of income and resources.

Burundi has faced a significant deforestation rate since 1990's due to various factors such as: demographic pressure, destruction of forest by bush fires, displaced people, illegal logging and drought induced by climate change is causing rapid deforestation of about 0.5 to 2% per year. In addition, 94.6% of the country's energy consumption is mainly based on wood, which is not sustainable in the long term.

While some countries such as Gambia or Madagascar benefit from reforestation programs, Burundi attracts less attention despite the fact that 47,6% (table 1) of the Burundian forest has disappeared over the past 20 years. Compared to Burundi, the situation is currently improving in Rwanda thanks to political stability. Indeed, Rwanda has also attracted various investors and new forests have appeared recently. Like Rwanda, Burundi does require the international assistance from international organizations.

Table 7: Top 5 countries with highest deforestation percentage in Africa (FAO, 2011)

		Total deforestation (%) since 1990	Deforestation/year (%) since 1990	Forest cover (ha) today	Forest cover (%) today
1	Rwanda	50.9	6.9	480,000	19.5
2	Burundi	47.6	5.2	152,000	5.9
3	Togo	43.6	4.5	386,000	7.1
4	Mauritania	35.7	3.4	267,000	0.3
5	Niger	34.9	1	1,266,000	1

Burundi is currently looking for solutions to improve their environmental, social and economical situation and believe that rattan and more specifically bamboo can solve part of the challenge, especially because the species can be utilized by most of the population as it does not require high level education. In addition, bamboo has great environmental benefits can be used to improve the environmental challenges that the country has to face. Local and International stakeholders are interested in new country area but one of the main issue with Burundi is data availability.

It is widely recognized that deforestation is the second most important source of greenhouse gases with 20% of global emissions. In recent years, large literature discussed the causes and drivers of forest degradation and deforestation, with a specific emphasis on developing countries for the REDD projects.

While there is a strong focus on developing projects for REDD initiatives, it is important to make a baseline survey to understand the social, economical and environmental situation to define future improvements and avoid side effects. When developing countries want to develop, they always face various dilemmas. Indeed, they require a lot of resources to ensure development, which has an impact on their environment but they also have to make sure that their resources are not disappearing, and that they do not reach the point of no return. This study can also be a support to complete a project documentation design (a deep analysis of the social, economical and environmental impact of any reforestation project), which is one of the requirements to claim carbon credits within the REDD initiative.

It is possible to claim carbon credits with bamboo plantations through the REDD initiative as the species is classified as a non-wood species under the Kyoto protocol while in reality it is considered as a plant. It appears that Bamboo's carbon sequestration rate outreaches the rates of most of the fast growing traditional trees under the similar or same conditions. In addition bamboo has very interesting characteristics for producing bio-fuel and durable products.

What are the Bamboo and Rattan species available in the country? Do the management practices need to be improved? How the local communities use the resources? How the resource utilization can be improved? What would be the overall benefits of new plantations? This report will provide background information to INBAR as well as to all local and international stakeholders interested in completing bamboo and rattan projects in the country.

In Chapter 1, we will briefly describe the methodology, which has been used to complete the study. The country's social, economical and environmental resources and challenges will be analyzed in Chapter 2. Chapter will provide information about the environmental findings on Bamboo and Rattan including the harvesting and maintenance practices. Chapter 4 will explore the social and economic benefits from bamboo activities. The final conclusion is given in Chapter 5.

General Information About The Study

Objective

The main objective of this assignment is to carry out a Production to Consumption Study for Burundi, with an analysis of the data collected and suggestions for interventions / follow up for projects in Burundi with a logical framework for the proposed intervention in the report.

In Chapter 1, we will briefly describe the methodology, which has been used to complete the study. The country's social, economical and environmental resources and challenges will be analyzed in Chapter 2. Chapter 3 is a summary of the data collected during the field trip and the final conclusion is given in Chapter 4.

Organisation

The following table describes the planning of the study. As we can see both field trips and ministries visits were planned. In addition, we took the opportunity to go to the main provinces where bamboo and Rattan can be found. There is a more important focus on bamboo as the resource is more widespread and used by the local communities.

Table 8: Planning to find information to complete the PCS study

#	Dates	Places
1	Mon. 19 Sept. 11	Data collection (ministries and agencies)
2	Tue. 20 Sept. 11	Visit of bamboo décor + CFA + nurseries + data collection
3	Wed. 21 Sept. 11	Field trip in the Mwaro province (Bamboo)
4	Thu. 22 Sept. 11	Field trip in the Bujumbura province (Bamboo)
5	Fri. 23 Sept. 11	Field trip in the Cankuzo province (Bamboo)
6	Sat. 24 Sept. 11	Field trip in the Cankuzo province (Bamboo)
7	Mon. 26 Sept. 11	Field trip in the Muramvya province (Bamboo)
8	Tue. 27 Sept. 11	Field trip in the Bururi province (Rattan)
9	Wed. 28 Sept. 11	Data collection in Bujumbura
10	Thu. 29 Sept. 11	Data collection in Bujumbura
11	Fri. 30 Sept. 11	Presentation

Itinerary (figure 1)

The following figure describes the itinerary we have followed. As we can see on the map, we were able to collect data in the main provinces where bamboo and Rattan can be found.



Figure 1: Itinerary in Burundi

Methodology

To achieve this objective, the methodology used was to first to conduct a literature review and gather general information (secondary data) on the country (geographical, topographical, climate,

demography, political, environmental). Background information is necessary to understand the country's challenges to face and the country's opportunities for new projects to come. Secondary data mainly come from ministries and agencies based in the country. Regarding the social and economic data, the country is mostly relying on data from the World Bank (CNUCED), IMF, DFID and ONUDI (Ministry of planning, 2011). The government has a service called ISTEERBU, which is responsible to consolidate national data (ISTEERBU: Institut de Statistiques et d'Etudes Economiques du Burundi).

Most of the information were not available online, thus it was necessary to go on the site and visit the various ministries. Indeed, secondary data were also more challenging to find due to the lack of budget for ministries to collect data on the field.

Primary data: Social and economic data have been collected on the site through interviews with the local communities. Questionnaires have been completed and translated into French for this study. Environmental data have been collected on the site through resource assessment and with the support of a GPS. There is no literature available about bamboo in Burundi and this study is thus, the first one in this field. Local interviews that took place in the remote areas of Burundi. The government has a service called ISTEERBU, which is responsible to consolidate national data (ISTEERBU: Institut de Statistiques et d'Etudes Economiques du Burundi).

General Information About Burundi

Chapter Introduction

Collecting general information about Burundi will help INBAR understand the main challenges that the country has to face. In addition, it is important to understand what resources are available and how they are utilized in order to find tailor-made solutions and improve the social, economic and environmental situation. Background information is also often used to implement new projects.

This chapter has a lot of energy information because INBAR works a lot with bamboo charcoal and the organization would like to put in place an electricity project, which will use bamboo as organic biomass. Thus, data can be used to make predictions and assumptions for future projects.

Environmental Information

Geography And Climate

The main physical features of the country include a variety of ecological regions. These include Imbo region, which comprises lowlands in the western parts of the country, Congo/Nile Crest region comprising of high mountain ranges, Kirimiro region made of central plateaus, Kumoso region comprising of low-lying terrain in eastern areas, the Bweru region made up of pen plains in the north and Bugesera region constituting lowlands in the northeastern parts of the country. More details are given in table 3.

Table 9: Ecoclimatic information in Burundi (CIE)

Ecoclimatic regions	Altitude (meters)	Average rainfall per year (in mm)
Western plain of IMBO	800 – 1 100	800 – 1 100
Western steep slope of Murirwa	1 000 – 1 700	1 100 – 1 900
Crest Congo-Nile (Mugamba-Bututsi)	1 700 – 2 500	1 300 – 2 000
The high central plateau	1 350 – 2 000	1 200 – 1 500
The depressions of Kumoso and Bugesera	1 100 – 1 400	1 100 – 1 550

Impact Of Climate Change

From 1999 to as of today, annual trends show that the rainy season is shorter and the dry season lasts longer. The results of climate change simulations show that in the period 2000-2050, we will expect an overall increase in rainfall, ranging from 3 to 10%, albeit rainfall will decrease from 4 to 15% in May (May is the end of the rainy season while October is the beginning).

Topography

Generally, Burundi lies in high altitudes that range from 774m to 2670m above sea level. The figure 2 shows the topographic feature of the country. The highest peak, Mount Heha is 2,685m highland lies to the southeast of the capital, Bujumbura. The Nile River's source takes place in the Burundi province, and is linked from the Lake Victoria to the headwaters via the Ruvyironza River Lake Victoria, which is also an important water source, which serves as a fork to the Kagera River. Located in much of Burundi's southwestern corner, the Lake Tanganyika is another major lake.

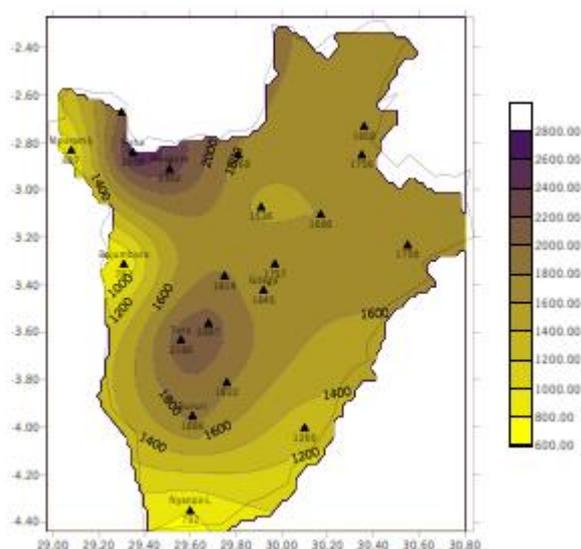


Figure 2: Topography features of Burundi

Soil And Vegetation

Burundi has faced significant soil erosion issues lately, and is as a result of the expansion of agriculture into marginal lands as well as overgrazing. Much of Burundi's soil is rich volcanic soil suitable for many kinds of agriculture. Some of the major environmental issues that Burundi is facing are soil erosion (caused by the expansion of agriculture into marginal lands and overgrazing), an inefficient protected area network, high population growth and density, deforestation (there is little forested land left), as well as the threat of the wildlife populations due to habitat disappearance.

Conservation efforts for fauna and flora are being hampered because of insufficient financial resources, lands as well as the poor enforcement of environmental legislature. Some of Burundi's major agricultural products are coffee, (tapioca); meat, sorghum, cotton, tea, corn, sweet bananas, manioc potatoes, milk, and hides. Coffee, tea, and cotton are grown for trade; especially coffee which is the chief commercial crop and export. Beans, cassava, corn (maize), rice, sweet potatoes, peanuts, peas, and sorghum are subsistence crops. Soybeans, oil palms, and sugarcane are grown as well.

Forest Resources

Burundi's original forest vegetation is mainly composed of closed sub-montane and montane rainforest along the Nile-Congo watershed, running the length of the country, and savannah woodland along the

Southeast border and areas surrounding Lake Tanganyika. Much of the forest and woodland has been degraded or cleared to expand agricultural lands. Remaining preserved forests are mainly in the north of the country in the south in the Buriri forest and around Kibila National Park. Common species include *Macaranga spp.*, *Polyscias spp.* and *Entadrophragma excelsum*. In addition, significant areas of bamboo forest *Yushania* (former *arundinaria*) *Alpina* can also be found in the high altitude regions. Eucalyptus plantations now cover a lot of areas. Indeed, *Eucalyptus globulus* can be found at the top of the mountains as well as *Eucalyptus salgina*, those species are predominant in the country.

In the savannah forest, we can find "miombo" woodland, the drier west comprises *Acacia* grasslands, and the south, it is associated with *Brachystegia-Julbernardia*. Burundi has a network of about 10 protected areas that include 3 national parks. In addition, about 12 percent of the country's forests are in preserved areas.

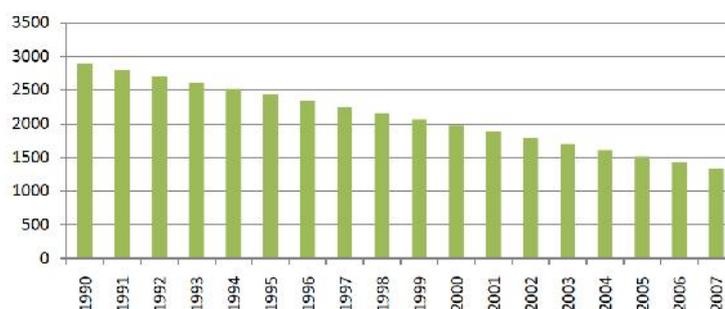
The demographic pressure, destruction of forest by bush fires, displaced people, illegal logging and drought induced by climate change is causing a rapid deforestation of about 0.5 to 2%/year. According to the World Bank (cf; graph 1) forest areas outside spaces agro forestry have been reduced by half between 1990 and 2007 that is to say, 289 000 ha in 1990 to 133,600 ha in 2007. The loss from bush fires is estimated at 4,000ha/year. Illegal logging represents about 2,000 ha/year while victims from the civil war would require more than 5,000 ha / year of afforestation. On the other hand, the exploitation of wood resources (fuelwood and timber) is greater than bush fires and illegal logging combined. The forestry and the agro-forestry contribute to about 2% of GDP and provide 6% of employment. It also plays an important role in conserving water and soil quality and to maintain biodiversity and hydrological equilibrium.

Land in Burundi is limited to 27,834 km² and as we can see in table 4, 900 000 ha of arable lands¹¹, 900 000 hectares of pastures and permanent crops of about 390 000 hectares (FAO, 2011). Forest area is limited to 172 000 hectares, thus only about 6.5% of the total lands. As the result we can say that the land in Burundi is very exploited.

Table 11: Land use indicators

Source	Indicator	Value	Updated year
FAO	Land area (1000 ha):	2 568	2011
	Arable land (1000 ha):	900	2011

Table 10: Trends of the forestry area in Burundi from 1990 to 2007 (km²) - (World Bank)



¹¹FAO defines arable lands as land under temporary crops (double-cropped areas are counted once), temporary meadows for mowing or for pasture, land under market or kitchen gardens, and land temporarily fallow. Land abandoned as a result of shifting cultivation is excluded.

	Permanent crops (1000 ha):	390	2011
	Pastures (1000 ha):	900	2011
	Irrigated land (1000 ha):	23	2011
	Forest area (1000 ha)	172	2011
WB	Forest area (1000 ha)	152	2011
FAO	Natural forest (%)	26	2000
	Public plantations (%)	45	2000
	Agro-forestry	28	2000

Social And Economic Information

Population

90% of the population is in rural areas and is unevenly distributed. The distribution has been influenced by internal migrations, as the population has been looking for safety, fertile lands and employment. The average household size in Burundi is 4.7. In Bujumbura, it is not allowed to have more than 5 children as a new regulation has been put in place recently.

Table 12: Population per province (ISTEEBU, 2008)

Provinces	Population	Area (Km2)	Density in 1990	Density in 2008	Population growth
Bujumbura	348 188	1 089	207.4	319.7	2.43
Bubanza	565 070	1 232	299.7	458.3	2.39
Bururi	570 929	2 465	159.4	231.6	2.10
Cankuzo	221 391	1 965	72.4	112.7	2.49
Citiboke	460 626	1 636	172.8	281.6	2.75
Gitega	715 080	1 979	285.1	361.3	1.33
Karusi	433 061	1 457	207.0	297.2	2.03
Kayanza	586 096	1 233	359.8	475.3	1.56
Kirundo	636 298	1 703	237.6	373.6	2.55
Makamba	428 917	1 960	122.8	218.8	3.26
Muramvya	294 891	696	632.6	423.7	- 2.20
Muyinga	632 346	1 836	210.0	344.4	2.79
Mwaro	269 048	7 99	****	336.7	****
Ngozi	661 310	1 474	247.0	448.6	1.75
Rutana	336 394	1 959	84.7	171.7	2.99
Ruyigi	400 818	2 339	302.5	171.4	- 9.66
Mairie de Bujumbura	478 155	87	2 604.9	5 496.0	4.24
	8 038 618	25 910	206.4	310.3	2.28

As we can see from the above table, the overall density of the population is 310 habitants/km², so Burundi is one of the most densely populated countries in Africa. However, as we can see in table 5, there are some differences among the provinces. In Kayanka (475), Bujumbura (458), Ngozi (449) and Muramvya (424), the density of the population is above the national average. Other provinces are far less populated, Cankuzo (112), Rutana (171) and Ruyigi (171).

The rapid population growth leads to an excessive fragmentation of land, the gradual disappearance of nature reserves, the exploitation of marginal lands and thus endangering the environmental balance. Thus, the size of the farm household was 1.04 ha in 1973 fell to 0.33 ha in 2000. In order to acquire a

land in the country, the buyer himself needs to find someone who is ready to sell his land as the government does not have a map, which gathers the available lands. The government has just created an economic zone of 200 hectares but the precise location remains confidential at the moment. To be an official owner, the buyer has to make a contract with witnesses and a “notable” (someone who cannot be corrupted), then go to the administrative department after engineers and notaries check the area.

Social indicators

According to table 6, In 2010, Burundi ranked 166th out of 177 when taking into account an HDI of 0.282 in 2010. Over 70% of the population lived below the poverty threshold (\$ 25 per month so just over 30,000 FBU). Expenditures on health and education are not very high (5.2 and 7.2% of GDP respectively) and 81.32% of the population lives under the poverty line.

Table 13: Human development indicators in Burundi (UNDP, 2010)

	Indicator	Value	Year of update
Health	Life expectancy at birth for women	51.4	2009
	Life expectancy at birth for men	48.9	2009
	% of undernourished population	63	2006
	Under-five mortality (per 1 000 births)	168	2008
Expenditures	Expenditures on health (% GDP)	5.2	2007
	Expenditures on education (% of GDP)	7.2	2008
Education	Adult literacy rate (% aged 15 and above)	59.3	2010
Poverty	Population living below poverty line	81.32	2006
Security	Refugee (thousands)	281.6	2008
HDI	Composite indicator	0.282	2010

The socio-political crisis of 1993 undermined the efforts of the government in its national social policy aimed at meeting basic needs such as access to education, health care, water and sanitation, the fight against HIV / AIDS. The infant mortality rate, which was 110/10000 in 1993, increased to 125/10000 in 1998. The immunization coverage rate was 80% among children in 1992 and fell to 60% in 1998. Malnutrition has reached alarming proportions (over 20%) and there has also been a sharp increase in infectious diseases such as pneumonia, various forms of diarrhea, tuberculosis, malaria and AIDS.

47% of households in Burundi had access to drinking water in 1997. In 2002, the government noted some improvements as the figure reached 49%. There are a lot of disparities from one city to another as well as in the countryside. While 75% of urban households can drink proper water, 43% of rural households have access to treated or non contaminated water.

By 2015, the government has the objective to reduce by half, the number of households, which cannot access drinking water. By 2020, the other objective is to improve Burundian habitats. In addition, it will be necessary to ensure the supply of energy to power water pumps while ensuring the conservation of the natural reserves in the country.

Economic information

Burundi's economy suffers from multiple failures generated by the crisis and civil wars. Since the adoption of the Structural Adjustment Plan (SAP) in 1986 until 1993, the growth rate of GDP was higher than that of the population (3.7% per year against 2.7%). GDP fell cumulatively by 20% over the period 1993 to 2000, due to insecurity and embargo.

The balance of payments is in chronic deficit (10.7% of GDP in 1990, 12.8% in 2004) due to the high level of imports and continued decline of world coffee prices. The deficit in the state budget is also chronic and is funded by the use of certificates of the Treasury (outstanding FBU 31.4 billion in 2004). Contributing to the increase in domestic credit, falling foreign exchange reserves of the country and the instability of the currency. External debt has become a burden and reached 49.9% of exports in 2008. At the end of 2007 the external debt was \$ 1499 million including arrears of \$ 45.9 million. However, Burundi reached the completion point of the initiative for Heavily Indebted Poor Countries (HIPC), which should enable the country to benefit from a debt relief of \$ 833 million. Additional financial relief is expected from IDA, the IMF (\$ 105 million) and the African Development Bank (AFDB). Inflation has not been fully controlled yet: the annual rate increased from 23% in 2000 to 8.3% in 2004 due to increased food production but was revived in 2008 with 24.5%. The purchasing power of the population may be affected again.

The Government has been able to achieve its investment programs, partly thanks to an external assistance of \$466 million from a European Union Allowance (EUA) in 2007, so \$54.8 per capita. This assistance is equivalent to 49.8% of GDP. The investment rate, meanwhile, declined from 18% of GDP in 1992 to less than 9% in 2000, reaching 11.3% in 2004 and 11.1% in 2008. The macroeconomic environment during the period 2006/2007 demonstrates an economic recovery. GDP growth is estimated at 5.1% while the inflation rate was controlled at 2.9% net. Based on this development, which raises hope, the Government is determined to preserve its policy to ensure macroeconomic stabilization.

Chapter Conclusion

Through this chapter, we came across a lot of information about Burundi, which shows that the country is one of the most underdeveloped in the world. Improvements were notified before 1993. However from 1993 to 2000, civil wars took place, which destroyed the government efforts to improve the social and economical situation of the country.

As of today, the economic profile of Burundi is mainly dominated by family-type agriculture and the few small enterprises require capital to take off. Domestic consumption is expected to increase as well as social improvements in the coming years due to the government efforts.

The country's energy is mainly based on wood (about 95%), which is not sustainable in the long term. Burundi is already facing a high deforestation rate and if we include the population pressure, the country requires urgent assistance.

Bamboo and rattan resources

Chapter Introduction

This study is the first one about Bamboo and Rattan in Burundi. While there is no new species in the country, we can find different species in different provinces. This is due to the various climatic conditions (rainfall, temperature and altitude). This chapter has the objective to describe the bamboo and rattan resources as well as give some hints about the maintenance and harvesting practices.

In the following map, we can see that bamboos are mainly available in the West and East of the country while Rattan is mostly available in the South. Other exogenous species such as *Bambusa vulgaris* and *Dendrocalamus asper* are not included in this map as they are available in very small quantities and are widespread across the country (figure 3).



Figure 3: Bamboo and Rattan resources per province

Bamboo Species

Naturally, two bamboo species are growing in the country. These two indigenous bamboo species are *Arundinaria alpina* and *Oxytenanthera abyssinica*. There are also one introduced exotic bamboo species namely; *Bambusa vulgaris*.

Arundinaria alpina

In Burundi, *Arundinaria alpina* grows in high altitude mountains (above 2 200 meters) and on volcanic soils. The species is mainly found at the bottom of the mountains where patches of bamboo forest grooves. Traditionally, farmers plant bamboo on small lands that they own. Those lands are generally not suitable for agriculture, but besides of its environmental benefits, bamboo can provide culms for livelihood improvement. This species is also available in many other African countries.

Bamboos are interconnected thanks to a network of rhizomes, thus the bamboo plantations remain productive even if the culms are cut. Also, bamboo's fast growth and root systems rapidly stabilize soil erosion and their transpiration characteristics facilitate in the watersheds stabilization. In Burundi, where soil erosion is significant, this species can represent a great support and small associations try to improve the situation by planting some of them along riverbanks.

Extract of the INBAR/UNIDO manual for bamboo plantations (2009)

The infrastructure of a nursery generally includes an office and meeting room for workers, a warehouse or storeroom, a potting shed, a compost area, lath houses, shade tunnels, and an open-air container ground. Tools such as spades, picks, pruning scissors, and wheelbarrows are essential equipment for manual operations in every nursery. A small building or shed is needed as a stock room for storing pesticides, fertilizers and tools. The stock room must be spacious enough to stock all materials. It should be well insulated to protect all materials from rain and excessive humidity. A fence should be erected around the perimeter of the nursery to protect the nursery area from domestic and wild animals. The fence should be about 2 meters tall and may incorporate the use of barbed wire for added security. Simple shade houses using palm or bamboo roofing are suitable in most cases.

Bamboo culms are harvested at any time of the year but at the beginning of the raining season, bamboo owners are more careful with young culms and it is more difficult to access the plantations at this time of the year. Harvesters try to collect culms that are less than two-year-old. Those culms can be used to regenerate new and stronger bamboo shoots. Most of the Alpina areas that were analyzed during the study were highly exploited and not well managed through regular and selective harvesting practices. In the absence of maintenance practices, the new shoot culms gradually become thinner and dwarfer. Bamboo management methods should be improved to ensure the sustainability of the species in the long term. So far, nobody has attempted to correct this situation since there is no any intervention on the ground by government side or NGOs.

It is assumed that many hectares of bamboo forests were gradually converted into agriculture use and eucalyptus plantations but it is very difficult to estimate the total bamboo forest coverage in the country. In addition, young bamboo culms are highly demanded where the resource is available to make bamboo baskets or other bamboo products (see chapter 4). The reason is that, young bamboo culms are easy smooth and flexible to weave different types of baskets. Thus, in many areas, bamboos are disappearing, which will have an impact on the local communities' livelihood.

Bamboos are mostly propagated using vegetative materials such as rhizomes and offsets in nurseries. However, it appears that the propagation practices can be improved. Indeed, it is necessary to have larger pots to ensure the development of the bamboos roots and rhizomes. In addition, space between each lines should allow the maintenance people to water the bamboo culms. Finally, it is preferable to have some roof protection to protect the pots.

Oxytenanthera abyssinica

Oxytenanthera abyssinica is widely distributed on lowland areas in the remote areas of the Cankuzo province (eastern part of the country near to Tanzania), Ruyigi and Karuri provinces. *Oxytenanthera abyssinica* bamboo species can be found with mixed woodland forests like in Ethiopia, mixed with some widely spread indigenous tree species and savannah grasses. However, there is no precise data about the surface area covered by *Oxytenanthera abyssinica*. In fact, in areas where we were visited bamboo forest is

This species has a lot of properties such as biomass production, adaptation in Savannah woodland and in semi-arid wooded grassland and does not require a lot of water as it grows with a minimum annual rainfall of between 350 and 800 mm. This solid bamboo grows in part shade to full sun (preferably full sun), grows on all types of soils from various types of parent rock, soil 6.5 pH - 8 pH, sandy loam to clay loam, sandy soils, and tolerates very degraded soils. It is also used for soil erosion control and the rehabilitation of degraded sites (combat of desertification).

In addition, due to its solid nature, this bamboo is known for its quality raw material for making household and commercial products, building, pulp and fencing poles. It is a plant with interesting nutritional value and its small bamboo shoots can be eaten as vegetable if cooked, while the leaves can be used as animal fodder (like it is the case today in the area).

The local communities do not realize the importance of bamboos, whereas they are used for many purposes. We interviewed many local communities leaders and relevant government experts and they believe that the resource is gradually disappearing but it is difficult to get documented data. Like *Arundinaria alpina*, *Oxytenanthera abyssinica* is not well managed, as there are no maintenance practices that are put in place.

In addition, harvesting is done randomly without taking the culms age into account. We have not found any *Oxytenanthera abyssinica* nursery during our study but generally it is possible to use seeds from Ethiopia. In addition, in the East part of Burundi (around the Cankuzo province), Eucalyptus is particularly susceptible to termites while Bamboo resists very well and plantations can be developed.

***Bambusa vulgaris* (Yellow)**

Bambusa vulgaris is a very common species for decoration purpose, even in Burundi. In addition, it can adapt many soil types, thus the species is widely distributed in many areas of the country.

It can be easily propagated used culms and branches, which is why it is such a common species. The Burundi's agro-ecological climate is ideal to expand *B. vulgaris* plantations. Also, it can produce a large amount of biomass evenly distributed throughout the year. The size of the clump is very big with an average 9-12 cm diameter and 15-17m high. One clump is consisting of more than one hundred culms and some of the culms we saw have already started to decay in the grooves. This species also require regular selective harvesting in order to expand.

Rattan Species

There is rattan in the Kigwena and Vyanda natural reserves and outside the protected areas, we can find rattan in the Murembwe valley and in the Mariza area (commune of Burambi). In provinces, we can find rattan in Makamba in Kazirabageni in the commune of Nyanza-Lac. In the Kigwena natural reserve (587 hectares) there is peri-guinean forest where we can find more than 300 species. We can find rattan in marshy areas, under other trees. The Vice chief of protected areas of Burundi, Munama Melchior, estimates that 10% of the area is covered by Rattan. In non- marshy areas, Rattan does not grow. In the Vyanda natural reserve (3 900 hectares), Rattan covers less than 5% of the reserve. The reserves gathers species such as: Chimpanzees (150), baboons, cephalodia, servals, chacals and there was one Leopard in 1990. Two other natural reserves exist in Burundi: Kikira (40 000 hectares) and Ruvuku (50 000 hectares).

In protected areas, Rattan cannot be collected, as there is a legal restriction. Before 2008, it was possible to collect Rattan legally. On the other hand, when rattan is matured it is better to collect it. Rattan is not endangered but is extremely overexploited. Only small rattan trees remain. The ministry starts to be sensitized to Rattan and they would like to associate Rattan to Bamboo when talking about reforestation projects. However, no specific plan has been made for Rattan.

An association was created to create rattan products but the government decided to stop it in 2008 in order to protect Rattan. Some seizures have been organized by the government to avoid illegal importations of Rattan. When it is the case, Rattan is confiscated and sold in auctions to the local communities.

Special attention should be given to identify the species and silvicultural management requires the participation from the local communities. The species are shade tolerant and climbers. They cannot grow in open areas without supporting trees. Rattan has to be harvested regularly in order to ensure the regular maintenance of the species. Part of the harvested resources can have some social benefits as Rattan is frequently used for craft activities. Therefore, intervention is required from experts, who have the experience and skills to manage Rattan.

Chapter Conclusion

At the moment, we can find bamboo and rattan in the country. However, bamboos and Rattan are threatened. Indeed, bamboo starts to disappear, as the propagation and maintenance methods are not appropriate the moment. If well managed, bamboo plantations can expand be expanded naturally. In addition, bamboo is often utilized by the local communities and local enterprises to make basic products (see chapter 4), which worsen the resource situation.

Other bamboo species can be introduced in the country such as the *Dendrocalamus asper*, which is a very good bamboo candidate for afforestation projects. Furthermore, the species can provide significant biomass, which is very interesting for bamboo charcoal or other furniture making activities. It could be one of the candidate bamboo species in the future for the further of bamboo afforestation in the country. This species can be propagated through genetic practices on large-scale plantations.

Overall, bamboo is already playing its role as a soil erosion regulator, especially along the riverbanks. Bamboos are often used to fight soil erosion (*Arundinaria alpina*), as well as desertification (*Oxytenanthera abyssinica*). Additional bamboo plantations should take place in those areas to ensure the resource availability and the price sustainability. Other benefits from bamboo are unknown by the local population and awareness should be increased about them as it can partly reply to the deforestation challenge that Burundi has to face.

On the other hand, Rattan is so exploited that it is difficult to find the species at the maturity stage. The government has started to put in place a protection system back in 2008 and we have seen the first results as small Rattan plants (under 2 years old) can be found in the natural reserves. Efforts need to be strengthened and it is also important to increase awareness with the local population to explain the importance of the protection practices.

Social and economic impact of bamboo & rattan

Chapter Introduction

The success of environmental projects is proportional to the social economic impact on the local communities in the field. Thus, it is very important to pay attention to the local communities' needs by involving them in all interventions on the field. Knowing the local stakeholders as well as the activities that are currently taking place is mandatory to ensure the success of any environmental projects. Involving the local communities will also allow knowledge transfer as well as increase awareness about the importance of the resources.

Social, Economic And Institutional Information About Bamboo

Bamboo Stakeholders

Institutions

At the moment, the Ministry of Environment is trying to define a reforestation policy program. So far, bamboo was not really included in the policy but some initiatives related to bamboo have started and the government is willing to introduce bamboo to avoid flooding issues and to protect the riverbanks.

At the national level, it is important to assess the amount of bamboo available in the country and try new species to develop the bamboo potentials. As of today, bamboo is only used for handcraft products and furniture but rarely for houses and is never used for irrigation, or scaffolding. At the moment, Eucalyptus is used for construction but can be replaced by bamboo, which in turn would greatly protect eucalyptus trees. The bamboo benefits are not well known.

In 2010, through the Clean Development Mechanism, three calls of tenders have been announced and the government has now started new bamboo plantations. However, it appears that local partners, who have been selected, do not know how to maintain and plant bamboo. The government would like more information about CO₂ storage potential and have documentation available in French to convince the local communities to plant and maintain bamboo. The government is also interested in bamboo charcoal.

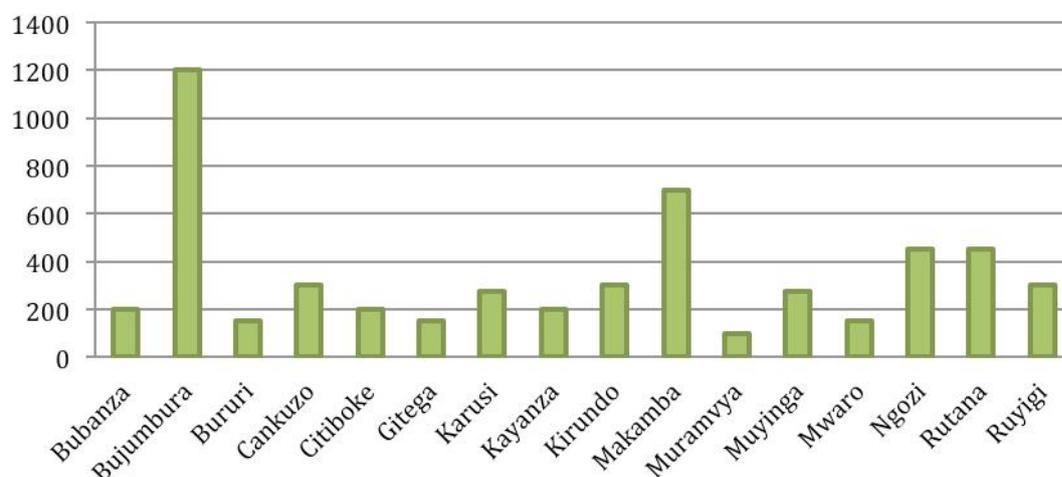
The objective of the ministry of trade is to use bamboo as an industrial tool in order to develop local economic activities. Bamboo is quite expensive as it is not abundant in the country and bamboo products are made in a very simple way. It is important to develop the technology to improve bamboo products. The other objective is poverty alleviation. Stems and not seeds are used for the plantations.

Within the National Institute for Environment and Nature Conservation (Institut National pour l'Environnement et la Conservation de la Nature: INECN), the forestry department has done some bamboo research and there is also a seed supply. This institute has also the responsibility to protect bamboo in protected areas and use fences as a protection. However, private stakeholders plant most of the bamboo. It appears important to intervene to help the local communities manage the bamboo in a more sustainable way. In order to improve research capacities, the National Institute of Agronomic Sciences (Institut National de Recherche des Sciences Agronomiques) could start working with bamboo too.

A national reforestation program (excluding bamboo) has started in 2009 in collaboration with the local administrations. 400 to 5 000 plantations per province of fruit trees and forest trees were planned. In 2009, the plantations started with the community but it was not very successful. In 2010, some precise locations were identified and 5 400 of forest trees were planted. Local associations supervised and managed the work. The government provided the tools and seeds needed. In 2011, no new plantations took place as most of the financial resources were used to maintain the plantations. While the government has just started a reforestation program, there is no long-term strategy yet. In addition, it appears that no much recent data about soil erosion is available.

A few main associations work in partnership with the government: IFDC did a 6 000 hectares plantation project: It is a regional organization working with Rwanda and Congo as well (6 000 hectares in each country), Eglise Anglicane (3 000 hectares) and Help Channel Burundi.

Public forest plantations per province in 2010 (ha)



Source: Ministry of Environment (2011)

Associations

The association « Act to promote bamboo and rattan » is the only one we have found in the country which promote bamboo. In December 2009, the association has been created with the objective to promote both Bamboo and Rattan. The members think the rational use of bamboo and rattan can help create a better environment in Burundi through environmental protection and livelihood improvement actions.

The objective is to fight the current constraints such as limited access to information on bamboo and rattan propagation and processing, limited information about the availability of the bamboo and rattan resources, lack of awareness about the value of the resource and Rattan disappearance because of the population pressure.

The association wants to use the following actions to promote Bamboo and Rattan:

- Organize and supervise local people and local associations regarding bamboo and rattan propagation; Create processing units of bamboo and rattan in all ecological zones;
- Supervise product makers and start actions to reduce local prices
- Use of bamboo for landscape restoration, to fight erosion, sequester carbon and protect drinking water sources
- Join international networks linking stakeholders in the area of bamboo and rattan to share international experiences

National Training Center (figure 4, 5 and 6)

A training center (CFA) created in 1985, exists in Bujumbura thanks to a bilateral cooperation with China. This training center is linked to the ministry of education. The experts are sent by the Chinese government, which also pays for the machineries. So far, 8 experts have supported the center, every time they stay 24 months. The government of Burundi provides the facilities.

The objective of this governmental cooperation is to train local people to make bamboo and rattan furniture in addition to wood and knitting activities. Regarding bamboo products, the center has 2 sections: Braiding of bamboo products (they only have one person for this section. Because of the lack of expertise, they had to stop this section) and production of bamboo furniture.



Figure 4: Training center in Kamenge



Figure 5: CFA buildings where trainings are given



Figure 6: Bamboo storage in the training centre

The center starts working on a new product only when they have a command from a customer who gives some cash in advance. It takes 2-3 weeks to do 1 sofa with 2 seats, 1 sofa with 3 seats, 4 chairs, 1 table and 1 repose pied) and it is necessary to hire 3 boys (mounting) and 4 girls (braiding). Total costs of the total is about 200 000 to 300 000.

Employees: They have 2 categories: first type get 35 000 FBU from the government + 25% of the sales and the second type have 35-38% of the sales without a fixed salary. In total, the center has 3-4 employees for bamboo and 3-4 employees for Rattan. Interns are trained for free. In exchange, they do not get a salary from the CFA and perspectives in the future are limited. Indeed, only 10% of the 300 graduated people have been hired by the center. The remaining 270 students have started some self-enterprises but it is difficult to monitor these activities. However, it appears that most of them are just unemployed. The center selects the new trainees who have at least completed their primary education and sometimes they have to pass a test too. They do not necessarily come from rural areas.

Private initiatives

Bamboo décor (figure 7) is one of the main shops where it is possible to find bamboo and Rattan products. While Dieudonné Havyarimana (the director) has been trained by the CFA in 1984 and he is one of the first suppliers of rattan products in the country. He has been elected “top 100 people who participate in Burundi’s economic development”. He has about 100 employees: 8 working full time with bamboo, 22 working full time with Rattan and 70 working full time with wrought iron (labour cost to process bamboo/rattan products is about 60 000 FBU – 70 000 FBU/month).

The shop is very modern and contains many furniture such as Bamboo and Rattan chairs, tables, beds and other household items. While the main shop is in Bujumbura, the capital, there are 2 other showrooms in Gitega and Ngozi. In the short-term, local people will be able to contract loans to buy products from Bamboo Décor as a partnership with a bank is about to be signed.



Figure 7: Bamboo Décor is well known in the country

Table 14: Public bamboo plantations per province

Province	Commune	Planned plantations	Area in hectare	Number of bamboo	Distance between each bamboo	Phase
Bujumbura rural	Mukike	5550	5	5 445	3	Nursery phase
	Mugongomanga	5550	5	5 445	3	
	Muhuta	5550	5	5 445	3	
	Mutambu	5550	5	5 445	3	
	Nyabiraba	2220	2	2 178	3	
	Isale	2220	2	2 178	3	
	Mubimbi	2220	2	2 178	3	
	Bugarama	2220	2	2 178	3	
Total		31080	28	30 492		
Ngozi	Tangara	2220	2	2 178	3	
	Ngozi	2220	2	2 178	3	
	Marangara	2220	2	2 178	3	
Total		6660	6	6 534		
Total on the plains			34	37 774		
	Rivers	Planned plantations	Area in km			
Mairie de Bujumbura	Ntahangwa	2000	2	3 200	2.5	
	Muha	2000	2	3 200	2.5	
	Kanyosha	2000	2	3 200	2.5	
Total rivers		6000	6	9 600		

Information About Bamboo Plantations

Bamboo National Reforestation Program

In 2011, the government has decided to start a bamboo reforestation program of 28 hectares divided into 8 communes. The locations of the plantations have been identified and the associations responsible of these plantations in each commune have just started the nurseries but we do not always know where they are and apparently implementing associations do not always know how to manage them successfully. It appears that the monitoring and management of the current bamboo projects can be improved.

In the valleys, bamboo are planted every 3 meters, so we could expect 1 111 bamboo/hectares. On the riverbank, bamboo will be planted every 2.5 meters in order to avoid further soil erosion. For every bamboo planted, each association will get 500 FBU/bamboo. Species concerned: *Arundinaria alpina*

In the Mairie of Bujumbura, we were able to visit a bamboo nursery with Fidele NAYIZIGIYE who is vice president of the association RAPACOBIDU (and lieutenant at the same time for the army). The objective of these nurseries is to plant 35 000 bamboo along the river to stop soil erosion. The methods used appear to be basic and can be improved. The association works in partnership with the government after winning a call of tender.

Generally, the population is against those projects, as they prefer eucalyptus. Indeed, the local communities believe that bamboo have to be planted in soil which contains of lot of water, which is not always easy to find and bamboo cannot be planted with other species, thus it requires lands that cannot be used for crops or livestock. In addition, they prefer Eucalyptus, as they believe it is the only species with which we can produce charcoal and this source of energy is very often used in the capital. The demand is so high that consumers from the capital go the Bujumbura province to buy the charcoal on the site. The local communities also think that Eucalyptus is much easier to plant, to manage and that it can be used to fight soil erosion in a more efficient way that bamboo. They use the leaves to fertilize the soil where potatoes grow.

Bamboo Plantation Project With FAO

Nzigiyimpa Leonidas who works for the INECN based in Bururi is currently starting a bamboo project in Bururi province. The implementing agency is INECN. The project has received financial support from FAO. While the province goes up to 2 300 meters of altitude, it is possible to find *Bambusa vulgaris* but in very small quantities.

The objective of the project is to complete 100 000 plantations of bamboo over 2 years. The first year, 50 000 plantations will be established (production of cuttings and not burst of strain). In addition, 50 000 other plantations will be completed in year 2. The concerned bamboo species are *Yushania alpina* (50%) and *Bambusa vulgaris* (50%).

Bamboos will be planted along 3 rivers (including Kayokwei and Ruvyironza) with 6 meters between each bamboo. The nursery will start in beginning of October 2011. The objective is also to integrate the population to the project, to increase awareness about the resources and to alleviate poverty by providing training to the local people. Leonidas has some expertise after following a training in Rwanda with the Rwanda Bamboo society (local NGO) but he would like to have some expertise to help him with the nurseries and to make bamboo products.

Two other projects can also be developed by INECN, but has not received financial support yet.

- Project 1: Multiplication and valorization of Rattan. Rattan is disappearing and most of Rattan that is being transformed in the country come from Congo or Tanzania. The objective is to create a network to exploit, transform and sell Rattan products with the financial support of

the “Forest Fund for the Congo Basin” financed by the Development bank of Africa but the answer received in June 2011 was negative and Leonidas does not know why.

- Project 2: In the Province de Makamba, the idea is to plant additional *Oxytenanthera abyssinica* as it is over exploited and the population needs this bamboo for everything (make local products, houses, etc...).

Private Projects

Some bamboos are planted in private areas. We have met one of the few people who plant bamboo to resell them. It is more a hobby than a full time job. The person planted 6 bamboo 10 years ago and his area is now plenty of bamboo. He planted new bamboo in November in the rainy season but believes more in Eucalyptus and has planted many of them. Eventually, he sells bamboo to people at 500 FBU/culm. In order to plant a new bamboo, he cuts the bamboo at 3 meters high, dig a hole with a hoe and apparently the new one are of better quality. He harvests the bamboo every 2 years to make fencings.

We also visited a private stakeholder interested in being involved in bamboo activities through a project called “bamboo plantation and bamboo transformation project for Burundi.” The idea is to use 5 000 hectares of deserted land to plant bamboo with the objective to fight local poverty and improve the local environment. The project is only in its planning phase and the owner will start with 25 hectares of bamboo that he already owns. This contact needs support to know which species to plant and how to train the local people as well. In addition to the traditional use of bamboo, Mr Bosco also wants to use bamboo to replace the traditional irrigation system or to treat polluted water, fight lake soil erosion and would like to export bamboo products within the east Africa community zone.

Some bamboo plantations projects are currently under analysis. Pascal Bizindasca is another private investor and has a bamboo project that is currently under analysis by the UNDP. The team from UNDP came last month and he is now waiting for the answer. The call of tender was about environmental protection.

Bamboo Harvesting Activities

Exceptionally, when one of the few suppliers requires a high amount of bamboo, a lot of farmers work together to harvest and transport bamboo to the trucks. Most of the time, farmers require less than 10 bamboos at one time and they are responsible for the harvesting themselves.

We have not found people who collect bamboo all year long. In the Bujumbura province, collectors prefer bamboos, which are flexible but do not break easily. He estimates the age of bamboo by looking at the leaves. If they start to be yellow and strong, that means the bamboo is 3 years old. Processors use 2 types of bamboo to make his baskets; 1-year-old bamboo for horizontal plait, and 3 years old bamboo for vertical plait. They prefer when there is a big distance between the internodes. They usually measure 15 meters but the size can vary.

Bamboo Supply Activities

Zacharie Nzobambona is one of the main bamboo suppliers in the country. He supplies the CFA (training centre) and some private companies that transform bamboo too. He mainly buys bamboo in the Bujumbura and Mwaro provinces. He has no difficulties in finding the bamboo as he comes from the area and knows everyone.

The price is very high as it also includes labor costs to collect, treat and load the trucks. The price rise from 500 to 1 200 FBU from 2004 to 2011 because of the rising transportation costs and a new tax has been introduced on products for training centers (sold price: 1 500 FBU/bamboo). On the other hand, bamboos appear to be easy to find and remain quite available. We believe that Zacharie also command

a lot of bamboo (he buys between 500 to 1 000 bamboo every semester), so the local people see him as a priority. He always chooses bamboo with big internodes with 3 to 5 years old, which tend to be yellow.

Bamboo is also bought in the region of Kumoso, Rumonge and Citiboke. Before it was also imported from Congo but with civil war, it is more complicated. The CFA pays 800-1000 FBU for each bamboo of 6 meters. During the raining season, it is difficult to find Bamboo as farmers are afraid that baby bamboos may be deteriorated. Mites can attack bamboo too. The price of bamboo is steadily increasing in some provinces, in Muramvya where bamboo is very often used, the price of bamboo has increased by 250% in 5 years and in Cankuzo, the prices of bamboo products have been multiplied by 3 to 5 years.

For farmers, two main reasons explain the rising prices: resource availability (Province of Muramvya) and cost-of-living increases (all provinces). For suppliers, prices also rise but for other reasons, such as fluctuating taxes (there is no fixed tariffs for Bamboo) or fuel Price variation. Thanks to transportation, they can access areas with plenty of bamboo. Bamboo decor buys bamboo from the Mugamba province (around 300 stems every 6 months). His buy price in Bujumbura is 1 000 FBU/stem (on the site 400 – 600). He has no problem to find bamboo.

Bamboo Markets

In the provinces, bamboo is always used to transport agricultural products and very often used for other primary needs (carpet, fencings, houses). In the Mwaro and in the Bujumbura provinces, bamboo is used to transport seeds, agricultural products, tea leaves, to reinforce houses or to make firewoods. In the Mugamba region, bamboo is widely used by Burundians and it is sometimes perceived as a criteria of wealth (APBR, 2009). The houses, yards, attics, are designed with bamboo. In the province of Cankuzo, people are also using bamboo to make products such as baskets, houses, fencings or vegetable matelas. Additionally, they can use bamboo as a garden stake for beans, fodder or even firewood. In the Muramvya province, a lot of bamboo products are produced for decoration and even people from Rwanda come and buy the products in this area.



Figure 8: Main places where we can find bamboo products

In the capital, bamboo is used to make furniture (tables, chairs, footrest) and to make decoration products too (lampshades, baskets). Bamboo products are sometimes not perceived as good quality in the capital and bamboo furniture remain expensive compared to other products. Indeed, the criteria for choosing a good bamboo are not known and the good treatment methods of bamboo are not widespread. Depending on which bamboo is used, the customer will be more or less satisfied. Now that Burundi has joined the east Africa community, some local people believe that it might be easier to sell bamboo products. In the past, mostly expatriates bought bamboo products but local people start to show their interests as well.

While some bamboo craft producers sell their products at home, it is possible to find bamboo products in 4 main locations (figure 8):

- In Bujumbura, CFA, Bamboo décor and GTS (another private initiative) are the main shops where bamboo products are sold in the capital.
- In the Bugarama market (Muramvya province) on the road to go to Rwanda, bamboo products have mainly a decoration purpose in this area.
- In the Rwibaga market (Bujumbura province), livestock is mainly sold but many bamboo baskets to transport seeds and agricultural products can be found too.
- In the Nyakararo market (Mwaro province), where apparently some bamboo products are sold too.

Value added	Producer's benefits	Seller's benefits
Bujumbura province	85%	8.5%
Muramvya province	50% - 80%	25% - 40%

Table 15: Value added estimation from bamboo products (Muramvya)

Producer									Seller		
	Number of bamboo	Price of bamboo	Work-load (day)	Costs	Sold price	Benefits (%)	Number of products per month	Monthly revenue	Buy price	Sold price	Benefits (%)
Small basket	1	1,000	1.0	1,000	3,000	67%	5	15,000	3,000	5,000	40%
Basket with stem	1	1,000	1.0	1,000	5,000	80%	6	30,000	5,000	7,000	29%
Big basket	3	1,000	1.5	3,000	6,000	50%	1	6,000	6,000	8,000	25%
Other basket	2	1,000	1.0	2,000	4,000	50%	3	12,000	4,000	6,000	33%
Huge basket	3	1,000	2.5	3,000	7,000	57%	7	49,000	7,000	12,000	42%



Figure 9. Products From The Mwaro Province: Basket to transport tea leaves – Figure 10. Products From The Cankuzo Province: Basket to transport various products



Figures 11, 12 and 13. Products From The Bujumbura Province: Basket with lid to transport and hide agricultural products on the head - Basket to store and transport seeds with cow dung as protection - Bamboo basket seller in the local market



Figure 14. Products From The Muramvya Province: Baskets sold in the Muramvya market; these baskets have a decoration purpose and are only sold in this area

Products From The Bujumbura City

Now that Burundi has joined the east Africa community, some local people believe that it might be easier to sell bamboo products. In the past, mostly expatriates bought bamboo products but local people start to show their interests as well. CFA, Bamboo décor and GTS (another private initiative) are the main shops where bamboo products are sold in the capital.

Bamboo Decor Products

Details about products sold in Bamboo decor: figure 15. Bamboo décor has stopped making a lot of bamboo products as it is difficult for the company to find good quality bamboo and to treat the resources

CFA Products

The main bamboo products that the center produces are chairs, sofa, footrest and lampshades. These are the local prices (figure 16):



Figure 15: Bamboo product sold in the shop Bamboo Decor in the capital. - Figure 16: Bamboo furniture in the training center's showroom

Table 16: Information about bamboo products

Places	Products	Price	Time spent	Bamboo needed
Province of Mwaro	Small Basket	5 000 – 7 000	2.5 day/pers	2
	Basket	1 000	0.5 day/pers	
Bujumbura province	Basket to transport agricultural products	6 000	TBD	
	Basket to transport seeds	6 000	2.5 days/pers	3
Cankuzo province	Basket	1 000	0.5 day/pers	
	Matelas	1000/m	2 meters/day/pers	
	Basket to transport seeds	5 500	2 days/pers	
Muramvya province	Small basket	3 000	1 days/pers	1
	Basket with stem	5 000	1 days/pers	1
	Big basket	6 000	1.5 days/pers	3
	Other basket	4 000	1 days/pers	2
	Huge basket	7 000	2.5 days/pers	3
Bamboo Decor	Chair 1	70 000	5 days/pers	8
	Shelves	500 000	12 days/pers	50
CFA	Chair	35 000	1 day for 1 pers	
	Small table	35 000	Not available	
	Sofa	80 000	2-3 days for 1 pers	
	Footrest	7 000	Not available	
	Lampshades	7 500	2 days for 1 pers	

Product treatment: To treat bamboo, businesses use Dursban in Bujumbura city while processors use Cléoline in the Bujumbura province. Farmers only use natural treatment such as cow dung for example (figure 17).



Figure 17: Basket with dung cow to protect the bamboo naturally - Figure 18: Tools to make bamboo product.

Bamboo Tools

The workers we met have used the following tools and they are changed every 3-5 years. Accessing the tools is not an issue and the quality is good enough to supply basic bamboo products (figure 18).

Table 17: Tool prices to make bamboo products in rural areas

	Liana	Knife	Serpe	Machete	Nail/needle
Mwaro	Free	1 500			1 000
Bujumbura	Free	1 500		3 000	1 000
Cankuzo	Free	200 – 300		2 500	
Muramvya	Free	1 000	3 000		

The following table gathers the tools used by the CFA. In addition, it gives information about the tools that are necessary to expand the training activities. Nowadays, the tools are not sufficient to provide the best education and the center is looking for external support to develop its quality/expansion.

Table 18: CFA Bamboo and Rattan tools

Material for CFA	Material needed	Price
Used tools	White glue used for furniture (rudibois)	300 USD (10 units)
	Saw	50 USD
	Electric saw	90 USD
	Price of an electric plane	10 000 USD
	Price of a sander (grinder)	1 000 USD
Tools that are not used yet	Knife planer	80 USD
	Planer combined with assembly support	15 000 USD
	Ball bearing	60 USD
	A circular saw machine	15 000 USD
	Router	8 000 USD
	Tour	2 000 USD
	Bandsaw	300 USD

Impact Of Bamboo On Local Livelihood

Bamboo is widely used to make houses, firewoods and fencings in all provinces where bamboo can be found. In the Mwaro, Bujumbura and Cankuzo provinces, bamboo contributes to the household revenue from 10% to 40%. On the other hand, from October to May, it is really difficult to find customer outside the harvesting period and a lot of producers stop working in this period (figure 19).

Families do not always own bamboo as their own land is used for other purposes, especially for farming and livestock activities. In the Mwaro and Bujumbura provinces, on average, 10 people per hill work with bamboo products.

People in this Cankuzo province mainly rely on agriculture for their subsistence but they use bamboo on a regular basis, which grows naturally in the area. Bamboo represents an additional source of revenue. Most of the bamboo is just collected from the nature but in some parts, it is not free and has to be bought to the local owner of the land. Bamboo is collected all year and is rarely planted. Bamboo are disappearing and is not available in sufficient quantity as the population and cultivated lands are growing. Most people rely on agriculture (90% of the revenue) and bamboo generates the remaining 10% revenue. Compared to Eucalyptus, bamboo has demonstrated a great resistance to thermites.

In the province of Muramvya in Bugaran, bamboo is also widely used by the local communities. We have interviewed local producers in collaboration with the local authorities. Producers entirely rely on bamboo products for their monthly revenue, the revenue is very stable and he does not depend on the harvesting period as the products have a decoration purpose. Customers pick up the products at the

producers' homes and give 85% of the amount in advance so the producer has no difficulty to find financing or to have new commands. Some people buy bamboo products for Rwanda.



Figure 19: Bamboo is used to reinforce houses' structures

Table 19: Summary of data collected in rural areas in 4 provinces

Provinces	Proportion of income	Periods	Age des processors	Estimated number of processors
Mwaro	10% - 40%	Harvesting	51	Average 10 by hill
Bujumbura			50	Average 10 by hill
Cankuzo			TBD	To be defined
Muramvya	100%	All year	49	To be defined

For producers, while basket prices are too low, bamboo resources start to decrease in many provinces. Some of the basket producers spend a lot of time finding and negotiating bamboo whereas this time should be spent on making additional bamboo products. For instance, it is the case in the Bujumbura and Mwaro provinces the missing revenue has an impact on their livelihood conditions.

In the Mwaro province, while the bamboo products are of very good quality, it takes about 2 hours to look for bamboo and 3 hours to come back with the resources. It takes 1-2 hours by walk to go to the local market and bamboo is transported on the producer's back. In the Muramvya province, the time needed to look for bamboo keeps increasing as well as the price, which might threaten local businesses in the long term.

While bamboo can generate local revenues for farmers in the harvesting periods, it is almost impossible to sell bamboo products in non-harvesting period (from October to May). The possibilities of bamboo are largely unknown and neglected. We mainly find bamboo baskets and matelas but there is no bamboo furniture in the provinces for example. Only people above 50 years old make bamboo products. The profession does not attract the youngest as they do not believe in the perspectives.

Table 20: Time needed to look for bamboo and sell bamboo products

Provinces	Time to look for bamboo (return time)	Time to sell bamboo products (return time)
Mwaro	5 hours	3-5 hours
Bujumbura	5 hours	3-5 hours
Muramvya	5 hours	Products picked up by buyers

Chapter Conclusion

Bamboo is used for a lot of purposes such as livelihood purposes through houses, fencings or even firewood. In addition, bamboo is always used to make local baskets to transport tealeaves and agricultural products, as bamboo baskets are very solid and light. Other products are sold in local shops but the design and the protection methods have to be improved. There are a lot of potentials to develop bamboo furniture making activities as people even come from Rwanda to buy the various products. Indeed, people making decoration purpose products are always have plenty of commands.

On the other hand, due to the overexploitation of Rattan, the local resource (from Burundi) cannot be used to make baskets or furniture, thus, we were not able to interview local people on their practices. A few enterprise have been set up but Rattan come from outside Burundi, thus the impact on local livelihood cannot be assessed. Nonetheless, we believe that illegal use of Rattan takes place.

Conclusion and recommendations

Conclusion

The population in Burundi does not realize how important bamboo is to the country. Some ministers and people from the Ministries have been impressed by the bamboo potentials following their visits in China at INBAR. Thus, they believe that bamboo plantations have to be developed and current bamboos have to be well maintained. In order to reach this objective, documentations in French should be widely available for the ministries and for local people.

Many families rely on bamboo in the provinces to ensure their livelihood. Bamboo baskets are always used to transport tealeaves and agricultural products as they are very solid and light. People in rural areas also use bamboo to improve their living conditions as well as to make products that can be sold to local markets.

The methods and the tools used to make products are very basic and can be improved. Bamboo products are popular for decoration purposes and some markets can be developed in this field if bamboos are in sufficient quantity. Indeed, the disappearance of bamboo has increase the bamboo prices in some areas which as an impact on the bamboo product producers' revenue. In addition, the time to look for bamboo has increased and less time can be spent on product making.

Bamboo furniture is often seen as expensive and sometimes is not of good quality. This is due to the fact that there are no known criteria to choose bamboo well and methods to treat bamboo are not widespread. Young people making bamboo products have difficulties to start their own businesses, as tools are too expensive for them. Most of the time, producers are above 50 and young people are not motivated to do the same jobs (except in the Muramvya province where the perspectives are better).

Regarding Rattan, information about the current resources is difficult to obtain. Rattan in the natural reserves is well maintained but the plants remain young. We have not found data about collected Rattan outside the natural reserves.

Rattan furniture is very expensive for local people but the quality is very good. However, it is difficult to import Rattan to Burundi in large quantity, which is a big limitation to the field development in the long term.

Recommendations

It is difficult to set up develop successful policies and strategy action plans for bamboo and rattan for short, medium and long term development and utilization program today. Indeed, it is difficult to have a precise idea of the amount of Bamboo and Rattan resources available. Software such as GIS can help governments towards this objective and should be one of the priorities.

Additional incomes can be created in various provinces by setting up a furniture-making local enterprise that will make bamboo furniture and handicraft products. Local communities should be trained to create non-timber products and bamboo housing materials and construction through the furniture-making enterprise with an emphasis on design and product protection practices.

New technologies transferred from other countries should be used for the development and utilization of Bamboo and Rattan. Local communities should be trained with an emphasis on protection, maintenance and harvesting in every province where bamboo can be found. This will allow the natural expansion of the species.

In addition, bamboo charcoal has not been introduced in Burundi yet. Other projects have been developed in other countries such as China, Ethiopia, Ghana and Vietnam but in Burundi, people rely more on Eucalyptus and are not aware of the bamboo charcoal benefits. Indeed, bamboo charcoal can be as efficient and even more depending on the technology used than traditional charcoal. If bamboo is used instead of traditional trees, forest reserves will be preserved. Bamboo charcoal is made of bamboo by pyrolysis process.

Fodder opportunities can also be expanded with cows and chickens. Indeed, feeding chickens on an organic diet containing fresh bamboo leaves results in them weighing up to 70% more than those fed on standard organic diets, according to results from INBAR's Philippines Action Research Site in Abra Province.

Analysis: For the bamboo sector development, what are the main Strengths, Weaknesses, Opportunities, Threats that we can identify based on the information we have gathered:

Topics	Strengths	Weaknesses	Opportunities	Threats
Geography / climate	Favorable climate and evenly distribution of annual rainfall, for bamboo and rattan forests	Desertification starts to increase progressively Land use depends on topography and land quality	Many bamboo species that are fast growing and can be harvested all year long can adapt to the climate, soils and rainfall conditions.	Climate change impact (dry season will last longer). Soil erosion, watershed deterioration, high deforestation rate, land degradation, biomass shortage
Present status of the Bamboo sector	Bamboo can be found in many provinces.	The management is not always efficient	The government wants to improve bamboo management	Bamboo is not well maintained and considered.
Population	There are a lot of young and motivated people.	Access to lands is limited. Skills to manage and use bamboo are limited. Poverty is increasing	Motivation remains. Poor people can be involved in SMEs	Access to financing is limited. Increasing poverty and unemployment rate.
Socio-economic situation	Livelihood is improved from bamboo baskets, firewoods, fodder handcrafts and construction materials	Bamboo perspectives are limited. Product designs are restricted and not always durable. People do not realize the importance of bamboo	They are a lot of opportunities to develop in this country (energy, resources & handcraft projects)	Political instability. Livelihood depletion and biomass shortage, migration of the rural population to urban dwellers.
People and bamboo/rattan interaction	The local communities always use Bamboo (30 000 baskets to transport tea leaves). Rattan can be used too.	Skills are limited. In the provinces, producers of bamboo products are mainly above 50 years old.	High potential for furniture making enterprises. Natural forests are disappearing and bamboo can improve the situation.	People do not know how to select bamboo and treat it in the capital.
Institutional situation	Bamboo. MEM, MEEATU and	The management of the nurseries/plantations	Bamboo projects are used to fight soil erosion and	The two indigenous bamboo species are about to

	NGOs are already researching and working with bamboo	can be reinforced. Lack of awareness about Rattan	desertification	disappear as well as Rattan outside the natural reserves. Implementing agencies have to be well supervised as they lack experience with bamboo.
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References

- Belcher, B. and K. Schreckenberg (2007). "Commercialisation of Non-timber Forest Products: A Reality Check." *Development Policy Review* 25(3): 355-377.
- Betti, J. L. (2007). *Strategie/Plan D'action pour une meilleure collecte des donnees statistiques sur les Produits Forestiers Non Ligneux au Cameroun et recommandations pour les pays de la COMIFAC*. I. A. e. I. P. d. C. Ministère Fédéral d'Allemagne pour l'Alimentation. Yaounde, Ministère Fédéral d'Allemagne pour l'Alimentation, l'Agriculture et la Protection des Consommateurs, COMIFAC, FAO: 170.
- Brendan (2007). *Genocide: modern crimes against humanity*. Twenty-First Century Books. ISBN 0761334211.
- Brink, M. (2008). *Bambusa vulgaris* Schrad. ex J.C.Wendl., Protabase. Louppe, D., Oteng-Amoako, A.A. & Brink, M. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands.
- CNUCED (2011) *Examen de la politique d'investissement pour le Burundi*
- Dumont, Henri J. (2009). *The Nile: Origin, Environments, Limnology and Human Use*. Springer. ISBN 1402097255.
- FAO (2011) <http://www.fao.org/countries/55528/en/bdi/>
- FAO (2007). *State of the World's Forests*, Rome.
- FORA (2006). *Developing Guidelines for Sustainable Management of Three Philippine NTFPs: Rattan, Honey and Almaciga Resin*. Non-Timber Forest Products Task Force, Los Banos, Laguna.
- INBAR/BOTA/ABS (2009). *Constructing and Conserving with Bamboo*. World Forestry Congress XIII, Buenos Aires, Argentina, FAO.
- Ingram, V. (2009). *The hidden costs and values of NTFP exploitation in the Congo Basin*. Paper presented at the World Forestry Congress XIII 2009 Buenos Aires, Argentina, FAO.
- Laird, S. A., R. McLain, et al., Eds. (2009). *Wild Product Governance: Finding Policies that Work for Non-Timber Forest Products*. People and Plants International Manual. London, People and Plants International
- MEM, Pierre Savary (2010): *Élaboration de la Stratégie sectorielle pour le secteur de l'énergie au Burundi*
- MEM (2011): *Élaboration de la Stratégie sectorielle pour le secteur de l'énergie au Burundi*
- Ministère de l'intérieur: *Recensement général de la population et de l'habitat: 2008 (2009)*
- Ministère du plan et du développement communal: *Economie Burundaise 2009 (2010)*
- Rangeley, Robert (1994). *International river basin organizations in sub-Saharan Africa* World Bank Publications ISBN 0821328719.
- Ruiz Perez, M., B. Belcher, et al. (2004). "Looking through the bamboo curtain: an analysis of the changing role of forest and farm income in rural livelihoods in China." *International Forestry Review* 6(3-4): 306. Ruiz Perez, M., F. Maoyi, B. Belcher, Y. Xioasheng, B. Mertens, L. Hua and X. Jinzhong (2009).
- Wilkinson, K. and C. Elevitch (2009) *Non-timber Forest Products: an introduction*. The Overstory Volume, 1 DOI

A sociological approach of Bamboo exploitation within Development - How the space of opinion reveals the “real-development” and its contradictions

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For more than 10 000 years, human being experienced its power on his environment and nature. During the Neolithic the agriculture and other mean helped him to live in a hostile environment. Since that time, the relation of human being with nature changed simultaneously but not identitically in different part of the world. From the 15th century, the western world has conquest many part of the planet. This how the western ideas of the domination of nature by science and techniques, has been spread and finally gave birth to the industrial revolution as a logical result of an historical process¹².

Today, the perception of most human being is influenced by this history. Their power on the environment, their understanding of exploitation, the way they see the evolution of the material is characterized by a belief they interiorized: There is one way of Development. Any project related to the domination of nature and its exploitation is perceived as a necessary step to reach Development. The use of bamboo, its transformation, its value and its worldwide evolution, brings the evidence that bamboo, despite its specificities, has already entered in the historical process.

Many populations have used bamboo for thousands years in different space-time. Bamboo is, as part of nature, a piece of our global history. But it is only in the end of the 20th century, beginning of the 21st that it is taking a serious place in what the called “Development”. Although, most of actors accept, as a fact, the logical links between the two words: bamboo-development. And of course, many Development projects, considering bamboo as a gold resource, are implemented in many part of the world ant they all are under different influences.

This working paper has the objective to bring to light that the exploitation of a material, considered as green like bamboo, can reveal the distortion¹³ within the praxis of Development. The distortion will be seen as a good angle to study this sector. Based on the viewpoint that Sociological theory describe common aspects of assorted social systems, to detect driving forces beyond social changes, and to explain causal relationship between different variables within a social process (Wolfgang Meyer 2002), this paper will try to put into perspective the main actors, levels and interactions that can facilitate the understanding of the social fact.

In order to do so, the actors would be scrutinizing from their space of opinion or/and exchange (Xavier Dufrenot, Sorbonne University, 2003). In another word, a dematerialized area that share, by their opinion, different actors engaged in the promotion of the material: Bamboo Non Governmental and International Organization, Development and Humanitarian NGOs, industrial companies, villages association, marketing company and workers. If all of them share at least one interest (bamboo

¹² Paul Bairoch, *Victoire et déboire, histoire économique et sociale du monde du XVI siècle à nos jours*.

¹³ The « distortion » might be understood from our reading of Paul Ricoeur analysis about ideology and utopia: It is an understanding of the reality influenced and motivated by a belief and interests that can be in contradiction with facts and other interpretations. (cf. Ricoeur, 186, 1997).

exploitation) their contradictions and their convergences, reveal how a certain idea of Development is legitimate by different means, their interactions and a history. The analysis of the link between bamboo exploitation and Development, through a sociological approach, could introduce a reflection on the role of the actors in their contribution to this greener that the use of bamboo is suppose to participate.

In his 1949 inaugural address¹⁴, the US president Harry S.Truman, used the expression underdeveloped area for non modern countries. According to him, the scientific advances and the industrial progress of the USA could benefit to ‘‘underdeveloped areas’’. By a syllogism Truman defined the developed world and the underdeveloped one.

This view is indirectly related to the concept of Modernity argued by Rostow who saw development as a process in 5 steps: The traditional society, the condition of the take off, the take off, the progress and the consumption society (Rostow 61-135). The thought of Rostow certainly reflects what, up to now, most actors of development and people understand by Development. It shows that there the western model of development is universal. Thus, all countries, nations and people must desire and imitate the model in order to satisfy their needs and frustrations.

In this idealistic point of view, the realization of Development recall the end of history in a Hegelian understanding like the one of Francis Fukuyama¹⁵. In other words, the acceptance of the economical (capitalism) and political (democratic) western model in all countries and for all, represent a philosophical end of history, by the fact that no other system will appear after. The history in that case is seen as being linear and having one destination: the satisfaction of all individuality through freedom to vote and freedom to consume. The capacity of consumption is considered as the indicator of the Development and the decline of poverty. The objective is to reach the right level of modernity, and in addition, to experience a democratic political system.

After 60 years of development, many schools of sociology and anthropology of development refute this idea. They argue that once you take this concept from its long-term history prospective and you confront it to reality, it falls for many reasons. For example, in 1999, the end of the USSR and the Cold War were supposed to send a wind of democracy and an economical boom on earth. In 2012 the results are under the shadow of doubts. In the other hand, they might accept that countries economy took off, but the link between the economical performance and democracy is still not proven as Fukuyama declared. In its 2010 Human Development Report, UNDP observed that: ‘‘ Even when countries progress in HDI¹⁶ (Human Development Index), they do not necessarily excel in the broader dimensions. It is possible to have a high HDI and be unsustainable, undemocratic and unequal just as it is possible to have a low HDI and be relatively sustainable, democratic and equal.

Despite this well-known observation, the most important unpredicted side effect contradicting this concept is the destruction of our environment and the global warming. The industrialization was supposed to be one of the key issues to reach the ideal stage of ‘‘Development’’. Unfortunately, the modern way of production and consumption showed an incredible ability to pollute the air, the water, the sea, and the soil, and finally a big part of the environment. For example, between 1960 and 1997, 10% of the Amazonian forest has been destroyed to be exploited (John R. MacNeill. 2010, 315). Anyone who adopts a skeptical understanding of these facts will defend the idea that we are in the period of change, and as always in the history, we will adapt. In that case, the idea of modernity

¹⁴ Halford Ross Ryan : The Inaugural addresses of twentieth-century American présidents, p.145. Greenwood publishing book. 1993.

¹⁵ According to Fukuyama Hegel idea of the end of the history is prophetic in many sense compare to Marx approach.

¹⁶ UNDP : 2010 Human Development Report- 20th anniversary édition. The Real Wealth of Nations : Pathway to Human Development, p.6

continues its recursive life with the risk that the adaptation, as the democracy for all, will be an ideal western prospective challenged by the time.

In another perspective, considering the speed of the destruction of the earth in the last 2 centuries, the continuous impact of human being action on nature, the risk on the environment and human being health, one can doubt the validity of the literal idea of modernity and Development. The best expression of this doubt is the emergence of the concept of sustainable development that finally considers and highlights the needs of the precaution principle. Thus, the classic concept of Development through the eyes of modernity seems to reach the end of its own history...if you are not a skeptical.

In opposition to the modernity approach, a current of thoughts raised in 1960: The post-developmentalists. These thinkers (sociologist, anthropologist, economist and social actors) from the North and the South of the hemisphere bring a critical vision of development. Their analysis, primarily based on the defeat of the development policies, want to give birth to innovative visions and concepts in economics, social welfare, cultural and ecological approach. People like Wolfgang Sachs from Germany, Esteva Gustavo from Mexico, Gilbert Rist from Switzerland, Arjun Apadurai an American-indian and the French Serge Latouche have opened new axes of reflections.

Rist, for example, developed the idea that development was a western belief that have contaminated the world but have not brought solutions to its own side effects. Wolfgang Sach and Esteva Gustavo argued that Truman called for a development race, which always keeps the same population behind, and this needs now to be questioned. Arjun Apadurai introduces the idea that globalization provokes heterogeneity and mixture, which links people but also create conflicts in a complex area of deterritorialized identities. In his side, Serge Latouche highlights that any approach and concept related to Development is based on the idea of growth economy, production, consumerism, with a trickle down effect that stays marginal. This is what he calls the “real development”, not a prospective development, but a picture of reality. According to Latouche, keeping the old idea of Development is keeping the destruction of nature on its path. This is why he developed the concept of De-growth and called for a new relation between human being and its environment, its economy, its social interaction.

In both of these concepts that help us understanding Development, the conclusion goes in the same direction: the heritage of the old idea of modernity and its implementation has an impact on the environment. This is irrefutable. The classic approach promotes the idea of sustainable development in order to face this challenge. In the other hand some of the post-*developmentalists* argue that there is an antinomy between the word Development and the word Sustainable. According to them, the roots and logics of Development don't change only with the addition of an adjective and limited projects. Therefore they also highlight that the concept of sustainable development has, itself, many interpretations¹⁷ that makes it even more opaque. This is why a specialist of Sustainable Development like Wofgang Meyer can observe that “there is up to now no general agreement on what “sustainable development” really should be and how it can be reached by global policy.

Indeed, everyone can estimate how much influence these concepts have on it own way of defining and understanding Development. Everyone can understand that Development is not only a simple word but and ideological acceptance, a social and economical model, it's a view on the futur, it's a will to create a common world. Thus, everytime one meets Development by promoting the exploitation and transformation of an element of our environment, by promoting new techniques and exploration of science, by promoting an economical paradigms link to the distribution of a new material, this one

¹⁷ According with Latouche, John Pesey from the World Bank, found thirty seven definition of sustainable development.

must question the kind of Development it is promoting. And if one makes the promotion of bamboo, it may need to analyze the impact of its promotion at different social and ecological levels.

It is right that Bamboo crossed history and was always close to the one who used it directly. It's a material used for housing, furniture's, kitchen utensils, pipes, baskets, written tablets in China. Some will say that there is 5000 use of bamboo. During the last 2000 years, bamboo was used out of modernity. This is why the industrial shape of bamboo is new¹⁸. If bamboo was commercialized all over the world during the 20th century, it mainly arrives at destination in a basic aspect. Craftsman made its transformation; the main users of bamboo were villagers, rurals, and famers. During centuries, bamboo was out of business. And we can say that, up to now, the same people use bamboo with a knowledge they have from the ancients. In rural India, a bamboo house doesn't need to be sustainable for 100 years, you can change the wall of your house after 5 or 10 years, the notion of durability is relative. What changed is that a material that crossed the concept of modernity so well, which didn't have so much value for decades, is becoming a lust resource.

The acceleration of the destruction of our environment, the impact of the chemicals in our agriculture, the global warming, the destruction of many tropical forests, brought bamboo as one of the new green material! But on the green market, bamboo is not only sold as an object but as a symbol. If it seems difficult to quantify the benefit of the symbolization of the giant grass, we know, at least, that all companies who sells bamboo, sells the symbol. We can also say that bamboo becomes a significant that signify much more than a useful material. On a website like alibaba.com, you can find 16 700 companies who sell bamboo items. All of them will speak about bamboo as a green symbol. They all promote the ecological character of bamboo in a way like:

“Bamboo is a fantastic resource that can be used for things such as building products, textiles, food, and medicine. This popularity can be attributed to the fact that bamboo is a very good material. It is naturally durable and is eco-friendly as well.”

But in addition to all the companies who sell bamboo and use the ecological symbol for marketing purpose, the image of bamboo starts to have its own symbolic life. Bamboo pictures, bamboo poles, drawings, painting, logos sell already more than bamboo, they sell the idea of ecology, green and, environmentally friendly. Take, for example, the famous green website “inhabitat”, if you look carefully at their logo, you will find out that it's a bamboo picture. This has reason, bamboo means green and environmentally friendly. If you look for bamboo product manufacturers on the Internet, you will get a result of more than 8 millions pages. Looking for bamboo logos, you will discover that a lot of companies, using this word, don't sell bamboo but services or other material. At least, most of them have a common sense: they appear as green and environmentally friendly entities. Then, Bamboo has a life in a dematerialized form : bamboo means green.

Based on the assumption above, we can infer than bamboo has 3 dimensions:

- An industrialized dimension that reflects the theory of the modernity.
- A symbolic dimension that shows the recognition of the environmental problem the world is facing, the globalization of the idea of the green economy but also the dematerialization of bamboo from a green material to a green symbol.
- The last dimension is a traditional use of bamboo, experienced by population from a large part of the world, who is considered outside of developed world or areas

¹⁸ Material like SWB (Strand Woven Bamboo), bamboo plyboard and fibers.

In fact these three dimensions are, together, simply the mirror of the “Real-Development”. Analyzing the evolution of the different kinds of exploitations of bamboo resources opens an interesting angle of analysis. This one helps to highlight the distortion between a hope in a greener and environmentally friendly world, and a reality that contradicts it. This distortion takes its roots in the contradictory interests of the actors who belong to the bamboo space of opinion¹⁹ and in the heritage of history.

For example, in its industrial “dimension”, bamboo is considered as environmentally friendly. We observe that the used of the raw material is enough to declare that the finished one is green. In that case, all companies who make bamboo products can use the green symbol for marketing. But most of the time, it is impossible to know at what degree the fabrication process is really respectful of the environment²⁰. Therefore, by their message, these companies call for a green industrialization. The question is: is it really happening or is it just a wish?

In its symbolic “dimension”, bamboo is definitely accepted as the representation of the green economy and a better care of our environment. Unfortunately, the significant signify a complex reality that is not visible. The symbol represent so many different aspects of bamboo that it seems to be more a catchall than a concrete significant. The word bamboo, as the image of bamboo, is vulgarized to primarily serve the marketing. The speech of consuming bamboo for a better environment seems disconnected of reality because of the complexity of the production of modern bamboo products. In fact, what bamboo signifies the best is not bamboo; it’s the environment, the need of a green economy. And if it’s not only use for business marketing, maybe the symbolic bamboo can contribute to a possible change of our perception of nature and a call for a Sustainable-Development?

Finally, with the traditional dimension, we know that million of people around the world are using bamboo without the mediation of the industrialization. They are most of the time far from the power of the symbols and the marketing ideas. They are often the most environmentally friendly user of bamboo. Bamboo is for them a natural resource that practically helps them to adapt to a place and respond to certain needs. This people who have, most of the time, a traditional use of bamboo, live at a certain degree in connection with the modernity. They are, like everyone, born in a world fragmented into rich-poor, developed-not developed. And with all, they share the global history of the ecology and the environment.

This is the same people some call for Development. Their possibility of development is often dependant of exogenous resources like NGOs, International Organization (United-Nations, World Bank) and private companies. Their local development doesn’t depend only on their capacity and creativity. At last, they belong to the “real-development” for decades, and for a lot of them, the modernity and its benefits seem to be a long way to go. A lot of them live in poor areas and contribute to the numbers given by the international organization to evaluate poverty. Thus, for the World Bank (2011 report), 1,3 billion of people live with less than 1,25 dollars per day, 1,1 billion of people don’t have access to potable water (WHO) and there are 925 million undernourished people in the world today (WFP). For many organizations, their chance is to have the opportunity to exploit the environment under the umbrella, one more time, of the Sustainable Development. Considering the fuzzy of the concept, there is certainly a need to define, in what sustainable development bamboo exploitation can participate? The fact is, that the interaction between the 3 dimensions, industrial, symbolic and traditional can easily unbalance the driving forces at the expense of the environment and community equilibrium. According to Meyer, “sustainable development is not restricted to the social

¹⁹ Understand bamboo space in a sociologic way as a area of tension, interaction, power between actors.

²⁰ We can also add to this argument the cost of the transportation on the environment

dimension of projects and programs and the economical and environmental impact must be measured by appropriate methods". The opposite is also true.

In conclusion

If poverty continues to decline, we all doubt that the Millennium Development Goal will be reached: ending poverty in 2015. But there is another reality; despite of the fight against poverty, we know that a Development for all is not possible if we maintain the same way of Development. Many analyses conclude that we would need another planet if everybody consume as they do in the western world. Of course, one can continue to be skeptical, but it is not sure that the belief in science, as we have since Descartes from the 17th century, will be able to respond to the emergency of the needs.

Therefore, considering the limitation of the "real-development" and at the same time, the needs of millions of people living close to green resources, the actors of Development, and the organizations who support them, might start to rethink the Development they want to promote. The transformation of bamboo, its economization and its production respond to different logics but evolve in the same space of interaction. Depending from which perspectives you inscribe your development, you will have different results and interactions. And when one call for local development, the question of the objective remains on hold. Would it be to get a raw material in order to develop an industry? Or to help a population to survive of the marginalization created by the modernity and the global market...and if so, for how long? In any case, the question of the environment and the social problems persists.

As the bamboo symbol is not sufficient to certify that a bamboo product is environmentally friendly, the word Development is not sufficient to believe that the exploitation of bamboo contribute to a Sustainable World. Yes, in the bamboo space of opinion, different interests of the actors reveal the distortion within Development. There is conflict of time, space and belief. For some there is an urgency to consider the ecological impact of any action, for others this message is mainly marketing and the time is not important; for some, the global and local are now in too much interaction to dissociate them, for other concentrating on the local is enough; finally, some continue to believe that modernity "will be" the solution by it own evolution, and some just doubt of this future. The development is facing an unexpected challenge that neither Truman nor Rostow could imagine from their space-time: the destruction of the environment require change. Thinkers like Paul Hawken, Amory and Hunter Lovins promote a Natural Capitalism, Serge Latouche campaigns for the de-growth of the world production and consumption, with the objective of a happy frugality. Hawken and latouche seat in the same space but at opposite side. Their contradictions reveal the need of new concepts but also the need of actions. It seems that the same is happening in the bamboo space and they need to be reveal. Oscar Hidalgo Lopez gave a social dimension to the exploitation of bamboo by designing affordable houses and since his first house; International Standards (ISO) has been validated. In the area of the environment and sustainability, an effort needs to be done by the actors in order to define more green standards in different frameworks (exploitation, industrialization, distribution). If not, bamboo will end as a green symbol and a simple material. About the Development, the "Sustainable Development", we certainly need to seat between Hawken and Latouche and take advantage of their contradictions. Considering the poverty, the importance of everyone environment, we might explore a new concept: "the realization". It might not be the time of the end of the history, but the time for the realization of the world!

References

- Bairoch (Paul), *Le Tiers Monde dans l'impasse, le démarrage économique du XVIIIe au XXe siècle* (troisième édition revue et augmentée), ed. Folio actuel, 2 000.
- Bourdieu (Pierre), *langage et pouvoir symbolique*, ed. Fayard, Paris, 2001.
- de Sardan (Pierre Olivier), *Anthropologie et Développement, essai en socio-anthropologie du changement social*, ed. Khartala, 1998.
- Dr Wolfgang Meyer: *Sociological Theory and Evaluation Research, an application an its Usability for Evaluation Sustainable Development*. University of Saarland, 2002.
- Fukuyama (Francis), *La Fin de l'histoire et le dernier homme*, D.-A. Canal (Poche - 14 septembre 1993)
- Guichaoua (André), *Goussault (Yves), sciences sociales et développement*, Paris, Armand Colin, 1993
- GWF Hegel, *Leçon sur la philosophie de l'histoire* Librairie philosophie J.Virin, 1963
- Juvin (Hervé), *Produire le Monde, Pour une croissance écologique*, Gallimard, Paris, 2008.
- Latour (Bruno), *Politique de la nature, Comment faire entrer les sciences en démocratie*, ed. La découverte, 1999.
- Marx (Karl), *Oeuvres*, edition Maximilien Rubel, Paris, Gallimard, t I et II, 2001.
- Morin (Edgard), *La méthode 1, La nature de la nature*, Le seuil, Paris, 2001.
- Origin and Development of Ecology/Shashi Singh Chauhan. Delhi, Vista International Pub., 2008.
- Ricœur (Paul), *l'idéologie et l'utopie*, ed. Seuil. 1997.
- Rist (Gilbert), *Le développement, histoire d'une croyance occidentale*, Presses de Science Po, Paris, 2001.
- Rostow (Walter W.) *Les étapes de la croissance économique*, Paris, Le seuil, 1963 ; édition anglaise : *The Stages of Economic Growth. A non-communist Manifesto*, Cambridge, Cambridge University Press, 1960.
- Sach (Wolfgang), « *global ecology, a new Arena of political conflict*, Londres, Zed Books, 1983.
- Sachs, *L'éco-développement : Stratégies pour le XXI^e siècle*, Syros, Paris, 1998.
- Simon Vélez, *Grown your own house*, ed. Buckminster Fuller, 1999.
- Zin (Jean) *L'écologie-politique à l'ère de l'information*, Editions è@e, janvier 2006.

Documents :

- UNDP Human development Report 2010-2011
- Global Footprint Network 2010 annual report
- United Nations, the Millenium Development Goal Report
- World Bank, World Development Report 2010-2011

Evaluation of Torrefied Bamboo for Sustainable Bioenergy Production

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Abstract

Bamboo is a potential sustainable biomass source for renewable heat and power production. Bamboo presents common fuel characteristics with other biomass feedstocks regarding heating value and chemical composition. Up to date, there are no studies on fuel properties of the bamboo specie *Guadua angustifolia*.

Bamboo is a difficult fuel and most thermal conversion processes have stringent fuel specifications, which are challenging to fulfil with biomass streams. Bamboo is tenacious and fibrous which makes it difficult and expensive to grind. Furthermore, the characteristics with regard to handling, storage and degradability are not favourable for biomass in general. The thermal pre-treatment torrefaction is a promising upgrading technology that can enhance the fuel quality by addressing these issues. During torrefaction, biomass is heated to 250-320°C in the absence of oxygen. At the end of the process the material is milled and compressed into pellets. In this way, the biomass becomes easy to grind, water resistant and has a high energy density. Alternatively, wet torrefaction (Torwash) allows for combined torrefaction and washing of the feedstock. Wet torrefaction, a form of hydro-thermal treatment, in addition to dry torrefaction removes salts and minerals from biomass, improving even more the quality of the product. This is in particular interesting for feedstock containing significant amounts of undesirable alkali components for combustion or gasification, as is the case of bamboo.

This paper presents an evaluation of the use of *Guadua angustifolia* as a fuel for heat and power applications. The results of biomass fuel properties and characteristics and quality improvement via dry and wet torrefaction are assessed. Torrefaction clearly shows the improvement of fuel properties and grindability of biomass. Wet-torrefied *Guadua angustifolia* is chemically an attractive fuel, with favourable fuel properties, e.g. the results showed a 98% of alkali removal, and the production of a grindable solid fuel.

Keywords

Bamboo, *Guadua angustifolia*, biomass pre-treatment, torrefaction, bioenergy, biomass/coal-cofiring, sustainable biomass.

1. Introduction

Bamboo is a potential sustainable biomass source for renewable heat and power production. Biomass is expected to play a major role in the transition to sustainable bioenergy production in the world. It is anticipated that in 2050 biomass could supply 30% of the total global energy consumption. Most of the energy will be produced via thermal conversion processes (combustion, gasification). The biomass used will be a combination of biomass residues, mixtures of biomass, waste and specially cultivated materials.

With view to maximizing the biomass share in the energy sector, biomass trade will become important, and regulations must be agreed upon so that the various biomass streams are produced in a sustainable way with positive social, economic and environmental impacts. Moreover, in order to simplify biomass trade and supply, the associated logistics and technology need to be optimized. Bamboo has the potential to be a sustainable biomass chain worldwide. The alternative production of bamboo as a bioenergy crop would generate local jobs and income for the poorest sectors in a rapid and continuous way. Natural areas of non-disturbed forest, generally associated to fragile ecosystems, could also be prevented from deforestation, which would guarantee their existence and allow the preservation of biodiversity in their areas of influence (Riaño et al. 2001).

Bamboo presents common fuel characteristics with many other biomass feedstocks regarding heating value and chemical composition (proximate/ultimate analysis). The quality and properties of biomass as energy source differ according to the specie, maturity stage, cultivation practices, (e.g. fertilizers application), etc.

The physical and chemical properties of unprocessed bamboo as a fuel alternative to coal do not meet in general with the stringent fuel specifications of most thermal conversion processes, as is also the case with most biomass streams. For blending biomass in coal-fired power plants and entrained flow gasifiers, very small particle size is required. Bamboo, like other woody and herbaceous biomass, is tenacious and fibrous, which makes it difficult and expensive to grind. The poor grindability of biomass is one of the limiting factors for the introduction of biomass on a large scale. Further, its characteristics with regard to handling, storage, degradability and energy density are not favourable when compared with coal. These problems can be addressed by pre-treating the biomass in order to increase energy density, grindability and storability. As a thermal pre-treatment option, torrefaction is a promising upgrading technology that can enhance the fuel quality by addressing these issues. Furthermore, wet torrefaction (Torwash), a form of hydro-thermal treatment, allows for combined torrefaction and washing of the feedstock and removes salts and minerals from biomass, improving even more the quality of the product. This is in particular interesting for (high moisture) biomass feedstocks containing significant amounts of undesirable alkali components for combustion or gasification, as is the case of bamboo.

Up to date, there are no studies on fuel properties of the bamboo specie *Guadua angustifolia*, as the reported studies on bamboo as energy source mainly refer to other species. *G.angustifolia* is a woody bamboo species, which is native to Latin America, particularly the regions of Colombia and Ecuador, although it grows in other regions. *G. angustifolia* is considered to be one the three largest species of bamboo and one of the 20most used worldwide (Londono 1998). In Colombia and particularly in the coffee region, *G. angustifolia* represents an important natural resource traditionally used by farmers to build long-lived products such as houses, furniture, handicrafts, veneers and flooring. (Camargo et al. 2010). A significant amount of it is not suitable for manufacturing products and is available from

processing sites and from forest resource management. These residues could be used for bioenergy production, providing a potential economic use for this material.

We have evaluated the properties of *G. angustifolia* as a fuel for heat and power applications. We carried out experiments using *Guadua* samples of 5 years age. The fuel was first subjected to ultimate and proximate analysis, and samples were subjected to dry and wet torrefaction. The results were evaluated comparatively to data available for other biomass species.

The paper presents the preliminary results and some early conclusions of the ongoing technical evaluation of the use of bamboo fuel in the power industry. This work forms part of the project “Torrefied bamboo pellets for sustainable biomass import from Colombia”. The project aims to assess the techno-economic potential and the sustainability of the bamboo specie *Guadua angustifolia* as a biomass supply chain for bioenergy production. The technical issues related to the final fuel application are of high importance in the assessment of the complete supply chain.

2. Bamboo as an alternative energy source

Recently bamboo has received increasing attention because of its easy propagation, vigorous regeneration, fast growth, high productivity and quick maturity. Bamboo is an efficient user of land and produces more biomass per unit area than most tree species. (Kumar, 2002).

The quality and properties of bamboo as a potential biomass source differ according to the bamboo specie, maturity stage, cultivation practices, (e.g. fertilizers application) production site, which will affect the final application or conversion method, that depends on the specific properties of the material.

Bamboo's proximate analysis and heating value is comparable to most woody biomass feedstock as well as most agricultural residues, grasses and straws as can be seen on Table 1. Bamboo data was collected from different sources (Chen et al. 2011; Kwong et al. 2007; Scurlock, 2000; Stanislav et al. 2010) and refer to bamboo species other than *G. angustifolia*. Other biomass data is from Verhoeff et al. 2011.

Table 1. Proximate analysis, O/C ratio and LHV values for typical biomass streams

Material	Ash (%)	C (%)	H (%)	N (%)	O (%)	LHV (MJ/kg _{daf})
Bamboo	0.8-3.5	44-51	5.1-6.1	0.07-0.78	40.9-46.5	17.1-18.7
Bagasse	3.1	46.6	5.7	0.2	44.5	18.2
Grass seed hay	10.6	42.4	5.8	1.6	39.6	18.1
Road side grass	23.2	38.4	5.3	2.0	31.1	19.2
Straw	10.6	42.2	5.7	0.4	41.0	17.3
Beech	0.3	45.9	6.2	0.4	47.3	17.7
Poplar	1.1	47.2	6.0	0.0	45.7	17.7
Willow	1.7	47.7	6.0	0.4	44.3	17.4
Larch	0.1	47.4	6.1	0.6	45.9	18.2
Pine	0.5	48.7	6.3	0.1	44.4	18.5
Spruce	0.3	50.4	6.4	0.0	42.9	19.7

The ash content of bamboo lies in between clean wood and herbaceous material. An example of ash composition is shown in Table 2. Bamboo composition presents critical fuel properties such as high alkali metal content which requires special attention for processing and combustion equipment.

Table 2. Chemical ash composition of bamboo based on high-temperature ash analyses (normalized to 100% ash), wt.%

	SiO ₂	CaO	K ₂ O	P ₂ O ₅	Al ₂ O ₃	MgO	Fe ₂ O ₃	SO ₃	Na ₂ O	TiO ₂	Sum
Bamboo	9.92	4.46	53.4	20.33	0.67	6.57	0.67	3.68	0.31	0.01	100

a. Thermal conversions options and relevant fuel properties

Bamboo, like any biomass, can be converted to heat and power, to liquid, solid or gaseous fuels and other chemical products through a variety of conversion processes. The available processing routes range from conventional uses of biomass such as firing for cooking and heating, to modern production processes like converting sugars into ethanol, to combusting and co-combusting biomass with coal for

power production, to further advanced technologies such as gasification and transport fuel production. The use of bamboo replacing coal and charcoal for heating is a common practice. However the use of bamboo for power generation is negligible or even inexistent. The use of biomass in the power generation industry today primarily refers to wood, energy crops (e.g. miscanthus and willow) and agriculture waste (e.g. straw).

The evaluation of any alternative feedstock for bioenergy applications requires careful consideration of the effects that feedstock characteristics and composition have on the conversion process.

The fuel properties are directly linked to its storage, transport and pre-treatment options, and can lead to significant logistical and cost components of the bioelectricity production chain. The physical properties (particle size, density and moisture content) as well as the chemical composition (elemental, ash and volatile matter) and energy content of a fuel affect its use in a thermal system. Properties such as moisture, density and volatile matter can well be influenced and controlled by established pre-treating biomass options. By drying, (partial) devolatilisation and subsequent compression and pelletisation, biomass renders more energy dense with properties similar to pulverized coal and an increased heating value.

In addition, the ash composition of the solid fuel determines its thermal conversion behavior; certain ash properties such as formation of low melting solutions can have detrimental effect on the process. The major inherent ash forming elements in biomass include Si, Al, Ca, Mg, Na, Fe, K, S, and P. Some of them, such as K/Na and Cl cause operational problems such as slagging and fouling to the power plant units. In particular the high potassium (K) content of bamboo increases the risk of slagging, fouling, corrosion and in fluidized bed systems also agglomeration.

b. Biomass pretreatment options

Biomass supply chains consist of the following elements: production and harvesting, transportation, handling, storage, sizing, pre-processing (drying and/or other pre-treatment), and feeding. Furthermore advanced pre-treatment options include torrefaction (dry and wet).

Torrefaction is a mild temperature treatment at a temperature level of 250 to 320 °C, at near atmospheric pressure in the absence of oxygen. The occurring decomposition reactions at this temperature level cause the biomass to become completely dried and to lose its tenacious and fibrous structure. In addition, torrefaction increases the calorific value and the biomass hygroscopic nature can be destructed to yield a hydrophobic material. In combination with pelletisation, it enables energy-efficient and cost-effective production of pellets with superior properties in terms of high energy density (1.5-2 times than conventional pellets), excellent grindability and water resistant nature (eliminating/reducing biological degradation and spontaneous heating, enabling outdoor storage). For the large-scale biomass import, which is relevant to areas with low biomass resources, transportation costs can be dramatically reduced.

Dry torrefaction does not affect the inorganic (ash) composition of biomass. Alternatively, wet torrefaction is a pre-treatment of biomass in presence of water at a high pressure and temperature. This process called Torwash allows for combined torrefaction and washing of the feedstock and therefore removes soluble salts and minerals from biomass, improving further the quality of the product. This is in particular interesting for feedstocks like bamboo that contain significant amounts of undesirable alkali and/or chlorine components that affect combustion or gasification. With alkali and chlorine removal, corrosion and bed agglomeration caused by high salt content during the combustion process are substantially diminished.

3. Torrefaction of Biomass at ECN

The available torrefaction facilities at ECN, allow for both the research investigating the most important aspects of torrefaction, as well as further development of the concept to a pilot-plant incorporating ECN's torrefaction concept.

At ECN several feedstocks were tested in the past, and included various herbaceous and woody (both coniferous and deciduous) biomass streams (i.e. bagasse, grass seed hay, road side grass, straw, pine, willow, poplar, larch and spruce) in order to get insights into the torrefaction characteristics of these feedstocks and the properties of the torrefied material produced. The milling behaviour was determined by grinding the different materials to various particle sizes in a lab-scale cutter mill, while investigating the power consumption during grinding. **Figure 1** shows some of the results of these size reduction experiments carried out on dried and torrefied grass seed hay (GS), road side grass (RG), straw (ST), beech (BE), willow (WI), pine (PI) and spruce (SP) as well as coal.

From a technical point of view, torrefaction has a similar impact for all relatively dry lignocellulosic biomass feedstock and it may be attractive for the upgrading of certain mixed waste streams as well. It can be concluded that for all the materials tested, it is very beneficial to torrefy the material before grinding. In all cases the power consumption is reduced drastically when the material is torrefied.

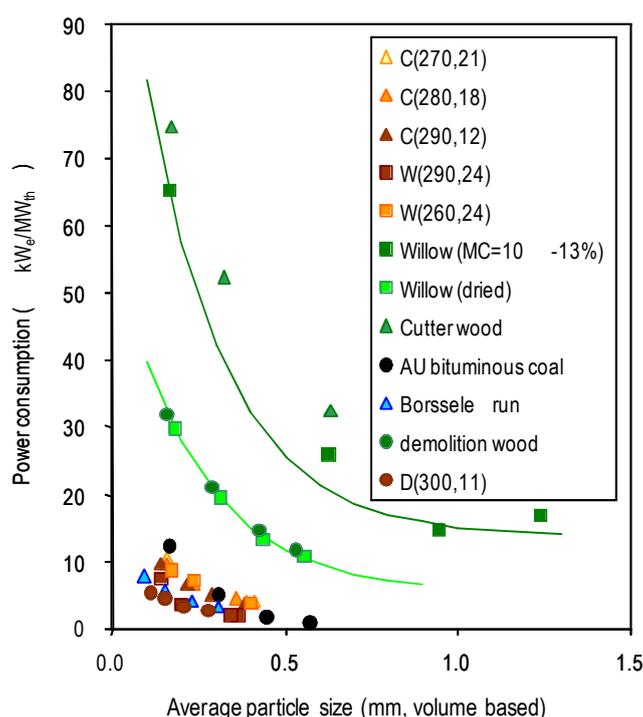


Figure 1: Power consumption as a function of final particle size (torrefaction conditions in brackets, temperature in °C, residence time in minutes) (Verhoeff, 2011)

4. Evaluation of bamboo as an alternative biomass fuel

Exploratory and conclusive experimental work is required to tackle technical aspects using any novel biomass fuel in the heat and especially the power industry. Biomass characterization and pretreatment tests were performed at preliminary stage.

Samples of *Guadua angustifolia* were received from a plantation in Colombia. The harvested *Guadua* was 5 years old. The material was sun dried in the harvesting location. The feedstock had a moisture content of 10-16 wt%, with particle sizes of 40x40x15 mm.

4.1 Chemical composition

The samples of *G. angustifolia* were analyzed for proximate, ultimate and ash composition. The results are shown in Table 3 and are compared to other solid fuels analyzed at ECN.

Table 3. Comparison of the ash composition of several biomass samples with *G. angustifolia* based on ICP ash analyses carried out at ECN

Fuel	Russian Coal	Lignite	Wood	Olive kernel	Cynara cardunculus	Shea meal	<i>Guadua angustifolia</i>
Moisture	10.4	48	7.1	5.78	11	11.13	16.4
Proximate analysis (% mass, dry fuel basis)							
Ash @ 815°C	8	27	1.44	6.29	5.1	5.41	6.3
Volatile matter	32	45	80	72	75	61.9	70
HHV (KJ/kg)	27800	15000	20093	20000	19000		18750
Ultimate analysis (% mass, dry fuel basis)							
C	68	41	50.25	48	42	49.4	47.1
H	4	2.4	6.13	5.75	5.5	5.35	6.05
N	0.87	1.1	0.37	1.1	0.55	2.61	1
S	0.35	0.67	0.026	-	0.15	-	0.125
Ash composition (mg/kg fuel, dry basis)							
O by diff.	11.6	31	44.2	38	43	40.05	44
Na (± 7)	405	775	191	1300	4100	179	111
Mg (± 1)	1277	6850	404	1800	1500	1937	405
Al (± 4)	16583	9000	474	1200	160	772	339
Si (± 90)	34841	20000	1331	6200	650	1861	12143
P (± 15)	386	250	122	620	910	1684	770
K (± 20)	2390	1600	984	8900	12000	20789	23029
Ca (± 20)	2750	110000	1919	13000	12000	2145	344
Ti (± 8)	622	395	96	76	8,6	47	12.5
Mn (± 6)	89	130	66	35	17	24	6.5
Fe (± 4)	6077	9700	301	1800	110	1095	140
Zn (± 1)	21	9.3	25	12	13	3.6	10.7
Pb (± 20)	10	~5	8	25	3,5	1.9	0
Sr (± 5)	183	170	11	15	59	18.3	6.9
Ba (± 5)	260	78	29	11	26	22.4	8
S	3500	6700	260	860	1500	2704	1284
Cl (± 20)	100	76	253	2000	2800	797	568

From the *G. angustifolia* analysis performed at ECN it is clear that the chlorine (Cl) content (600 mg/kg) is higher than average wood (100 mg/kg), and less than the average herbaceous crops (as

cynara). The potassium (K) concentration in *G. angustifolia* (23000 mg/kg) is much more than in wood. Most other ash forming components are lower than in herbaceous crops and more comparable to wood with a noted exception of silicon (Si).

From the above information it is obvious that *Guadua's* physical and chemical composition may place it well among the grass and woody biomass. This renders it attractive as a fuel, but the high K and Si content must be kept in mind.

4.2. Torwash results of *G. angustifolia*

The samples were subjected to Torwash experiments in a 20 l autoclave under elevated pressure and 200°C. The produced material was mechanically dewatered. Table 4 shows the results from the Torwashed product characterization compared to the untreated or raw *G. angustifolia*.

	Proximate & ultimate (% mass, dry fuel)		Ash composition (mg/kg fuel, dry fuel)	
	Raw	Torwashed	Raw	Torwashed
ash @ 815°C	6,3	4,5	Na	111
			Mg	405
			Al	339
HHV (KJ/kg)	18750	20000	Si	12143
			P	770
C	47,1	50	K	23029
H	6.05	5,8	Ca	344
N	1	2,7	Ti	12,5
S	0.125	0,026	Mn	6,5
			Fe	140
			Zn	10,7
			Sr	6,9
			Ba	8
			S	1284
			Cl	568

The heating value of the torwashed *G. angustifolia* was 11% higher than of the raw material. The pre-treated material contains lower concentrations of all its inorganic components, except for Si and Ca. The removal of K is 98% and of Cl is 78% while the removal of Na was 73%. This is a dramatic improvement of the inorganic elements critical for utilization as a fuel. Typically, 500 mg/kg alkalis (K and Na) can be interpreted as a limit for fuels that will not cause any problem in co-firing.

Additional to alkali removal, the Torwash treatment gave very good and promising results:

- Torwash breaks down the fibrous structure of the material and makes milling possible.
- A series of single test pellets was made with a material density of 1200-1300 kg/m³, which indicates that a somewhat higher density than regular torrefied pellets is possible, exceeding the material density and energy density of regular wood pellets.
- As a result of the Torwash treatment, mechanical dewatering is easy. On lab-scale, the resulting cake had a moisture content of only about 30 wt%.

Note that these results are preliminary and need confirmation in larger scale tests.

5. Conclusions and outlook

Bamboo presents common fuel characteristics with other biomass feedstocks regarding heating value and chemical composition. Bamboo is tenacious and fibrous which makes it difficult and expensive to grind. Furthermore, the characteristics with regard to handling, storage and degradability are not favourable for bamboo as is the case of biomass in general.

The hydrothermal treatment (wet torrefaction of Torwash), removes salts and minerals from biomass, improving also other qualities of the product, such as grindability and moisture content. This is in particular interesting for feedstock containing significant amounts of undesirable alkali components for combustion or gasification, as is the case of bamboo.

Samples of *Guadua angustifolia* were received from a plantation in Colombia. The harvested *Guadua* was 5 years old. Biomass fuel analysis and pretreatment tests were performed.

From the fuel characterisation results we conclude that *G. angustifolia* is a potential solid fuel due to its elemental composition and high heating capacity. Properties are similar to those of clean wood rather than other herbaceous feedstocks, except for alkali content, which in bamboo is quite high. When the material is hydrothermally pre-treated with wet torrefaction (Torwash), it is possible to eliminate two of the main characteristics that may prevent bamboo from being co-fired: first, the high alkali content as it removes 98% of alkali (K and Na) and second it breaks down its fibrous structure, making milling possible. It is also expected to be easy to pelletize Torwashed bamboo.

The preliminary results form the basis for the complete technical assessment of using *G. angustifolia* as an alternative energy source. Further research in this project includes specific conversion and combustion tests.

The ultimate goal of the technical assessment is to address the suitability and options to adapt this promising fuel to the existing power industry. This requires in-depth knowledge of the fuel behavior in thermal conversion systems as well as optimum pretreatment conditions and techniques. ECN expertise on torrefaction and combustion are of key importance in successful integration of bamboo in the bioenergy market in Europe.

The technical issues related to the final fuel application will be part of the techno-economic and sustainability assessment of the complete supply chain. The techno-economic and sustainability analyses of this testing biomass chain will allow for innovation and technology development, while promoting the marketing of sustainable biomass.

Acknowledgement

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Our gratitude goes to the partners in the project: Technological University of Pereira (COL), The Colombian Bamboo Society (COL) and Imperial College London (UK).

References

- Camargo, J.C.; Moreno, R.; Villota, N. 2010. Sustainable management of guauda bamboo forest, Colombia. ETFR News. Vol 52. Tropenbos International, Wageningen, the Netherlands.
- Chen, W.H.; Cheng, W.Y.; Lu, K.M.; Huang, Y.P. 2011. An evaluation on improvement of pulverized biomass property for solid fuel through torrefaction, *Appl Energy*, (88), 3636-3644.
- Kumar, A.; Ramanuja Rao, I.V.; Sastry, C. 2002. Bamboo for sustainable development. Proceedings of the 5th international bamboo congress and the 6th international bamboo workshop. San José, Costa Rica
- Kwong, Ph.; Chao, Ch.; Wang, J.H.; Cheung, C.W. Kendall, G. 2007. Co-combustion performance of coal with rice husks and bamboo. *Atmospheric Environment* (41), 7462–7472.
- McNeil Technologies, Inc. 2003. Biomass Resource Assessment and Utilization Options for Three Counties in Eastern Oregon. Oregon Department of Energy.
- Riaño, N.; Londoño, X.; Gómez, J.; López, Y. 2001. Preliminary data on quantification of the carbon sump effect by guadua (*Guadua angustifolia* Kunth). Proceedings of the International Workshop on the Role of Bamboo in Disaster Avoidance. International Network for Bamboo and Rattan (INBAR)
- Scurlock, J. M. O. 2000. Bamboo: An overlooked biomass resource?, *Biomass and Bioenergy* (19), 229-244.
- Stanislav, V; Baxter, D.; Andersen, L.K.; Vassileva, Ch.G. 2010. An overview of the chemical composition of biomass, *Fuel* (89), 913–933.
- Verhoeff, F; Pels, J.R.; Boersma, A; Zwart, R.; Kiel, J. 2011. *ECN torrefaction technology heading for demonstration*. Proceedings of the 19th European Biomass Conference and Exhibition (EU BC&E), ICC Berlin, Germany.

Accelerated and graveyard tests applied to bamboo strips treated by pressure and by immersion process

Antonio Beraldo

Abstract

Despite several advantages showed by bamboo if compared to conventional materials applied in building constructions, most of the bamboo species experience pronounced decay by biological organisms, as fungi and insects. So, researches aiming to develop efficient treatments applied to bamboo and to bamboo-based materials are very important to enhance its service life. In this research work it is reported the behavior of giant bamboo specie (*Dendrocalamus giganteus* Munro) strips treated with pressure and immersion process, employing two chemical solutions: CCB (chromium, copper and boron) and ABB (sodium octaborate). Scanning electronic microscope (SEM) analysis permits an advance the understanding of the interaction between the anatomical bamboo elements and the chemical products distribution pattern. Accelerated (56 days) and graveyard tests (30 months) indicated the best performance of the strips treated with CCB against bamboo decay.

Keywords

chemical treatment, SEM, CCB, ABB, sodium octaborate

Introduction

Bamboo is a renewable and low cost raw-material exhibiting interesting physical as well as mechanical properties, showing many possibilities of applications. Nevertheless, one of the most important drawbacks showed by bamboo is the short service life of the most of the species (Ashaari and Mamat 2000).

Several researches were developed aiming to enhance bamboo resistance against decay as reported by Hidalgo-López (2003). Compared to the wood, bamboo anatomical structure differs mainly by the absence of cell rays which inhibits a homogeneous preservative products distribution of the across the culm thickness (Kumar et al. 1994; Liese 2003; Gonzales and Gutierrez 1995; Beraldo and Ferreira 2008).

Lee et al. (2001) employed CCA as preservative and reported that “was much more difficult to penetrate bamboo than Southern pine under the same treatment conditions”.

Among the treatment process applied to bamboo, sap displacement and immersion/diffusion were the most employed, mainly in rural areas. Several products were tested aiming to enhance bamboo service life, as inorganic solutions (CCA –chromium, copper and arsenic, and CCB oxide – chromium, copper and boron), as reported by Yang and Hui (2010).

Despite its great porosity, bamboo cells sometimes are blocked by air bubbles (Gonzales and Gutierrez 1995) thus decreasing the treatment effectiveness.

Besides the type of process employed, others several parameters are considered important for evaluating the treatment performance: specie considered, age, position of the specimen in the culm (bottom, middle or top), time after felling, specimen’s geometric characteristics (culm or split), environmental conditions (season, temperature and relative humidity, soil type), time period and exposure type (protected or not).

Suprapti (2010) evaluated the resistance of three Indonesian bamboo species against fifteen types of fungi attack. Results showed that bamboo performance depends on the fungi genus (soft-rot, white and brown rot). The bamboo decay grade is related to the specific enzymes action on the bamboo cell wall compounds.

2. Methodology

2.1 Harvesting, manufacture and bamboo strips treatments

Strips (2.0 cm x 30.0 cm x 1.5 cm) were cut from culms of 6 years old giant bamboo (*Dendrocalamus giganteus* Munro) (Figure 1a and 1b). Ten strips were obtained from each culm and one of them was prepared for Scanning Electronic Microscope (SEM) analysis. The others strips were air-dried and stored in a plastic box, carefully protected by a nylon net, aiming to avoid previous borer attack (*Dinoderus minutus* Fabricius). After drying to 15% average moisture content, two process treatments (by pressure or by immersion) were applied to the bamboo strips. Selected chemical solutions were: MOQ OX50 (chromium 63.5%, copper 26.0% and boron 10.5% – CCB oxide form) and sodium octaborate tetrahydrate (boric acid 40.0% and borax 60.0% – ABB).



Figure 1. a) Culms from *D. giganteus*; b) Bamboo strips.

Strips treatments were carried through the following protocols:

a) By pressure: full cell; initial vacuum (660 mmHg) during 30 min; average pressure of 10.5 kgf.cm⁻² during 60 min; final vacuum of 660 mmHg during 5 min. MOQ OX50 (CCB oxide) and sodium octaborate (ABB) retentions were 4.0 and 6.5 kg of active elements per cubic meter as employed by Lee et al. (2001).

b) By immersion: strips were soaked in chemical solutions for one and two weeks; retentions – CCB: 4.0 and 6.5 kg of active elements per cubic meter and ABB at 8% of concentration. Ashaari and Mamat (2000) reported that even a simple immersion in water could easily decrease starch content in the bamboo strips.

As comparison, *Pinus* sp stakes (5.0 cm x 50.0 cm x 2.5 cm) were treated only by pressure with the same retention values of the bamboo strips (4.0 and 6.5 kg of active elements per cubic meter).

2.2 Retention analysis

Retention of chemical elements copper, chromium and boron were analyzed by atomic absorption spectroscopy using Varian Spectra A220 equipment, according to AWPA 11-93. Contents of elements presents in bamboo were expressed by kilogram per cubic meter of treated bamboo sample.

2.3 Accelerated test

Treated bamboo strips were submitted, after drying, to the accelerated tests at the Technological Research Institute (IPT – SP).

a) Fungi decay. The IPT Method n° 1157 – Part D5, 1980 – “Accelerated laboratory test for the determination of preservative efficiency against soft rot” was carried. Specimens were placed in a controlled room until attaining mass constancy and then weighted. Bamboo splits were then soaked into a non sterile garden soil with moisture content adjusted to 180% of its water retention capability. Bamboo strips remained in that controlled room for 16 weeks. At the end of that period, specimens were removed, carefully cleaned, and after mass stabilization they were again weighted. The efficiency of the treatment employed process or those of the product were carried by the evaluation of the mass loss (%), when compared with the initial ones. It was expected a strip mass loss to occur due to the soil microorganism activities.

**Eucalyptus grandis* stalks were also evaluated as reference (control).

b) Termites attack. The method applied was an adaptation of the “IPT Reference Method –IPT Publication n° 1157 – Part D2, 1980 – “*Termites attack resistance determination*”. Specimen’s dimensions were approximately 7.0 cm of length and 2.3 cm width; thickness was variable according to the position occupied by the strip in the culm. It was employed six replications of 40 termites (39 workers and 01 soldier) from *Cryptotermes brevis* specie (Kalotermitidae family), a dry-wood decay insect. Bamboo specimens were laterally recovered with aluminum paper letting only the upper surface exposed to the termites attack.

Test was conducted in a controlled room during 44 to 48 days. At the end of the test, it was evaluated the degree of attack provoked by the termites: **0** (none), **1**(light), **2** (moderate), **3** (hard) and **4** (deep). Percentage of died termites was also read.

**Pinus elliottii* stakes were also evaluated as reference (control).

2.4. Graveyard test

Test was conducted according to the ASTM D 1758-02 and AWWA E7-07, with respect to the field of installation, the distance between the specimens, the procedure and the interval of evaluation. The Campinas State University campus soil was classified as a red distroferic latosol, showing high clay content. Bamboo strips were randomly distributed in the field and buried into the soil at the half of its length, i.e. approximately 25 cm (Figure 2).

For almost all of the bamboo strips it was observed in a few days a quick superficial attack, denoted by green- like spots on the inner region. In a few weeks, that surface becomes completely dark (Figure 3). However, it was expected that preliminary attack to be not properly related to the fungi action, which needs a longer period of exposure.



Figure 2. Strips distribution in the field.



a) One week b) 2 months c) 3 months
Figure 3. Fungi decay along the exposure period.

After 6 months of exposure, strips were evaluated by 5 persons according to the AWPA recommendations. Firstly, it was applied a small impact with the foot on the strip at the contact zone with the ground; if a fail occurs and the strip breaks it was attributed grade “0” and the strip was considered as “destroyed” and it was discarded for further evaluations. If the strip did not break, it was carefully cleaned with a metallic blade and observing a possible degree of decay and/or termites attack, according to the grade showed at Table 1a and 1b, respectively. Strips evaluation was conducted until 30 months of exposure at 6 months of interval between the evaluations.

Table1. Scheme for bamboo strips grading.

a) Soft-rot decay	Classification	b) Termites attack
Description of the condition	Grade	Description of the condition
without decay or with superficial decay	10	without attack, or with 1 to 2 bites
0% to 3% of decay	9	0% to 3% of attack
3% to 10% of decay	8	3% to 10% of attack
10% to 30% of decay	7	10% to 30% of attack
30% to 50% of decay	6	30% to 50% attack
50% to 75% of decay	4	50% to 75% of attack
destroyed	0	destroyed

2.5 Scanning Electronic Microscope (SEM)

Specimens were prepared at Structural Constitution Laboratory (LCE), from Materials Engineering Department (DeMA), at São Carlos Federal University (UFSCar). Firstly, specimens were recovered with a thin gold layer (10 nm), in a Sputter Balzer equipment. Electron dispersion spectroscopy (EDS), performed at a XL 30 TMP – Philips equipment, was employed aiming to detect the occurrence of natural bamboo chemical elements or those belonging to the preservative solution retained in the bamboo cells. However, because it's small atomic number, boron could not be detected by this technique. So, only chromium and copper, from CCB solutions, and sodium, from ABB solutions, could be detected in some regions of the specimens (Figure 4a).

3. Results and discussion

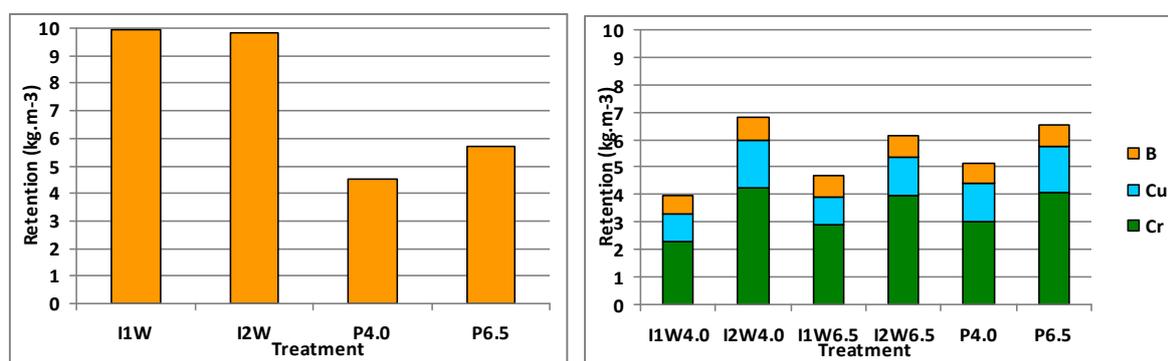
3.1 Chemical elements retention on bamboo tissues

Strips treated by immersion with ABB solution (8.0%), during one or two weeks (I1W and I2W), shows high boron content (10 kg per cubic meter), denoting the easiness of the boron absorption by bamboo tissues. According to Gonzales and Gutierrez (1995), *Guadua* culms, treated by Boucherie process, were totally impregnated with boron. However, there is an important difference with respect to the correct preservative content into the bamboo cells, because strips treated by immersion or by pressure process are also recovered by a chemical products layer.

Higher the pressure greater boron retention (P4.0 and P6.5), but retention was lesser than those obtained by immersion process (Figure 5a).

For CCB, strips treated by immersion or by pressure process show small boron and copper content variability according to the period of immersion or with the concentration of the solution when pressure process was applied; however, it was noted that chromium content depends on these parameters – for two weeks of immersion (I2W4.0 and I2W6.5) or when concentration was of 6.5 kg per cubic meter (P6.5), chromium content has increased (Figure 5b).

However, for *Phyllostachys pubescens*, Lee et al. (2001) reported that the retention of CCA was only 22-23% of the targets similar to those employed in this work. Authors suggested that higher concentrations, longer treatment period and/or higher pressure probably yield better results in terms of chemical retention in bamboo cells.



a) ABB solution: boron retention. b) CCB solution: boron, copper and chromium retentions.
Figure 5. Chemical elements retention on bamboo tissues.

3.2 Traceability of chemical elements by Electron Dispersion Spectroscopy (EDS)

3.2.1 CCB solution

Boron has a small atomic number and cannot be detected by the equipment employed in this investigation. Nitrogen was eliminated at the analysis because it shows high content in all of the specimens (~ 80%, in the most of the cases).

Some chemical elements detected in the analysis were not from the treatment itself, but it was originated from the soil particles (K and Fe) or from the soil fertilizers (Ca and Mg); chlorine was originated from the soil but also from public water treatment. Silicon belongs naturally to the bamboo structure, and its content is expected to be higher at the outer region of the culm.

For a bamboo strip treated by pressure with CCB (at 6.5% concentration), the percentage of chemical elements retained in the specific bamboo anatomical regions (vessels, fibers and parenchyma cells) and on three others selected regions of the specimen (a, b and c) as shown at Figure 6. These regions (a, b and c) were randomly chosen at the fiber transverse direction in image magnification of 50X. However, in some cases there was a great probability of vessels occurrence in these regions, enhancing the average content with respect to those regions without vessels.

Due to their greater dimensions, vessels are more easily fulfilled by chemical elements (Gonzales and Gutierrez 1995; Beraldo and Ferreira 2008).

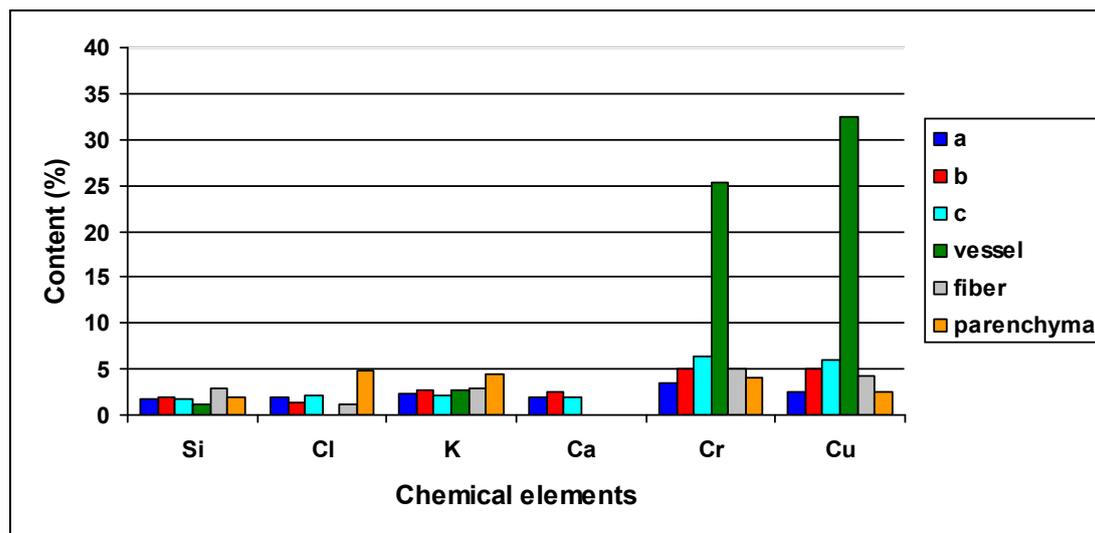
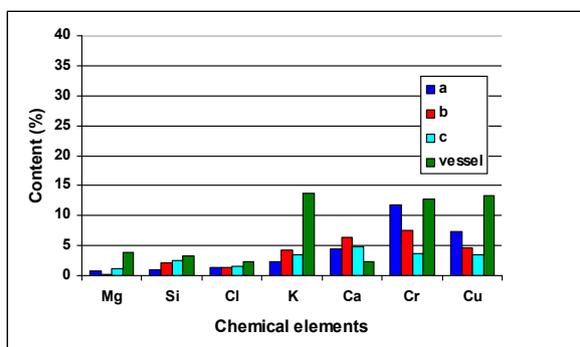


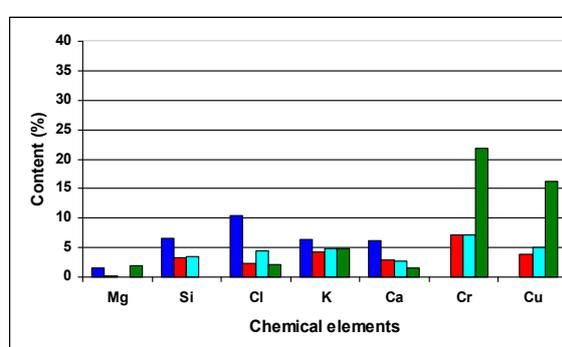
Figure 6. Percentage of the chemical elements distributions (Pressure - CCB solution at 6.5%).

a) Immersion treatment

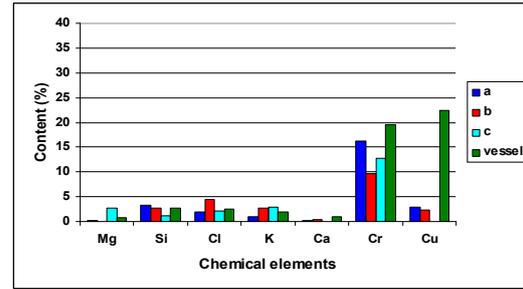
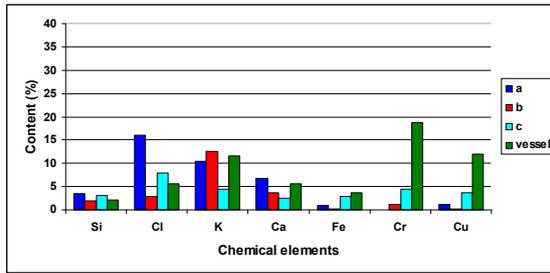
For the same concentration, the longer the period, the higher the chemical elements percentage was noted (Figure 7). Also, there is a tendency of the higher the concentration more efficient was the treatment. However, some results were unexpected which can be attributed to the type of selected region for the analysis; the absence of chromium and copper in some regions **a** indicated that region was near of the outer region, with high silicon content and occurrence of vessels with small diameters. In this case, the deposition of the copper did not occur.



a) One week – 4.0 kg.m⁻³



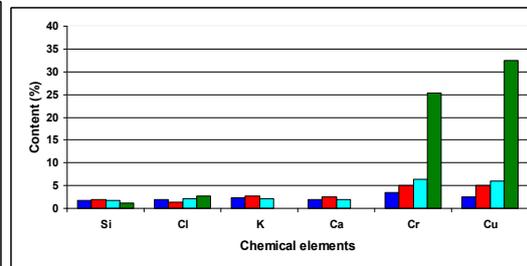
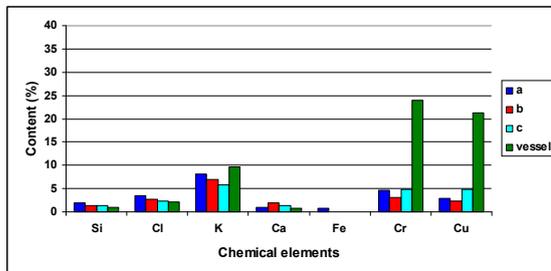
b) 2 weeks – 4.0 kg.m⁻³



c) One week – 6.5 kg.m⁻³
 d) 2 weeks – 6.5 kg.m⁻³
Figure 7. Percentage of the chemical elements distribution. Immersion. CCB solutions.

b) Pressure treatment

It's clear that higher chemical elements content occurs only at the vessels (Figure 8). That fact corroborates to the great difficulty to carry homogeneous treatment on bamboo even by pressure. The absence of ray cells combined with a considerable vessel obstruction in the aged bamboo inhibits the flow toward the parenchyma cells.



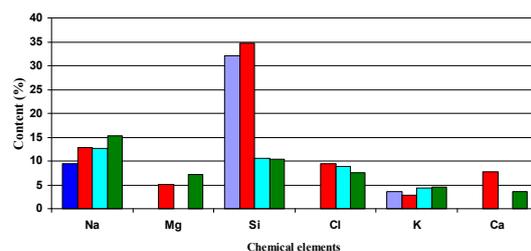
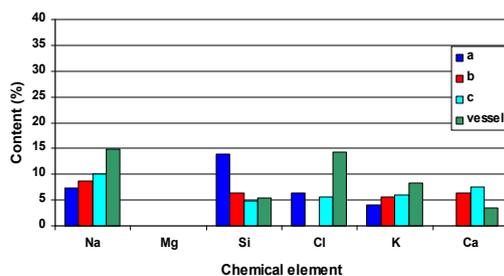
4.0 kg.m⁻³
 6.5 kg.m⁻³
Figure 8. Percentage of the chemical elements distribution. Pressure. CCB.

3.2.2 Sodium octaborate (ABB) solution

Due its small atomic number, sodium shows greater mobility as compared with heavy metals (chromium and copper). So, it was expected the ABB treatments to show better performance in terms of distribution homogeneity into the bamboo cells.

a) Immersion at 8.0%

Apparently, this treatment produces more homogeneous results, showing the average sodium content of 10% (Figure 9). Treatment duration (one or two weeks) did not play an important role, probably due to the great mobility of the sodium and by the high higroscopicity of the bamboo cells. Some analyzed regions clearly belong to the outer culm regions (high silicon content), at the regions **a** (one week, Figure 9a) and regions **a** and **b** (two weeks, Figure 9b).



a) One week
 b) 2 weeks
Figure 9. Percentage of chemical elements distribution. Immersion. ABB at 8.0%.

b) Pressure

These results were similar to those obtained with the strips immersion in ABB solution at 8.0%. However, in this case sodium distribution was more homogeneous (average of 12%), mainly for strips treated with 6.5 kg of active elements per cubic meter (Figure 10).

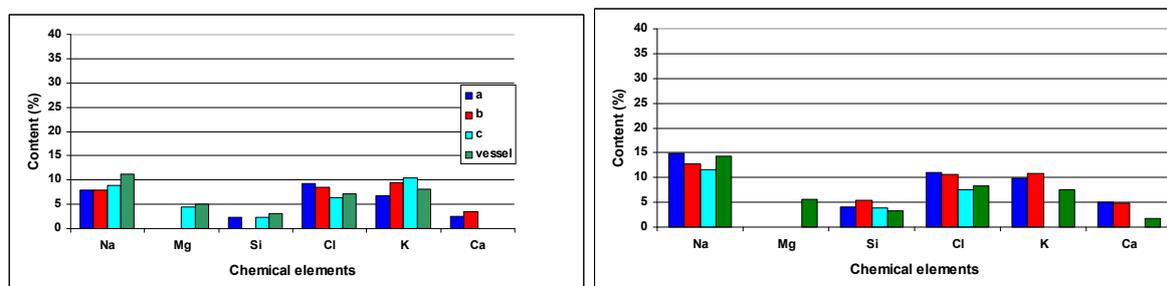


Figure 10. Percentage of the chemical elements distribution. Pressure ABB.

3.3 Accelerated tests

3.3.1 Termites attack

After 44 to 48 days, reference and treated bamboo strips did not show a great degree of attack by *Cryptotermes brevis* specie. Even when strips experienced certain attacks, their degrees fall at level 1 (light). In the other hand, stakes of *P. elliotii* (reference ones) showed a severe attack (average 3.75) and termites mortality was 53%. For bamboo strips treated by immersion or by pressure, termite's mortality was 88% and 77%, respectively. It seems that, for this termite specie, specialized in dry-wood attack, bamboo shows a natural resistance, probably due to its fibers lignification that occurs in great degree at seasoned culms of 6 years old.

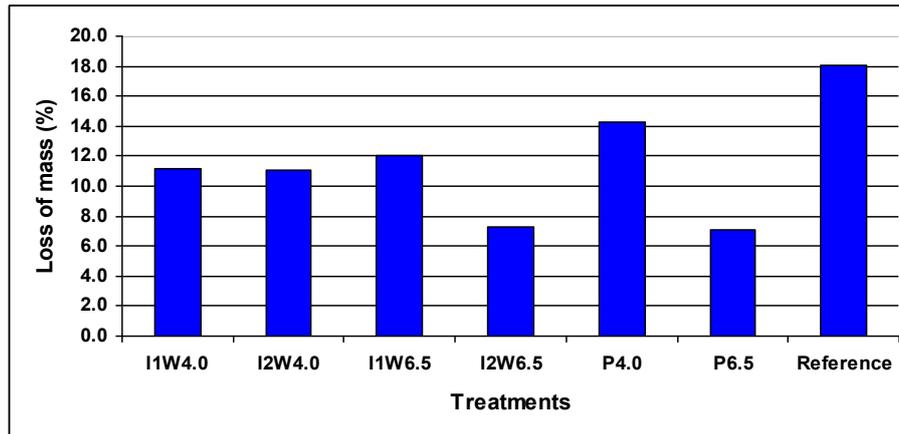
Other possibilities for the apparent resistance of the bamboo strips were the short period of exposure, the employed termite specie and a bamboo structural composition. Ashaari and Mamat (2000) reported that termites prefer cellulose than starch.

3.3.2 Soft-rot decay

Averages mass loss for bamboo strips treated with CCB or ABB are shown on Figures 11a and 11b, respectively. For those specimens treated with CCB, strips mass loss was significantly lesser (6.5 to 14.0%) than the reference ones (18%). This fact is less evident for those bamboo strips treated with ABB, except for the treatment I2W8.0 (immersion during 2 weeks in 8% ABB concentration). That fact can be explained because boron leaches when exposed to the wetted places, as the soil utilized in this controlled room.

Ashaari and Mamat (2000) reported that for three bamboo species mass losses ranged from 13.2% to 15.9%.

As comparison, the mass loss of *E. grandis* as reference was 14.4%.



(a) CCB solutions.

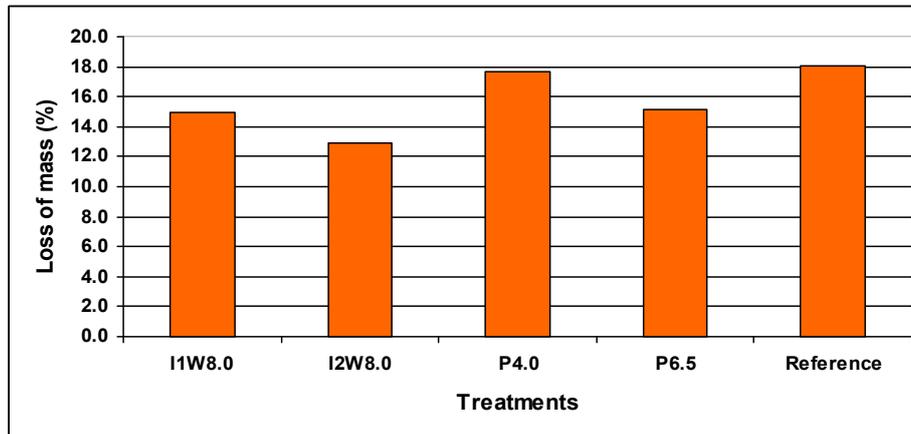
I1W4.0 = Immersion, 1 week, 4.0 kg.m⁻³I2W4.0 = Immersion, 2 weeks, 4.0 kg.m⁻³P4.0 = Pressure, 4.0 kg.m⁻³I1W6.5 = Immersion, 1 week, 6.5 kg.m⁻³I2W6.5 = Immersion, 2 weeks, 6.5 kg.m⁻³P6.5 = Pressure, 6.5 kg.m⁻³

ABB solutions.

I1W8.0 = Immersion, 1 week, 8.0%

P4.0 = Pressure, 4.0 kg.m⁻³

I2W8.0 = Immersion, 2 weeks, 8.0%

P6.5 = Pressure, 6.5 kg.m⁻³.

Figure 11. Loss of mass (%) of the treated bamboo strips.

Statistical analysis showed significant differences at 95% of probability level by Tukey's test, among the treatments applied to the bamboo strips. So, it was possible to range the effectiveness of the treatments with respect to the mass loss of the bamboo strips.

*“=” means that there is not statistical differences between the treatments at 95% probability level.

- a) CCB – pressure, 6.5 kg.m^{-3} = CCB - immersion, 2 weeks, 6.5 kg.m^{-3} .
- b) CCB – immersion, 2 weeks, 4.0 kg.m^{-3} = CCB – immersion, 1 week, 4.0 kg.m^{-3} = CCB – immersion, 1 week, 6.5 kg.m^{-3} = ABB - immersion, 2 weeks, 8.0%.
- c) *E. grandis* = ABB – immersion, 1 week, 8.0% = ABB - pressure, 6.5 kg/m^3 = CCB - pressure, 4.0 kg.m^{-3} .
- d) ABB - pressure, 4.0 kg.m^{-3} = Reference (without treatment).

Except for the treatment with ABB, by pressure, 4.0 kg.m^{-3} , all of them showed superiority when compared to the reference ones (range d). CCB based treatments shows clearly the favorable effect of the solution concentration, when the pressure method was applied, or then the positive effect of a longer period of the bamboo strips immersion (2 weeks).

For the better situation, ABB solution was ranged in the second category (b), denoting for that product, and the importance of a higher immersion period of (2 weeks). This fact can be attributed to the degree of maturity of the bamboo culm employed in this investigation, which inhibits the performance of the chemical solutions flow across the vessels, normally blocked for seasoned bamboo culms, as reported by Liese (2003).

3.4 Graveyard test

3.4.1 Soft rot

At the first evaluation (6 months), bamboo strips treated with CCB (Figure 12) and with ABB (Figure 13) showed a good performance when compared to the reference ones (grade 9.5). Only one strip treated with ABB by pressure (4.0 kg.m^{-3}) broke at the foot impact (grade 0). As comparison, the grade of Pinus stakes as reference was 9.0 (Figure 14). However, after this period, bamboo decay was clear and at 18 month of exposure, all of bamboo strips reference failed; Pinus stakes as reference also fails at 24 month. Performance of the strips treated with CCB was superior to those treated with ABB; the same behavior was observed for Pinus stakes. Pressure treatment (apparently without difference with the pressure adopted) still protecting Pinus stakes.

Bamboo strips treated by immersion at higher concentration during 2 weeks (I2W6.5) showed better performance; in a less degree, the protection of the strips by immersion during one week at the same concentration (I1W6.5) was similar to those of the pressure at 6.5 kg.m^{-3} .

For bamboo strips it is clear the non efficiency of the ABB (both by pressure and by immersion process) - after 24 months of exposure almost all of the strips fail; also for Pinus stakes, ABB treatment was similar to those of the reference ones.

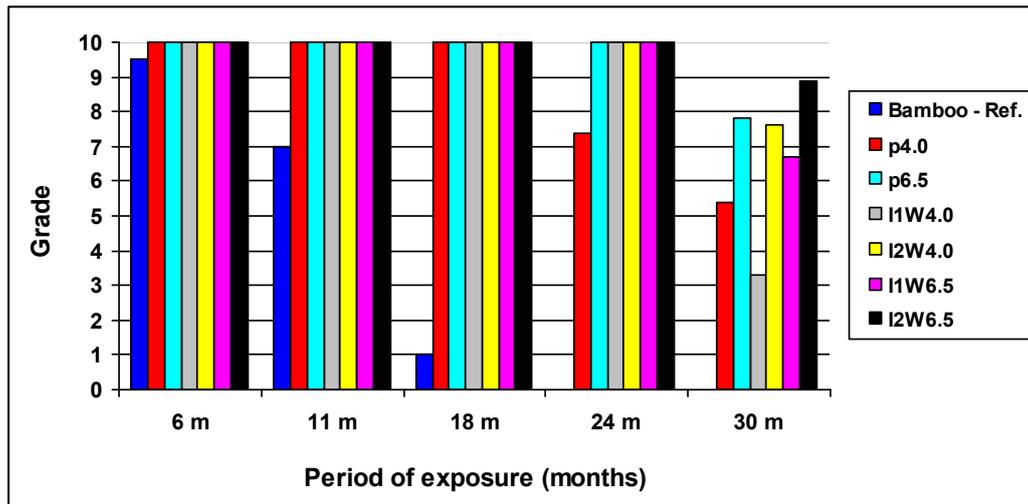


Figure 12. Performance against soft-rot of bamboo strips treated with CCB.

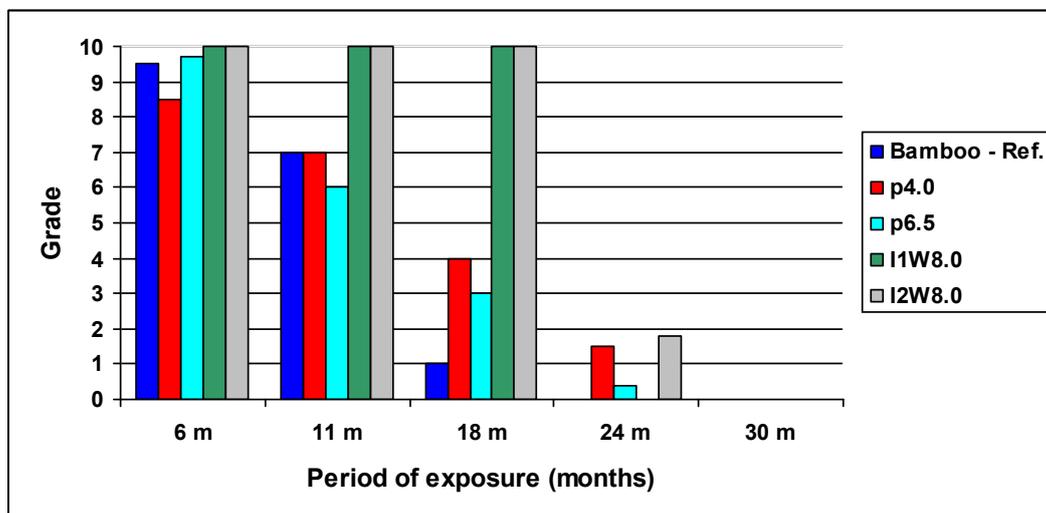


Figure 13. Performance against soft-rot of bamboo strips treated with ABB.

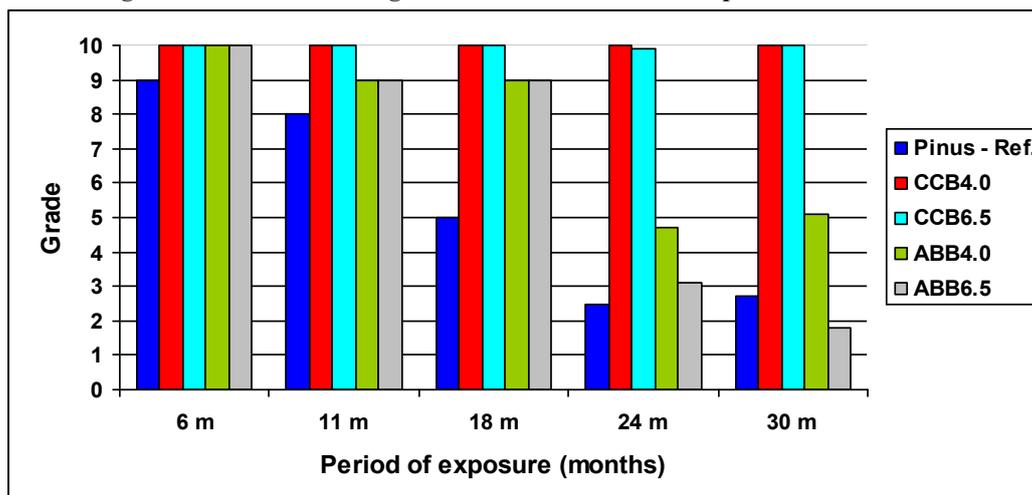


Figure 14. Performance against soft-rot of Pinus stakes treated by pressure with CCB and ABB.

3.4.2 Termite attack

After 6 months of exposure, none termite attack was observed (Figures 15 and 16). For bamboo and *Pinus sp* (Figure 17) references grades were of 9.7 and 9.5, respectively.

These results cannot be directly compared with those obtained from accelerated tests performed at controlled conditions. At the moment of the graveyard test, the soil has a high moisture content, that probably inhibits the attack of the termites, but, on the other hand, this condition can enhance soft-rot action.

Although, termites living in the field were not taxonomically identified, species were different from those employed at the accelerated test.

After 18 months, bamboo reference and those from strips treated by immersion with CCB at small concentration fail; after 30 months, only the strips treated by pressure and by immersion at high concentration combined with 2 weeks of immersion remained in the field.

After 30 months, except for the strips treated by pressure with ABB, all of the others fail. In a global way, CCB showed better performance than ABB for bamboo and for *Pinus* protection against termites attack.

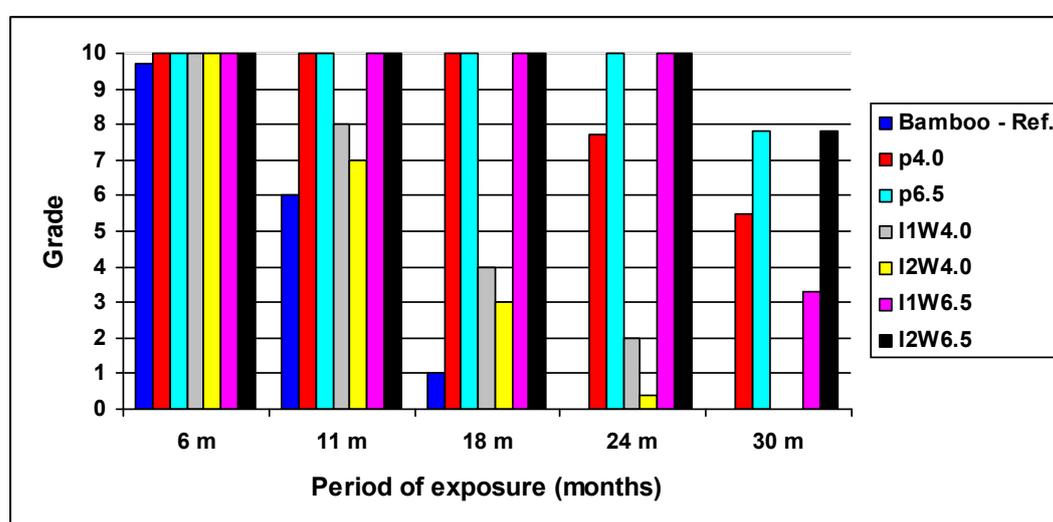


Figure 15. Performance against termite attacks on bamboo strips treated with CCB.

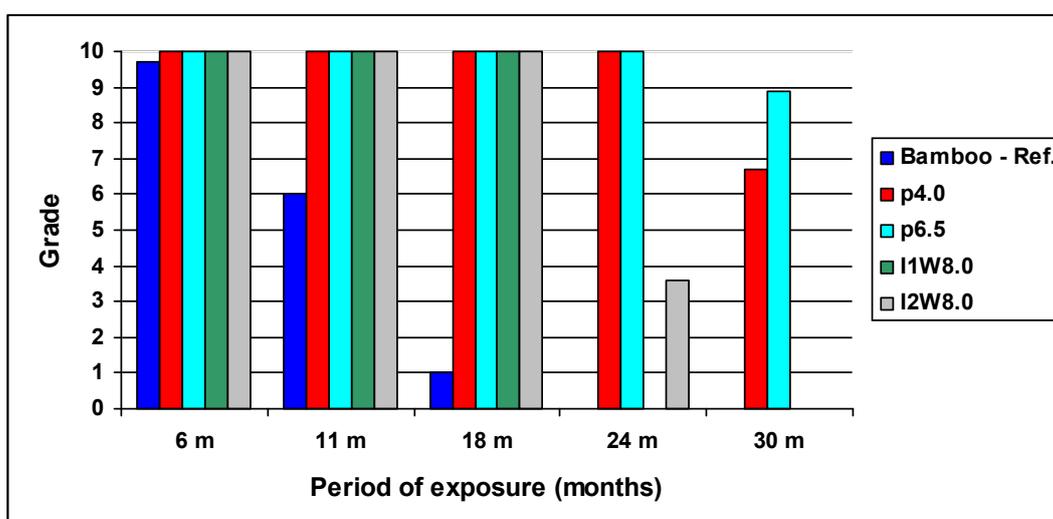


Figure 16. Performance against termite attacks on bamboo strips treated with ABB.

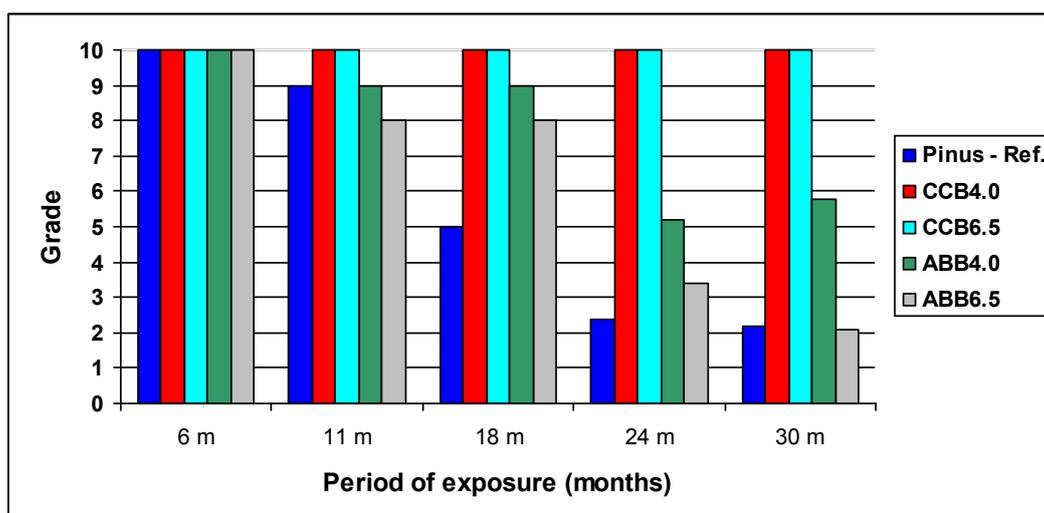


Figure 17. Performance against termite attacks on Pinus stakes treated by pressure with CCB and ABB.

4. Conclusions

- Accelerated test was proper to detect bamboo decay by fungi and to range the treatments by grades;
- Bamboo strips treated with CCB solutions showed better performance than those treated with ABB solutions;
- Immersion during two weeks with CCB at 6.5% was the most indicated treatment for bamboo strips
- SEM analysis confirmed that, in terms of efficiency, vessels were the most important anatomical bamboo element. Mainly for CCB solution, higher chromium and copper concentrations were observed in the vessels.

5. Acknowledgements

To the Brazilian National Research Council (CNPq), by the financial support; to the Montana Química, by the technical support.

6. References

- American Society for Testing and Materials. Standard D1758-02: Standard test method for evaluating wood preservatives by field tests with stakes. ASTM, 2004 – Wood. v. 04.10.
- American Wood Preserver's Association. Standard A11-93: Standard methods for analysis of treated wood and treating solutions by atomic absorption spectroscopy. In: AWWA: The Book of Standards, 1995.
- American Wood Preservers Association. Standard E7-07: Standard method of evaluating wood preservatives by field tests with stakes. In: AWWA: The Book of Standards, 2008.
- Ashaari, Z.; Mamat, N. 2000. Traditional treatment of Malaysian bamboos: resistance towards white rot fungus and durability in service. *Pakistan Journal of Biological Sciences*, 3(9), 1453-1458.
- Beraldo, A. L.; Ferreira, G. C. S. 2008. Chemical products traceability on treated bamboo. *Journal of Bamboo and Rattan*, 7(3/4), 177-182.
- Gonzales, G.; Gutierrez, J. A. 1995. Bamboo preservation at the Costa Rican National Bamboo Project. Vol. 3. Engineering and Utilization. In Rao, R. I. V.; Sastry, C. B. *Bamboo, People and*

- Environment. INBAR, Proceedings of the Vth International Bamboo Workshop and the IV International Bamboo Congress, Ubud, Bali, Indonesia.
- Hidalgo-López, O. 2003. *Bamboo the Gift of the Gods*. D'Vinni Edit. Bogotá, Colombia.
- Kumar, S.; Shukla, K. S.; Dev, T.; Dobriyal, P. B. 1994. Bamboo preservation techniques: a review. In: International Network for Bamboo and Rattan and Indian Council of Forestry Education. Proceedings...
- Lee, A. W. C.; Chen, G.; Tainter, F. H. 2001. Comparative treatability of Moso bamboo and Southern pine with CCA preservative using a commercial schedule. *Bioresource Technology*, 77, 87-88.
- Liese, W. 2003. Protection of bamboo in service. *World Bamboo and Rattan*, 1(1), 30-33.
- Suprapti, S. 2010. Decay resistance of five Indonesian bamboo specie against fungi. *Journal of Tropical Forest Science*, 22(30), 287-294.
- Yang, Y.; Hui, C. 2010. *China's Bamboo. Culture/Resources/Cultivation/Utilization*. INBAR. Bamboo and Rattan Research Institute, China Southwest Forestry University.

Bamboo Training and Development Centre in Mbeya, Tanzania: the Experience of an Architecture Student

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Polytechnic University of Madrid, UPM (*1)

Abstract

In 2010, Jaime Espinosa, by then student at Polytechnic University of Madrid, stayed in Tanzania, developing his final degree project on bamboo as a building material, awarded by a scholarship given by the university as part of its Final Projects in International Cooperation for Development Programme, with Ardhi University in Dar-es-Salaam as local counterpart for academic issues. Most part of field work consisted on collaboration between the author and a local women's cooperative society called Mbeya Bamboo Women Group, who were interested in learning how to preserve and handle bamboo round poles as building and furniture making raw material. The whole process of the treatment learnt by the author during his independent research, which took place mainly in Colombia, was followed and learnt by the members of the group, now able to replicate it. Treated bamboo was used for building a drying shed to store the material and for manufacturing six different bamboo furniture pieces prototypes, from armchairs to double school desks.

Moreover, a research project on mechanical performance of *Arundinaria alpina* bamboo samples being tested destructively was carried out by the author and personal from the National Housing and Building Research Agency in the laboratories of this official organism. Used specimens were taken from treated material in MBWG's workshop. A half of the specimens were destroyed by compression and the other half by shear, both efforts parallel to the fibres of the specimens and using the same test machine. The influence of the project in the academic sphere was palpable in a lecture given by the author in the Mbeya Institute of Science and Technology, and in the research material exchanged between the author and senior researchers at the University of Dar-es-Salaam.

Keywords

Tanzania, *Arundinaria alpina*, treatment, capacity building, furniture, research.

List of Abbreviations

ARU	Ardhi University
ETSAM	Technical Superior School of Architecture of Madrid
GTZ	German Office for Technical Cooperation
IHSS	Institute for Human Settlements Studies
INBAR	International Network for Bamboo and Rattan
MBWGMbeya	Bamboo Women Group
MIST	Mbeya Institute of Science and Technology
MoU	Memorandum of Understanding
NHBRA	National Housing and Building Research Agency
TAFORI	Tanzanian Forestry Research Institute
UDSM	University of Dar-es-Salaam
UPM	Polytechnic University of Madrid
UTP	Technical University of Pereira

1. Introduction and Background

Within the frame of Final Projects in International Cooperation for Development Programme by UPM the author of the present paper, Jaime Espinosa, by then student in ETSAM, spent around eight months in Tanzania, carrying out a practical implementation of his knowledge related to bamboo treatment and processes, most of it acquired previously in a research trip to Colombia, financed by UPM as well. After few years of theoretical research by the author (INBAR 2008; Janssen 1981; McClure 1953), that first field experience in Colombia during three weeks allowed him to follow one of Jörg Stamm's (*²) workshops in Ecobamboo, next to Cali, Valle del Cauca region, and to attend as part of the audience to the Second International Congress on Bamboo Modern Structures, celebrated in Bogota, in addition to visiting some of the largest structures reached up to date using structural round bamboo *Guadua angustifolia*.

In Tanzania, the author was welcomed by the MBWG to implement technical processes concerning bamboo preservation and its manufacture as material used in building and furniture production works (*³). MBWG is a cooperative society based on local women management and their work, physically located in Uyole, a village sited five kilometres far from Mbeya, a city in the Tanzanian Southern Highlands reachable by the road from Dar-es-Salaam to Lusaka, located approximately nine hundred kilometres far from Dar-es-Salaam. In their workshop facilities, the members of the group remove thin slices from fresh bamboo culms in order to be woven, creating a matt which may adopt a wide range of different sizes and shapes. This mat is used to make art crafts, handicrafts, ceiling boards among others high-quality finishing items.

Considering administrative terms, the author belonged to UPM but he was hosted officially by the ARU in Dar-es-Salaam while he stayed in the country. It was signed MoU between both universities, as a point of reference and written support for their potential academic exchange. This agreement meant an important goal for the project, as the programme purpose is to strengthen high-education institutions through international cooperation.

There was close contact with other relevant institutions in the same level going from holding an informal meeting to giving a lecture for professors and researchers. These institutions were the IHSS (which belongs to ARU), the UDSM (particularly the College of Engineering and Technology) and the MIST (where the mentioned lecture was given by the author). A different project spin off from collaboration between the author and MBWG which consisted on destructive load-bearing tests to *Arundinaria alpina* samples in the NHBRA facilities, a governmental institution sited in the city of Dar-es-Salaam.

2. Raw Material: Cut, Seasoning, Preservation and Dry

2.1. Setting up Basis and Scope of the Cooperation Project

After three weeks in Dar-es-Salaam, since mid-February until beginning of March 2010, the author moved to Mbeya, a city hosting around 500,000 inhabitants, even though statistics are not really trustful in sub-Saharan countries. In Dar-es-Salaam, the most populated city of the country, which also hosts most of official buildings, the chemical products needed for optimal preservation chose for bamboo where purchased: borax and boric acid. MSc. Arch. Ms. Elinorata Mbuya, professor in ARU and researcher in IHSS, was the counterpart supervisor for the UPM programme that the project was taking part in and was being financed by. Even though at the beginning of the project there was not still an agreement between UPM and ARU, a MoU was finally signed by both parts, enabling other students at UPM to develop their final projects in Tanzania with ARU as counterpart within this framework programme.

In Mbeya, different meetings were held between the MBWG members and the author, considered as a guest member of the group by the other members. During these meetings, key points related to the aim, the scope and the goals for the cooperation project were discussed and voted. An estimate to reach those objectives was approved. The group was interested in making bamboo furniture. Until then, the members of the group were skilful and experienced in bamboo woven mat art crafts and handicrafts, including baskets, bowls, ceiling boards, etc. even a furniture set of armchair and table of wooden structure covered by the mat and finished with rattan. They knew bamboo furniture was valuable for tourism industry, wealth particular costumers and local authorities looking for inexpensive ways to furnish primary schools (INBAR 2008).

Conclusions reached during these meetings were: 1.- to get raw material, African mountainous bamboo called *Arundinaria alpina*, from the forests between Isyonje and Isongole, 2.- to follow preservation processes for the bamboo to be used: seasoning in the forest, immersion in borax and boric acid solution, 3.- to dry in contact with direct sunlight and by the shadow, 4.- to use part of treated bamboo in the construction of a shady drying shed inside MBWG facilities, and 5.- to use the rest of the treated material in the manufacture of furniture pieces as prototypes to be replicated by group members by their own after the cooperation project period.

2.2. Getting Bamboo from the Forest

With the purpose of following bamboo cut labours and its seasoning evolution for three weeks, the author moved to Isyonje, a small village in the mountains located around twenty kilometres southern Mbeya. There, the author got in contact with local authorities and a group of people was assigned to carry out cut and lift works, from a bamboo forest located between Isyonje and Isongole, a neighbour small village. In order to make clearer explanations about how to cut and handle properly bamboo from the resource of the raw material (a forest in this case) the author edited a brief guide in Swahili, official national language in Tanzania, titled '*Namna ya Kukata Mianzi kwa ujenzi na vyombo vya nyumba*', 'How to Cut Bamboo for Building and Furniture' in English (Figure 1).

In that document were explained, in a colloquial manner, technical aspects to take into account in order to avoid moisture, fungus, insects and other undesirable pathologies in the material. The rules explained are based on research results achieved during last 25 years in Colombia, concerning several technicians from different fields. Although every experience is valuable, it is a must to remark the astonishing precision of cooperation projects between the UTP and the GTZ. Explanations headed to Tanzanian local population interested in learning the techniques regarding proper time of the day to cut bamboo, how to choose the right age for optimum mechanical performance, where to cut exactly every bamboo stem and how long should first season last at least (Figure 2). Three weeks after cutting sessions (discussing information in the paper and solving inquiries of locals) bamboo stems were considered seasoned enough, as the preservation process was going on in MBWG's workshop facilities (Stamm et al. 2001).

Namna ya kukata mianzi kwa ujenzi na vyombo vya nyumbani



1. Chukua panga lako na uende polini baada ya saa sita mchana. Kwa sababu, utomvu wa mianzi asubuhi hupanda juu, hivyo si vizuri kukata mianzi muda wa asubuhi.

2. Tafuta mianzi ya miaka mitatu, minne au mitano. Mianzi midogo hupoteza majani yake. Mianzi mikubwa huwa na madoa meupe mengi. Mianzi bila majani lakini ikiwa na madoa machache inafaa.



3. Ukikata mwanzi angalia kukata juu ya fundo kwa sababu szahina halitachukua maji kwa mvua, hivyo litafaa sana.

4. Kutengeneza mwanzi juu ya shina lake simamisha wima, hivyo maji ndani ya udongo hayawazi kuingia katika mwanzi.



5. Subiri wiki tatu kwa sababu utomvu ni mtamu hivyo unavuta wadudu; baada ya wiki tatu utomvu unakuwa mchungu, hivyo wadudu hawaharibu mwanzi.

Figure 1. Namna ya Kukata Mianzi, How to Cut Bamboo



Figure 2. Seasoning in the forest

2.3. Treatment in the Workshop

Preservation process followed in the project, after cutting proper culms properly and seasoning for three weeks, continued in MBWG's workshop consisting on the next basic steps: to wash every stem using scourers and water in order to remove lichens and fungus may be in the outer skin of the bamboo (Figure 3) so the preservative solution would be more easily absorbed, to open every cell of every stem so inner water come out and preservative solution will go in during immersion (Figure 4),



Figure 3A. Washing bamboo stems – B. Bamboo stem before washing – C. Bamboo stem after washing

to dry by direct sunlight in order to reduce moisture content. In summary, main criteria for bamboo stems were: 1. Age of the stem: between 3 and 5 years (Liese and Weiner 1996), presenting lack of leaves along the stem and with the presence of some lichens on its surface; 2. Altitude above sea level where they were grown: over 2,000 m.a.s.l.

Although seasoning is the key step in preservation process and the only step in traditional cases scenarios, in Mbeya we combined three weeks seasoning with one week more immersion in borax and boric acid solution at 4% concentration (Liese 1990): 2 kg of borax and 2 kg of boric acid for every 1,000 litres of water (Figure 5). There was an old water tank in the workshop facilities which was out of use for a long time, so it was cleaned and set up to be used as treatment tank by immersion of bamboo stems (Figure 6), pouring solution in the tank and putting something heavy over the stems in order to keep all of them completely submerged.



Figure 4. Opening bamboo cells



Figure 5. Mixing borax and boric acid solution



Figure 6A. Pouring solution in the tank – B. Bamboo submerged in the tank

After one week submerged in the tank, bamboo stems were removed from it and were placed leaning on the wall of the workshop receiving direct sunlight (Figure 7), getting dry with the only inconvenient of turning every stem a bit from time to time in order to avoid cracking while they were reducing their moisture content. Bamboo stayed there for four weeks before being kept in the shady drying shed. Actually, along its fourth week leaning on the wall, around a third of the total volume of bamboo was used for building mentioned drying shed. This building process is going to be explained in detail now on.



Figure 7. Direct sunlight drying

3. Construction of Bamboo Drying Shed

3.1. Previous Planning

Once the material was ready to be used (*⁴), there were selected from the whole volume disposed the most straight bamboo stems, paying attention to their curvature and to the ends of the required pieces with a closed knot, as this means one important step on protection by design because closed knots avoid insects going inside hollowed structural elements as bamboos are. Said required pieces were survived following a design based on independent frames to be made separately and then assemble them on definitive site. Its general layout (Figure 8) consisted on two vertical frames similar to each other, holding with their lowest side a grid slab working as floor, supporting two roof trusses linked by single rafters. Due to this planning works, the whole shed was built up in only five days, even though there were no carpenter or builder working with the team. Moreover, the only tools available consisted on a handsaw, a hammer, a spanner, bamboo splitters and an electric drill with different drill bits (Figure 9).

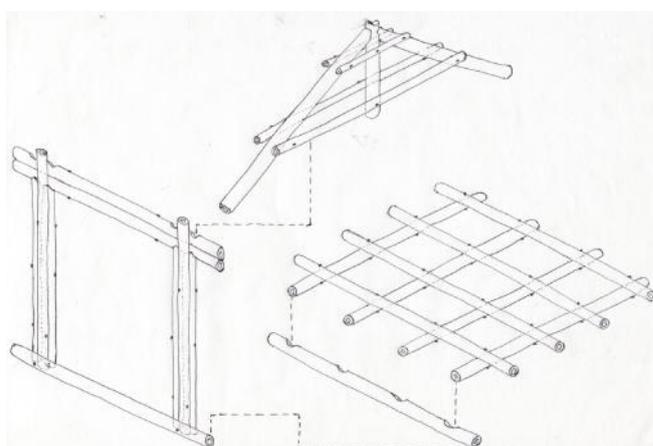


Figure 8. General design of drying shed – Figure 9. Available tools

Even though all the tools needed were there to be used, there was an uncertainty along every day of work: ‘Is power supply going to be working the whole day through?’ Some days the workshop was running out of supply from the general line and there was no generator for its own use, and the real uncertainty was about if that day power supply was going back or not. Before starting building process or even preliminary works, there were held inside workshop facilities some capacity building sessions, from theoretical and intuitive terms to practical handling of available tools (Figure 10).



Figure 10. A. Capacity building, discussions - B. Capacity building, handsaw practice - C. Capacity building, electric drill practice

3.2. Foundations

To meet design requirements, it was necessary to establish four independent foundation pieces, setting up a square on the ground using Pythagoras theorem. Every foundation piece, summing up four in total, was made placing a plastic bucket with a hole previously drilled at the bottom so a two feet length corrugated steel bar (half buried in the ground, half free vertically in the air) may be hosted its uncovered half inside the bucket and then concrete was poured in, waiting for it to harden.

Afterwards, buckets were pulled up so the half buried steel came out of the soil and the whole piece was turned upside down. Then, every bucket, working as shuttering, was separate being pulled up. These prefab-footings were half buried in a previously dug hole, leaning on big stones. Right level was achieved by using air-bubble-in-water method. Fulfilling the rest of the hole with compact earth, foundation works were finished.

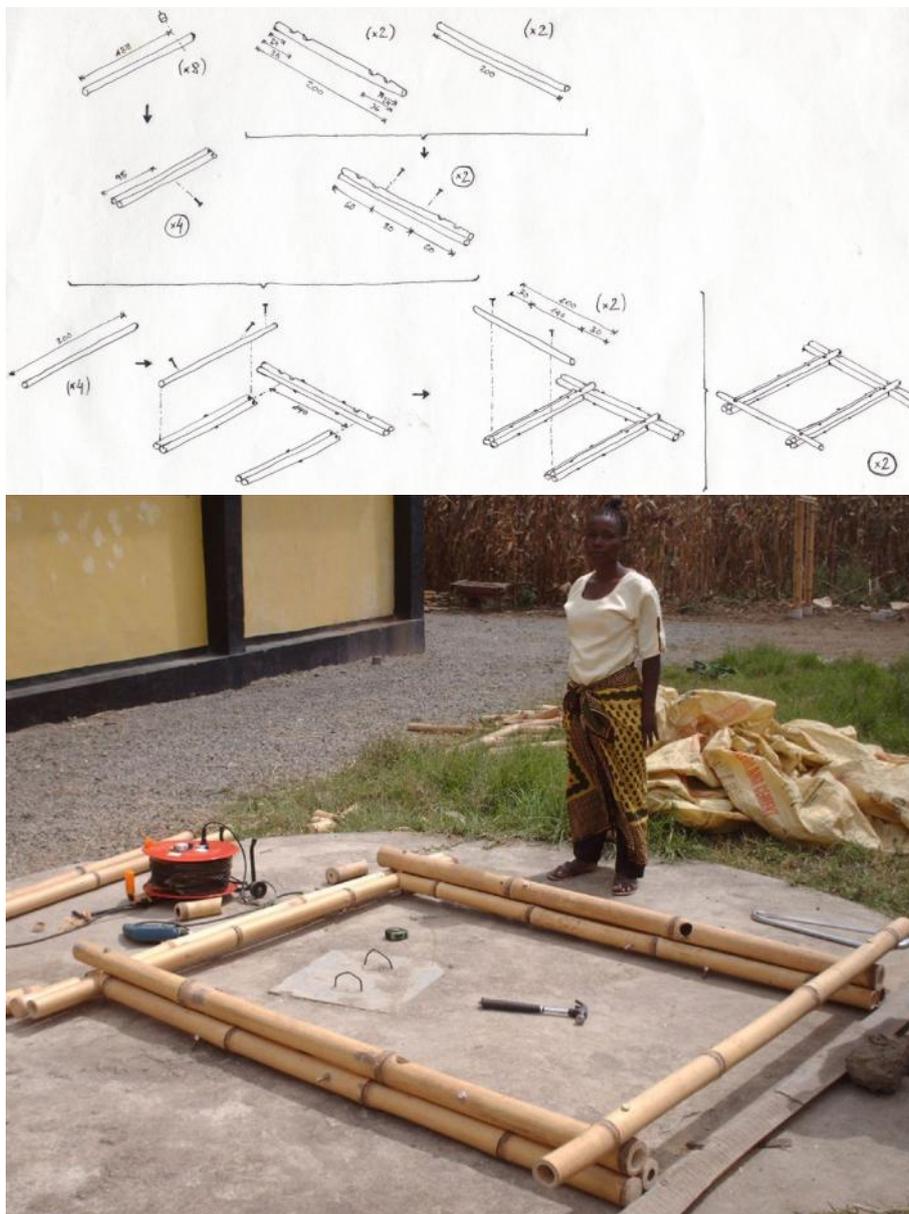


Figure 11A. Vertical frame diagrams - B. Vertical frame construction

3.3. Frames Prefabrication

With the aim of saving time during building process and to make this more efficient in terms of material, precision and safety, frames were made previously as independent pieces to be assembled later on. There were two different kinds of frames, each one made twice. There were two vertical frames (Figure 11) which vertical elements were composed by three bamboos, two of them of the same height and aligned so they supported the upper horizontal element, composed by two parallel bamboo of the same length, being prepared to receive roof trusses. The third vertical bamboo was going to be placed between horizontal elements of the trusses. At the bottom of the frame, another horizontal element linked both vertical elements of the frame, supporting as well the slab which will take part of the floor. Roof structure consisted on two similar trusses (Figure 12) which were made also independently and then assembled to make up the whole roof structure, which was lift up at once by every member of the group present that day in the workshop, using arm's power only, and using an empty drum as temporary support. Particular design of the joints was planned following the conclusions reached by previous research studies (Janssen 1981; Jaramillo and Sanclemente 2003) which tested different possibilities of joining depending on the design.

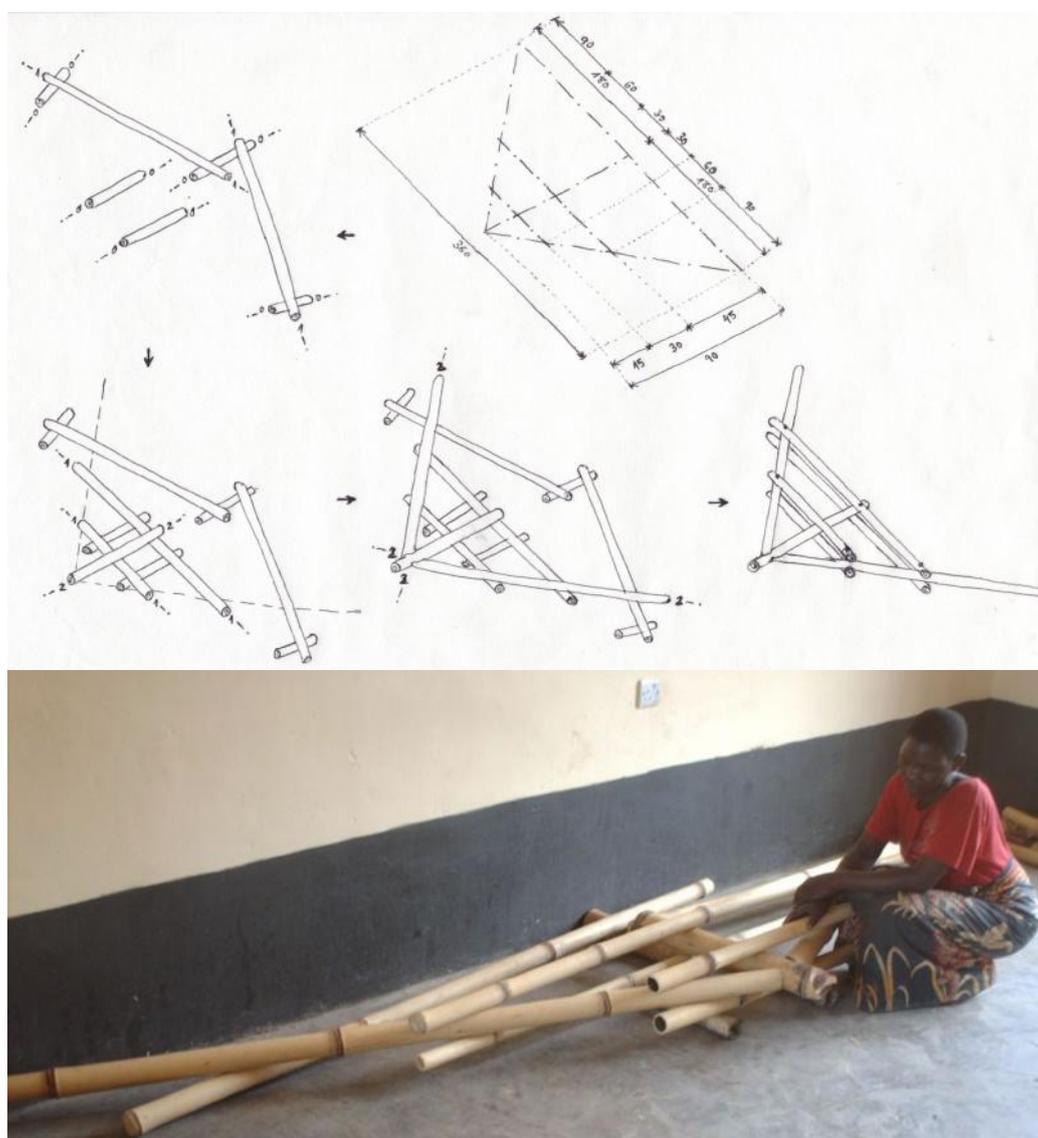


Figure 12A. Roof trusses diagrams - B. Roof trusses construction

Plenty of obstacles were faced and shorted out by this modest building project: limited tools and materials, inexperienced workers, irregular labour conditions and so on. Despite of these difficulties, in just three days all prefabricated frames and trusses were done. This means a clue about how easy and fruitful may result working bamboo with building endings. Joinery was based on screwed steel bar with washers and nuts in both ends (Figure 13). Concrete was poured inside bamboo cells which hosted steel bars coming from the foundations to gain stiffness in these points.



Figure 13. Joinery between bamboos

3.4. Assembly and covering

Planning based on prefabricated elements reduces timing and allow one or two women to carry every piece on their own, due to lightness of the material and how friendly it is for every kind of worker to use it (Hidalgo-López 2003). Placing frames up to previously arranged foundations and lifting up the whole roof structure, as it has already been explained, in addition to cover the shed with corrugated sheets painted to be protected against weather, took only two days more. In summary, the whole building process, except foundations hardening, took six days (*⁵), from Monday to Saturday (Figure 14). The following week would start furniture prototypes manufacture.



Figure 14A. Foundations - B. First vertical frame - C. Second vertical frame - D. Floor grid slab - E. Roof structure - F. First roof sheet - G. Second roof sheet - H. Working drying shed.

4. Bamboo Furniture Manufacture

4.1. Design and Planning

Every prototype was designed considering reduction in costs and time production of working with simple pieces (equivalent to prefabricated frames in smaller scale) during furniture manufacture process, in addition to comfort, endurance and aesthetic criteria. Some drawings were done in order to make clearer this process in every case (Figure 15). There were conceived a sitting room set (single armchair, triple armchair and a sitting room table), a dining room set (chairs and dining table) and a double school desk. Some tasks were common for every prototype though some were specific for one or several cases (Figure 16). This fact was taken into account in order to organize work as serially as possible, with its implicit benefits.

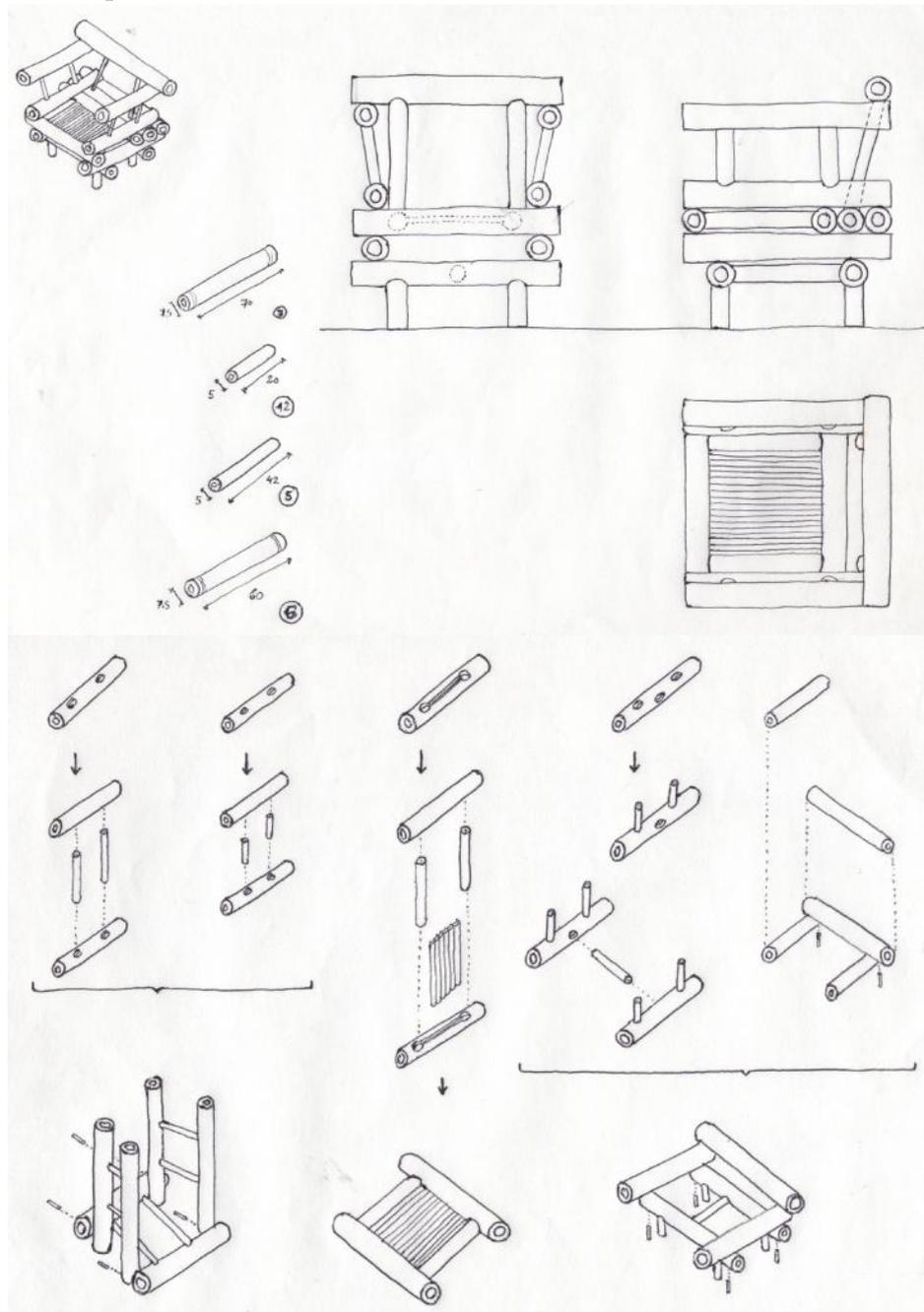


Figure 15A.Single armchair plans - B.Single armchair diagrams



Figure 16A. Teamwork - B. Assembling pieces

4.2. Manufacturing Prototypes

Due to most of labour was handmade, the rate of imprecision and risk undertook by manufacturers, especially in a beginning stages, is really high. To minimize these inconveniences, it is completely necessary to purchase proper equipment that ensures safety in the process and precision, leading to final high quality products. Although prototypes were done successfully (Figure 17), primary security rules and devices must be implemented to continue the project. In middle-long term, it would be better to acquire basic machines that would deal with unforeseen situations and time of production.



Figure 17A. Single armchair - B. Triple armchair - C. Sitting room table - D. Dining table - E. Standard chair - F. Double school desk

To make joints stronger, pegs out of bamboo were used, together with glue. Finishing was linseed oil (as non-acrylic pattern, let bamboo keep on transpiring) after covering flat surfaces with woven bamboo mat, speciality by the group members. There was edited an model of furniture order form (Figure 18), in case production would meet demand, as every furniture piece done was very popular between costumers. Potential rent of the business was proved as added value in furniture out of natural round bamboo stems covered by woven bamboo mat, everything finished with linseed oil pattern, achieve easily three hundred percent gross profit margin (*⁶).

Mbeya Bamboo Women
 Multipurpose Co-Operative Society, Ltd.
 Uyole, TZ (on main road to Mbeya)
 Tel. 754-028 009, PO Box 1425 Mbeya



Bamboo Furniture Order Form

The following furniture pieces may be ordered.

Prices listed (wholesale/retail) are based on pick up from our workshop in Uyole, TZ.

Delivery costs to other locations are extra.

Wholesale prices apply to orders of 10 or more items.

An advance payment of 1/3 of the order amount is required.

<u>Items</u>		<u>No. ordered</u>	<u>Total order</u>
	Single coach	Retail	95,000 TSH
		Wholesale	76,000 TSH
	Triple coach	Retail	185,000 TSH
		Wholesale	148,000 TSH
	Small table	Retail	85,000 TSH
		Wholesale	68,000 TSH
	Dining table	Retail	135,000 TSH
		Wholesale	108,000 TSH
	Chair.1	Retail	45,000 TSH
		Wholesale	36,000 TSH
	Chair.2	Retail	35,000 TSH
		Wholesale	28,000 TSH
<u>Date of order:</u>		<u>Total order:</u>	TSH
		33% advanced:	TSH
<u>Contact details:</u>		Balance due at time of shipping:	TSH
Name:			
Address:			
Tel.:			
Signature:			

Figure 18. Furniture order form

5. Technical Research on Bamboo in Tanzania

5.1. National Housing and Building Research Agency

While the author was collaborating with the MBWG, a visit by INBAR members from their headquarters in China took place. They came together with officials at different Tanzanian governmental institutions, such as the TAFORI and the NHBRA. The member of this institution, whose name is Eng. John Twimanyee, invited the author to visit NHBRA facilities in Dar-es-Salaam because they were very interested in doing research on bamboo.

It was arranged a micro-research project on load-bearing performance, through destructive tests. In Mbeya, the author selected and carried samples of treated bamboo which had been used during the collaboration project between him and the women's group. In total, thirty six specimens, 18 cm long each one, from eighteen different bamboos stems (Figure 19). Once in NHBRA's facilities, one piece of every couple was kept for future experiments and the other piece (still representing eighteen different stems) was cut in two halves, 9 cm long each one. A half was tested in compression parallel to the fibre of the specimens (Figure 20) and the other half in shear parallel to the fibre (Figure 21) as well. Both tests were done using the same machine (Figure 22) by *Seidner*, with additional metal pieces for the second type of test described.



Figure 19. Bamboo specimens

The moisture content of three samples was recorded through dry process in oven during one day, being weighted before going inside of it and afterwards. There was a great audience following the whole process paying attention, including Eng. Twimanyee among other engineers and technicians helping in the materialization of the research (Figure 23).

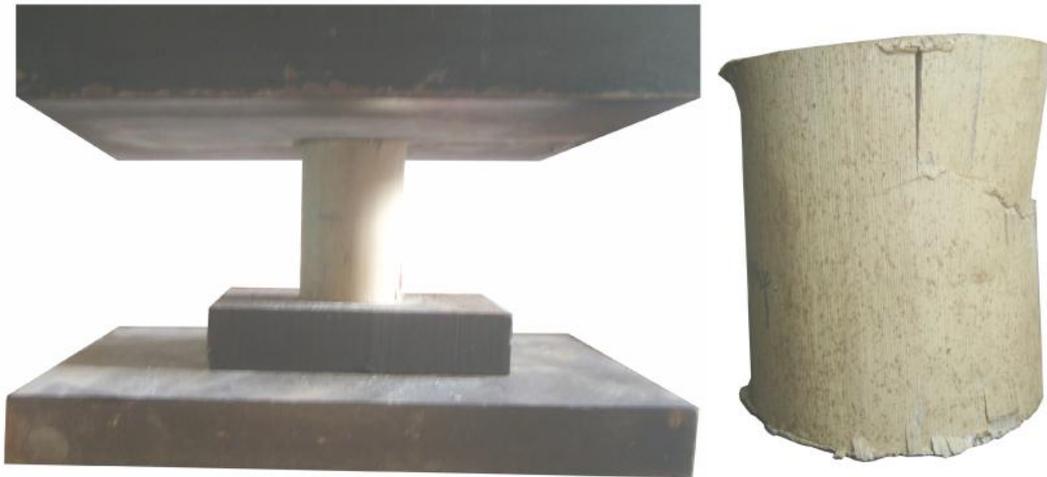


Figure 20A.Compression test in process - B. Compression sample tested



Figure 21A.Shear test in process - B. Shear sample tested



Figure 22.Test machine

5.2. Academic Sphere

5.2.1. University of Dar-es-Salaam

During author's stay in Mbeya, he met PhD. Eng. Leonard Mwaikambo, leader researcher at the UDSM, whose research work and tuitions are related to bio-composites characterization and comparative analysis. Dr. Mwaikambo invited the author to visit UDSM laboratories and see the state of the projects. Bamboo was starting to be taken into account as a material to be included in their curricular lines soon.

Until then, their work had been focused on sisal fibres to make rigid panels and cashew nut shell oil as natural glue to bring different materials together, for instance in multilayer composite materials. The visit was really interesting, meeting up and exchanging opinions with research staff members, including Prof. Lugoye (who had come back recently from Brazil, getting in touch for the first time with bamboo construction advantages nowadays), while knowing facilities and laboratories at the College of Engineering and Technology in the UDSM.

5.2.2. Mbeya Institute of Science and Technology

Due to its proximity to the resource of local raw material known, *Arundinaria alpina*, the author got in contact with the MIST. He held a meeting with the Principal, Prof. Joseph Msambichaka, in which was arranged a lecture about bamboo as building material to be given in MIST theatre with researchers, professors, engineers and architects at this institution as audience and the author as guest lecturer (Figure 24). Every assistant followed with attention the lecture and asked their questions in the end, mainly focused on where exactly where located the resources.

5.2.3. Ardhi University – Institute of Human Settlements Studies

Before being selected for the Final Projects in International Cooperation for Development Programme by UPM, the author contacted MSc. Arch. Ms. Elinorata Mbuya, professor at the ARU and researchers at the IHSS, institution affiliated to ARU. She accepted to be counterpart supervisor for the collaboration project between the author and the MBWG. It was one of the requisites to take part in the programme, however, to establish an official link between UPM, as sending institution, and hosting institutions, in this case ARU. This link was fortunately achieved formally through a MoU signed by representative personalities from both institutions. After author's stay in Tanzania finished, another student at UPM has been selected to continue the programme at ARU with a different project, in a different field.

5.2.4. Polytechnic University of Madrid

Ten months after coming back from Tanzania, the author get grade in Architecture with line of specialization in Project and Urban Landscape. During these months, in addition to having the chance of giving special lessons on bamboo as building material as invited lecturer in the Department of Construction and Architectural Technology at ETSAM-UPM and in other departments, he was invited to expose the project in Tanzania displaying a document in the Fifth National Congress on University and Cooperation for Development, celebrated in Cadiz (Spain), explaining graphically the achievements of the experience in Tanzania.



Figure 23.NHBRA staff members



Figure 24.Author's lecture at MIST

6. Conclusions

The value of the experience is not measurable, starting from the human scale until the technical approach. The members of the MBWG and the author shared an experience with no precedents for any of them in mutual learning and cultural exchange. In capacity building terms, however, the projects may be improved because the group did not gain the whole autonomy required to keep on manufacturing and marketing bamboo furniture, as the participants agreed as a goal for the project. Even being a pilot project with no continuity, as soon as the group will be able to afford expenses in order to go through safety, precision and secure marketing lacks up to date, they will be capable to carry on their own profitable business on this field.

In academic terms, a MoU was signed by UPM and ARU to enable formal exchange of students, researchers and material. The author proposed to Principal J. Msambichaka at MIST to sign a similar agreement between his institution, the UDSM and the ARU because they have already started academic exchange which is not reflected in an official document. Research on bamboo samples of *Arundinaria alpina* are one of the first tests done to this particular specie and may result in longer term collaboration between the author and the NHBRA with the aim of enhance bamboo as construction material.

References

- Hidalgo-López, O. 2003. Bamboo: the Gift of the Gods. Oscar Hidalgo-López Editor, Bogotá, Colombia. 553 p.
- INBAR (International Network for Bamboo and Rattan), 2008. Country Strategic Opportunities Paper – Tanzania. INBAR, Beijing, China. 31 p.
- Janssen, J. 1981. Bamboo in Building Structures. Doctoral Thesis at Technical University of Eindhoven. Eindhoven, the Netherlands. 237 p.
- Jaramillo, D.; Sanclemente, A. 2003. *Uniones en Guadua con Ángulos de Inclinación entre Elementos*. Final Project in Civil Engineering at Universidad Nacional de Colombia. Bogotá, Colombia. 95 p.
- Liese, W. 1990. Preservation of Bamboos. Federal Research Centre for Forestry and Forest Products. 165-172.
- Liese, W.; Weiner, G. 1996. Ageing of Bamboo Culms. Wood Science and Technology, 30. Springer-Verlag.
- McClure, F.A. 1953. Bamboo as building material. U.S. Department of Agriculture: Foreign Agriculture Service, Washington D.C., U.S.A. 49 p.
- Stamm, J.; Lehmann, H.; Aristizabal, V. 2001. *Guía para la construcción de puentes en guadua*. Proyecto U.T.P.-G.T.Z. JM Calle, Bogotá, Colombia. 48 p.

List of footnotes

- (*¹) Jaime Espinosa belonged to ETSAM-UPM as grade student since 2002 until 2011, when he became an Architect and Urban Planner. In the current moment, he is following postgraduate course on Human Settlements in Third World awarded by a scholarship by UNESCO Cathedra in Basic Habitability at UPM.
- (*²) Jörg Stamm, who has worked as international consultant for the United Nations Organization, is probably the most experienced and recognized expert in bamboo construction, particularly in bridges projects, field in which he has reached over 30 metres free span bamboo structures, combining European carpenter's tradition with vernacular knowledge on bamboo.
- (*³) The author knew about the MBWG by 2008 INBAR Annual Report, where an article about Paulina Samata, the group leader, and the other bamboo women's group appeared. In September and October 2009, due to a travel help in cooperation for development projects, the author had the big chance to meet the MBWG for the first time, before starting the project explained in the present paper, which lasted since February until August 2010.
- (*⁴) It is recommended to follow a period of shady drying after sun drying stage, around two weeks more if it is natural ventilated, to reduce moisture content down to 10% in order to lift it with low risk of cracking. In this case, bamboo was used directly after sun drying stage because it was going to be locally.
- (*⁵) Six days dedicated to prefabrication of frames and roof trusses, assembling, floor slab and covering works; foundation consisted on four prefabricated concrete pieces with a corrugated steel bar coming out from it. Those pieces hardened and also were placed properly before this considered six-day period.
- (*⁶) Although MBWG members expressed their thankfulness and satisfaction for the experience shared with the author, it would be necessary to make a little investment to purchase tools, items for labour safety and to establish fluent marketing and delivery channels with clients.

Weaving with Bamboo

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Abstract

Sri Lanka is a lower middle-income nation with less job opportunities in rural areas where more than three quarters of the country's population live. Many migrate to urban areas or sometimes abroad for menial jobs. Young mothers have left their families seeking foreign employment risking their own safety and family's wellbeing. The issue of economic disparity between the rural areas to urban areas has to be addressed to up lift the living conditions of the people in Sri Lanka.

Handloom industry was well established in Sri Lanka not so long ago, providing job opportunities to at least 200,000 people in rural areas, mostly women. The weavers worked from their homes, minding their family. The Weaving Centres provided them with cotton yarn and other accessories and supported these weavers. As the cotton growing was not successful in Sri Lanka, due to not having the raw material, cotton, the mills that spun yarn for Textile Industry closed down. The system collapsed.

Kandygs, a successful handloom manufacturer and an exporter, conducted a pilot project in *Ihala Madampella* a traditional handloom village in *Gampaha* District. Commencing in April 2011, 70 weavers were selected for the pilot project. The time this project started, the weavers were making a low quality handloom product by using a sewing thread that was not suitable for weaving. The selected weavers were given a basic training on designing quality control and a basic knowledge of costing and marketing. Within this short time, a Handloom Center in their own village was started to co-ordinate weavers with designers and buyers. Through the Center dyed bamboo yarn was supplied with contemporary designs. In six months' time a very successful Bamboo textile products exhibition was held in Colombo, giving weavers and their products the exposure to a niche market.

The trial marketing that was carried out locally and abroad recognized bamboo yarn as the key for uplifting an entire industry that would not only stipulate an income, but will improve the lifestyle of people. Bamboo farmers' contribution towards the backyard economy will help the country further whilst growing bamboo will help the planet.

Introduction

Sri Lanka

Sri Lanka, known as the “Pearl of Indian Ocean” is an island situated in South East Asia, with an area of 65,000 square km. The island consists of coastal plains, with mountains rising in the central part of the country up to 2524m. Sri Lanka is positioned just above the equator, endowing a tropical climate with ocean winds and considerable humidity with an average yearly temperature of 28°C to 31° C.

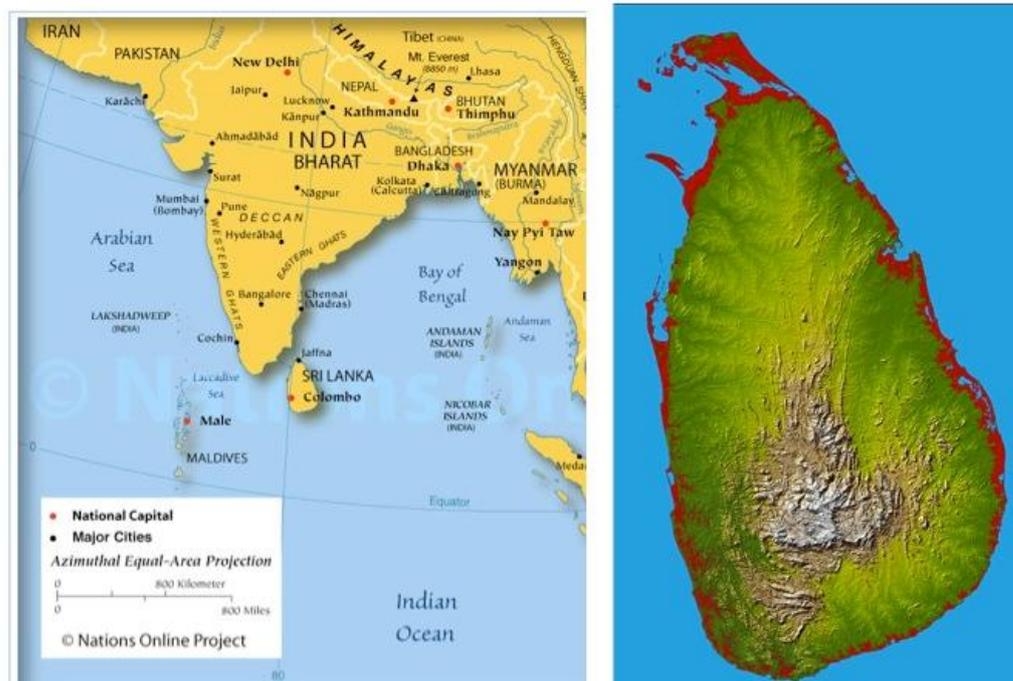


Figure 1: Map of South East Asia - Figure 2. Topography of Sri Lanka

People

Modern Sri Lanka emerged 64 years ago from four centuries of colonial rule under the Portuguese, Dutch and British successively. The period of foreign domination had an adverse impact on the social and cultural fabric that had developed over 2500 years of civilization. However, the British by the time they left in 1948, had developed a good transport infrastructure, which comprised road, rail and ports to support the export dependent economy, which they imposed on the country.

Sri Lanka has a population of 20.2 million, with the highest density in the western coastal region. Sinhalese constitute the largest ethnic group with 74%, followed by Sri Lankan Tamils (12.6%), Sri Lankan Moors, Burghers and Tamils of Indian origin who were brought into the country as indentured labour on British colonial plantations.

Sri Lanka is a multi – religious society, with Buddhists making up 70% of the population. Buddhism was introduced to the country in the 2nd Century BC.

Economy

According to the Department of Census and Statistics the economic output of Sri Lanka as measured by Gross Domestic Product (GDP) for the second quarter of 2011 is Rs. Million 686,928 registering an 8.2 percent growth. The per capita income is the second highest in the South Asian region, making Sri Lanka one of the fastest growing economies of the world. During the period of 2005 to 2011, poverty has dropped from 15.2% to 7.6% and the unemployment from 7.2% to 4.9%.

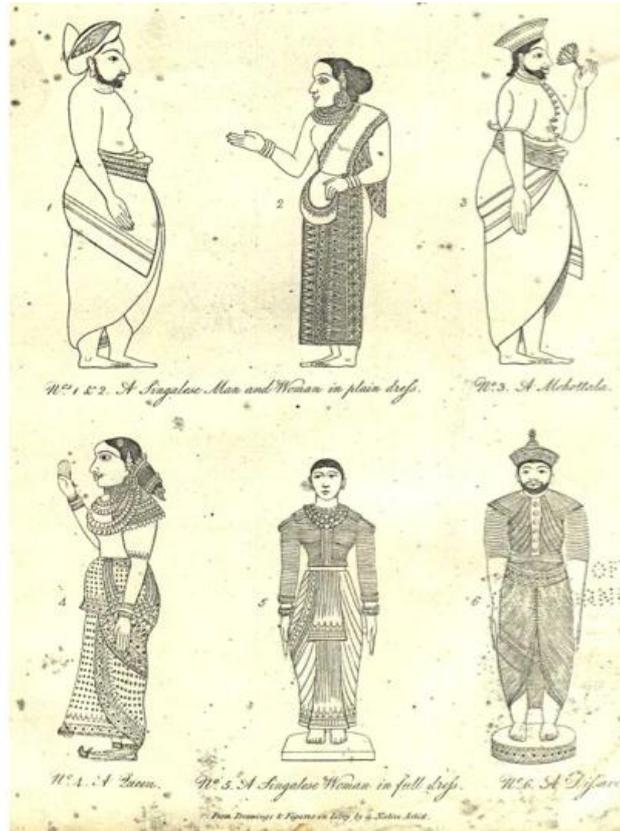


Figure 3: People of Sri Lanka

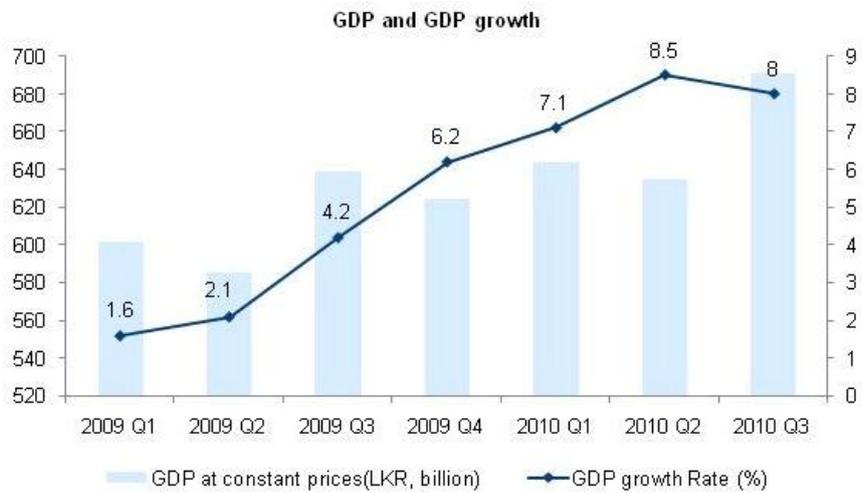


Figure 4: GDP Growth Rate

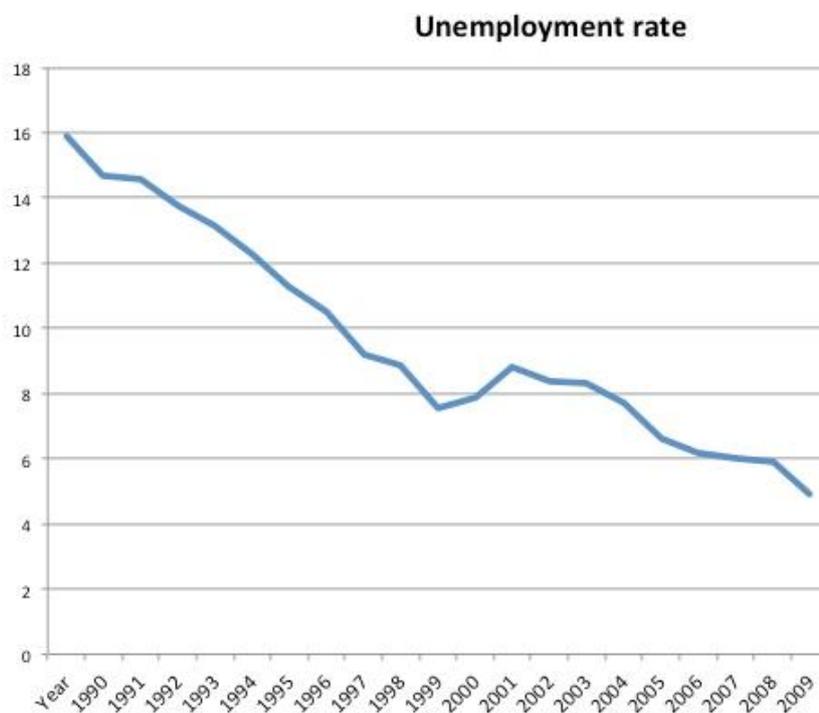


Figure 5: Unemployment rate

Since the free market economy was introduced in 1977, the nation moved towards an industrialized economy. Private enterprise was promoted and now the private sector accounts for 85% of the economy.

‘Ceylon Tea’ being one of the most well-known teas in the world, is still an important agricultural product in addition to rubber, coconut, paddy and sugar. Textile and the Apparel sector are the leaders of the industrial sector, which accounts for 28% of GDP. Service sector makes up to 60% of GDP developing rapidly and absorbing modern technologies.

Government of Sri Lanka is encouraging private and foreign investment to improve from its present 22% of GDP level to 28% of GDP in order to attain the anticipated accelerated economic growth. The government hopes to support this by continuing the development of infrastructure and improving regulatory arrangements and enhancing public sector services.

Development Challenges

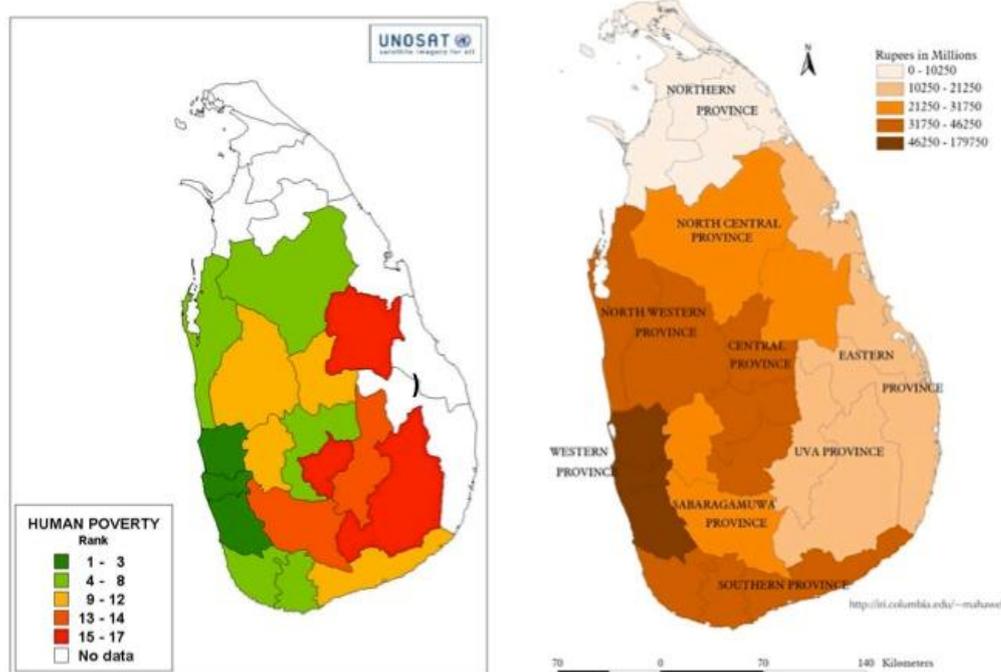
Sri Lanka is a lower middle-income nation, even though the country has achieved high economic growth and performed well against most Millennium Development Goals. However, there are serious economical disparities between and within provinces. While poverty is on the decline overall (from 28.8 per cent in 1995/6 to 8.9 per cent in 2009/10), it is still persistently high in some parts of the country.

"... The Millennium Development Goals set timebound targets, by which progress in reducing income poverty, hunger, disease, lack of adequate shelter and exclusion — while promoting gender equality, health, education and environmental sustainability — can be measured. They also embody basic human rights — the rights of each person on the planet to health, education, shelter and security. The Goals are ambitious but feasible and, together with the comprehensive United Nations development agenda, set the course for the world’s efforts to alleviate extreme poverty by 2015. "

United Nations Secretary-General BAN Ki-moon



Figure 6: Recent infrastructural developments in Sri Lanka. Sri Lanka's longest bridge, the Kinniya bridge in the Eastern province. Source: Sri Lanka news online



In Sri Lanka, 85.49% of the population lives in rural and estate areas even though urban migration occurs constantly. (Census & Statistics, 2004). The main occupation of these rural and estate areas is predominantly agricultural and other traditional livelihoods. They are vulnerable to climatic conditions and do not always receive adequate facilities for satisfactory regular incomes.

With a literacy rate of 92.5% and a youth literacy rate of 98% Sri Lanka has one of the most literate populations amongst developing nations and is one of the few countries in the world that provides free education from primary to tertiary stage. Educational attainment index in all three sectors, urban, rural

and estate shows an increasing trend. According to available statistics literacy rate is 94.5 in the urban sector and 92.3% in the rural sector (Dept. of Census & Statistics 1999, Central Bank of Sri Lanka 1999).

The Vision

Rural and Backyard Economy

“Effective integration of rural economy with emerging economic sectors in urban centers is being established with policies to enhance productivity and competitiveness of SMEs. The recent initiative of promoting household economic activities through the *Divi Neguma* programme particularly strengthens the backward integration with rural economic base. The *Divi Neguma*, the one million home economy (backyard economies) programme, has been designed to encourage a wide range of economic activities spanning from vegetable/fruit cultivation, livestock, environment friendly living arrangements, home based industries, services and backyard economic activities. This programme will help insulate households from various market vulnerabilities on their living, promoting healthy family lifestyles and organic agricultural production.”

Fiscal Management report 2012 Ministry of Finance

Revival of Handlooms in the rural areas to increase Rural and backyard Economy towards the anticipated Economic Growth in Sri Lanka

Handlooms in Sri Lanka

History

According to *Mahawamsa*, the chronicle of the of present civilization of Sri Lanka, the Sri Lankan history begins in 543 BC with landing of Prince *Vijaya* who is met by a princess of the land, princess *Kuveni* who had been spinning cotton. One of the earliest recorded accounts of the ancient Textile Industry in Sri Lanka from the first century A.D is a reservoir named after weavers in recognition of their service. Therefore it is assumed that handlooms have been around since the beginning of the civilization and thrived at a later period.

Prior to the introduction of free market economy in 1977, handlooms contributed immensely for the country's economy, producing many products for the local market. It is recorded to have around 120,000 handlooms in 1970's. (Department of Textiles) During that period, the raw material for handlooms, cotton yarn, was spun in Sri Lanka, as there were number of Spinning and weaving mills. Cotton for the mills were either imported or grown in Sri Lanka.

At present the numbers of handlooms are as low as 11,358.

	Looms in working condition		Looms in non-working condition	
	2002	2010	2002	2010
Government sector	5179	3454	2463	1811
Private sector	3358	2809	2160	2641
Co-operate sector	1136	497	425	146
Total	9673	6760	5048	4598

Figure 9: Number Handlooms in Sri Lanka

Source: Department of textiles

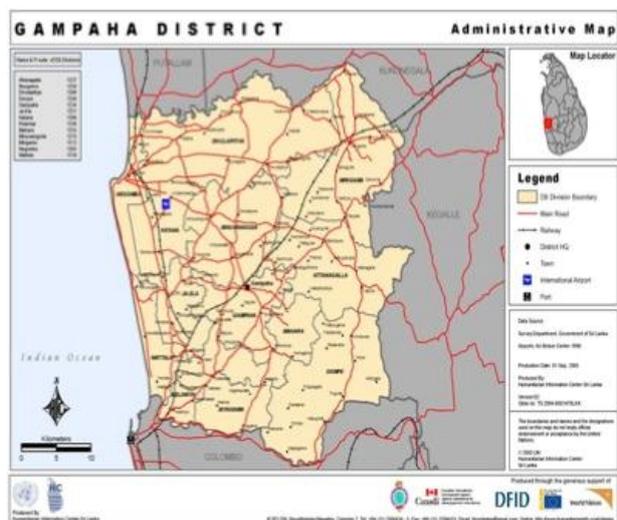


Figure 10: Map of Gampaha District

Source : department of Survey Sri Lanka

Figure 9. Number of Handlooms in Sri Lanka – Map of Gampaha District.

Case Study

I was only 10 years when my parents started a small business with 10 handlooms and had grown up with the rhythm of the handloom and the exotic colours that's use to make this very characteristic fabric. Working as a textile Designer for the last 25 years, with a work force 300 people and seeing the potential for this handmade product, I really wanted to study why the handloom industry perished in our country.

We did not have to travel further than 50km from Colombo, the financial capital of Sri Lanka to come to this beautiful village resplendent with paddy fields. *Ihala Madampella* is in *Gampaha* District, belongs to *Divulapitiya Pradeshiya Sabha* (Rural council). Divulapitiya has 217 villages with around a population of 150,000 people and 40,000 families.



Figure 10. Handloom village, *Ihala Madampella*

Divulapitiya Textile weavers were the forerunners of the Textile community, prior to 1977, free market economy. There have been a large number of handlooms and 11 power loom factories in this area. A textile cooperative society ran one of the power loom factories, distributing raw material and accessories to smaller scale handloom weavers and power loom weavers. With the help of this project, many weavers developed and became owners of small and medium scale businesses.

The textiles that were produced at that time were mainly for the local market, which did not face any competition prior to open economy. Therefore the design and quality of this product was not competitive enough to face the imported textiles that flooded the country.

Today most of the weavers' co-operative societies are closed down and the number of weavers involved has dropped to 1244.

We visited them at their homes. Most were family based with both husband and wife sharing the work, which started 40 years ago, may be with 10 looms and now sometimes just one. It was really encouraging to see younger generation's involvement with parents' work. Still in many homes the excess looms are removed and kept stored in the limited space of the house.

Most of them have a handloom or sometimes up to 5 outside their homes in a shed. Some of the looms

are in the only room they have. Winders and other accessories are kept in the outside verandah. The little space that's left in the house is used as a storeroom for the yarn. Some of these small time weavers have a power loom or two as well in their home garden factory.



Figure 11: Handloom weaver of Ihala Madampella



Figure 12: Handloom weavers

When the textile industry was flourishing in Sri Lanka, the yarn was produced in many spinning mills. Therefore the raw material required for the handlooms were available abundantly. With the closing

down of the spinning mills the yarn required for the industry was imported from the regional cotton growing countries. This was too expensive and not freely available to the rural weaver.

When we visited our weavers in *Ihala Madampella*, they were using a twisted polyester mix coloured yarn out of a yarn bobbin. Being curious about the source of the yarn, which was obviously a yarn used to sew but not to weave, we were informed that the yarn is ex stock from the garment industry. The most innovative raw material the rural weavers are using is the yarn from the collars and cuffs of knitted garments that were thrown away as seconds from the garment factories. The thread from the collar or cuff is unraveled and wound on to pirns. These pirns are used to weave the weft of the woven cloth.

A dealer would come with whatever the seconds or excess thread, he could get hold of from the garment factories, hand them over to the weavers, who would weave a low quality fabric on a handloom or power loom that has no appealing colour or design sense. The dealer comes back to buy back the product at his price of course.

Our rural weavers products are mostly sarees, bed sheets and serviettes that are taken to the wholesale market in Colombo and in turn sold mostly at the tea plantations amongst the labour.

The main glitches in this system were, apparent. The weaver, completely depending on the dealer who brings thread to them was not getting the right price to the product. When I inquired about costing, an older lady told me “ No, I haven’t quoted any cost for my weaving, because, this is something I do from home amongst my house work”.

The thread, which was supplied haphazardly, was not suitable for handloom weaving. The product has a low quality, which can never claim the appropriate price. The weavers or the textile village has no exposure to the market to know exactly, to fathom the developments in design, quality or to identify suitable products.

We decided to tackle these challenges immediately. Commencing on the 30th of April 2011 initially 70 weavers were selected. Academy of Design, a well-recognized design school in Colombo started training our weavers in colour co-ordinations and patterns. Department of textiles sent trained teachers for reiterate them in structures and quality control. Costing and other basics in business management were offered.

Most importantly, with the patronage of ministry of Economics a Handloom Center was started to co-ordinate weavers with designers and buyers in future. Through the Center dyed cotton yarn was supplied to the weavers. In six months time a very successful exhibition was held in the village at the Center.

Following this, Academy of Design gave the opportunity to the weavers to participate in Design festival; an Exhibition and a fashion show were held in a Hotel in Colombo.

Weaving with Bamboo

Kandygs, a successful manufacturer and an exporter of handloom fabric and products, started working with bamboo yarn, more as a design experiment. The work was done with viscose bamboo yarn, and tried out dyeing processes. As the protein content and the cellulose content of bamboo fiber may vary from the processing method of bamboo fiber, the methodology for dyeing has to be selected prudently. Dyeing results may vary from viscose bamboo yarn to linen bamboo yarn.



Figure 13: Opening of the new Handloom Centre in Ihala Madampella, under the patronage of the Ministry of Economic Development - Figure 14: Fashion show at the Sri Lanka Design Festival 2011

At Kandy's dye house the yarn is dyed with reactive dyes at 60°C-80°C using a fixing agent. The colourfastness was tested with the fabulous colours that were achieved. The absorption of dyes in bamboo yarn seems to be much better than cotton and has a sheen even better than mercerized cotton's. The coloured viscose bamboo yarn was then used to experiment on various qualities of textiles at the Kandy's weaving center.

Natural dyeing was also established at Kandy's. Barks, leaves or roots of different plants that could be found locally, are used in this traditional dyeing system. A subtle and somber colour range was achieved which too has a natural sheen. Textiles that were made with this yarn were used to make a variety of soft toys.

The fabulous fabric that was produced is soft to the feel, drapes well and has a natural sheen. Permeable, cooling doesn't stick to the body and absorbs water well, making it comfortable to wear, durable and washable. After a successful experiment, bamboo yarn was introduced to our rural weavers to revive the handloom industry with a vision of alleviating poverty from Sri Lanka. These villages had been predominantly handloom areas not so long ago.

The weavers accepted the challenge well. Some were a bit reluctant at the beginning, not being sure of continuous supply of this new yarn. Most were excited to work with the yarn that looked glamorous and, now are very happy to continue as weaving is easier as the yarn is strong. A collection of dress fabrics was woven; sometimes 100% viscose bamboo with open weaves and cotton warp (vertical yarn in the weave) and bamboo weft (horizontal yarn). Shawls, scarves, throws and summer blankets were woven. Products like cushion covers and quilts were made out of the fabric. A joyous collection of soft toys was added.

Kandy's first exhibited these products in February 2011 at Maison Et Objet, a trade fair in Paris, later a fashion show at the Sri Lanka Design Festival. December 2011, a complete collection of products out of viscose bamboo textiles was exhibited in Colombo, introducing bamboo textiles to Sri Lanka.



Figure 15: Kandygs at Maison, Paris, February 2011



Figure 16: Fashion show with Naturally Dyed Viscose Bamboo fabric at Sri Lanka Design Festival



Figure 17: “Weaving with Bamboo” Exhibition in Colombo

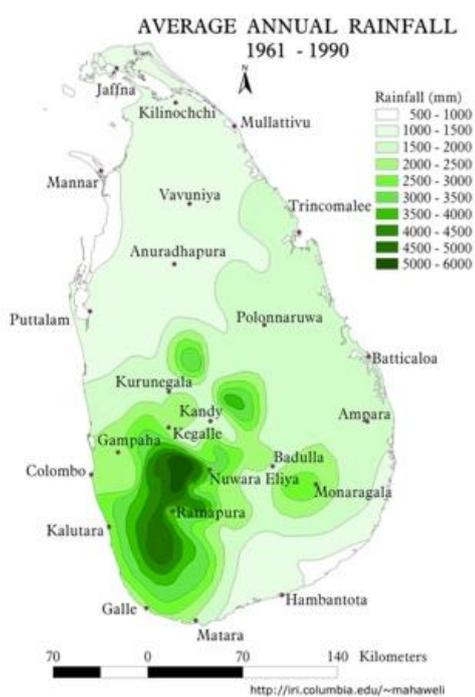


Figure 18. Annual rain fall in Sri Lanka (Source: The Sri Lankan Consulate in Los Angeles) – Figure 19. Map of rivers and river basins in Sri Lanka (source: waterwiki.net)

The Future and the challenges

Bamboo plant is a sustainable and versatile resource, able to grow even in diverse climates and the fastest growing woody plant. As they are grown densely they don't require much land. The water-use efficiency is good in bamboo plant and its extensive root system prevents soil erosion.

Banks of many rivers, which covers a large part of Sri Lanka, constantly requires remedies to land erosion. Growing bamboo has been a traditional method to protect riverbanks, which unfortunately has not been done systematically.

Growing suitable varieties of bamboo for textiles on riverbanks not only help us to solve a persistent problem of land erosion, mud slides and flooding but give us the opportunity to grow our own raw material for a latent industry with the potential to uplift the rural economy. For its long-term survival the cottage level handloom industry cannot be completely depend upon from imported cotton yarn. Growing cotton in Sri Lanka on a large scale has proved in the past not viable as cotton plantations require large amount of land and more importantly divert huge quantities of water from food production and export crops, given that 15, 000 to 20,000 liters of water are required to produce just one kilogram of cotton.

Bamboo, when harvested, regenerates without needing to replant, therefore harvesting will not hamper the land-use. After one-time planting little care and maintenance required. Bamboo being a good "carbon sink" and generating more oxygen than equivalent stand of trees, help the entire planet. One of the most important advantages of growing bamboo is, no fertilizers or pesticides required and this is a huge benefit for using bamboo as an organic textile.

Sri Lanka has 103 perennial rivers, locally known as Ganga, Aru, or Oya. Of these, 23 river basins are larger than 500 km² covering approximately 73.2% of the island. The longest river *Mahaweli* covers a distance of 335 km. Bamboo occurs naturally on riverbanks all over the country. Many species are identified and some bamboo culms are cultivated for building construction and production of other utilities. Though Sri Lanka is a small and populated island, the coverage from riverbanks is more than enough to grow bamboo to have Sustainable supply of raw material for Textile Industry. The challenge is to grow bamboo by the rural people who has access to riverbanks or any other water way. It is necessary to distribute right varieties of bamboo shoots for planting with home garden concept. In this way a new sector of jobs can be established as 'bamboo farmers' ensuring them a regular income for their harvest.

Conclusion

To restore the handloom industry in Sri Lanka to its former glory and provide rural weavers a sustainable source of income, the country has to move away from its total dependency on imported raw materials.

Bamboo provides an opportunity for Sri Lanka to contribute to the world economy in an environmentally friendly and sustainable way by creating products that are not only beautiful but helps to save our planet.

Research Progress in the Utilization of Bamboo - Resources in China

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Abstract

Bamboos are naturally distributed in the tropics, subtropics and even some areas of temperate zones. It grows faster than almost all the trees on earth and is an important supplement of wood biomass. Although bamboo has many traditional applications in many countries, modern technology is further widening its utilization. This paper first gave a short overview of China's bamboo resources and industry. More detailed information was then given on the research status of bamboo resource utilization of China in five areas, including bamboo based composites, bamboo fiber based materials, environmental-friendly protection of bamboo, bamboo houses construction techniques, and chemical utilization for bamboo resources. Most of the above researches were finished or joint finished by International Center for Bamboo and Rattan (ICBR), a China national-level institute focused on research and development of bamboo and rattan.

1. Overview of China's bamboo resources and industry

Bamboo is one of the most important non-timber forest resources in the world, growing faster than almost all the trees on earth. Compared with trees, bamboo is an excellent renewable resource with faster growth, better strength and properties of fiber (Jiang, 2007). According to China's Seventh National Forest Inventory (2004-2008), the nationwide pure bamboo forest area is 5.38 million hm², with the area of Moso bamboo (*Phyllostachys pubescens*) at 3.8683 million hm² accounting for 71.89%, and that of mixed bamboo forest at 1.51 million hm²; the number of bamboo culms amounts to 82.9 billion, including 9.157 billion of Moso bamboo and 73.743 billion of other bamboo species (SFA, 2009)

China's bamboo industry has a strong competitiveness in the international market. Thousands of China's bamboo processing enterprises enjoy total annual output of over 12 billion U.S. dollars. China is the biggest bamboo products exporter over the world with export value in 2010 at 1.86 billion U.S. dollars to more than 30 countries and regions.

Over 2,000 people are engaged in bamboo research in China. According to available statistics, a total of over 450 bamboo products and patented technologies enjoy intellectual property rights, as well as over 500 practical techniques on bamboos utilization being developed, resulting in significant ecological, economic and social benefits.

2. Research of bamboo resource utilization in China

Over the past 10 years, the Chinese government has been steadily increased its investment in scientific research on bamboo, which accelerated the utilization of bamboo resources. Bamboo products contain more technology than ever before. This paper briefly introduces some new progress in the researches of bamboo utilization in China in the past ten years, some of which have already been piloted or entered into industrial production, while some are still at the laboratory stage.

2.1 Bamboo based composites

Bamboo can be utilized as an alternative resource to wood for engineering materials due to its high strength and hardness, and superior flexibility (Liese, 1987). Composites production is the most important way for the consumption of bamboo resources, among which bamboo mat plywood, laminated bamboo strips lumber have long been industrialized in China, which are mainly used for concrete molding in construction, floorings of heavy vehicles and containers. In 2008, China has a production of 3.66 million m³ of bamboo plywood valued at 3.4 billion U.S. dollar. During the past 10 years, extensive researches have been conducted in China in order to add the value of bamboo based composites. Several new bamboo based composites, such as advanced bamboo-wood composites for wind turbine blade, bamboo structural lumber for construction application, bamboo scrimber for indoor and outdoor floorings et al, have been developed and commercialized.

2.1.1 Advanced bamboo wood composites for wind turbine blade production

Wind blades are the key parts of wind turbine, functioning to transform wind energy to electricity. Wind blades are mainly manufactured with glass fibre reinforced plastic (GFRP). It has been estimated 30 thousands of wind blades are needed annually, and this number will increase at a speed of 20% per year (Huang et al, 2007). Since wind blades are rather huge, normally have a diameter longer than 40 m, the production of blades will consume a large quantity of GFRP. It has been known GFRP is incapable of decomposition in the natural environment, so the large scale application of GFRP wind blade will inevitably cause serious environmental problems in the long term (Jiang et al, 2006).

The scientists from International Center for Bamboo and Rattan (ICBR) found ways to produce advanced bamboo wood composites for wind blades (Figure 1). They significantly improved the performance stability of the composite through mechanical grading and precise processing of bamboo strips, vertical resin immersion and other techniques. Furthermore, plasma surface treatment of bamboo, the addition of thin wood veneer between bamboo strips significantly improves the bonding performance of the composite. Several special techniques and equipments were also developed to manufacture full scale bamboo wood composites with length longer than 12 m. ICBR and Denmark RiSO National Lab have jointly finished the performance evaluation of bamboo wood composites for wind blades. The test results show the specific tensile strength, modulus and compression strength of the composite, the remain fatigue strength after high frequency are more than 22 GPa, 180 MPa ,136 MPa and 40% respectively, all of which are comparable to those of GFRP. Furthermore, the production cost of bamboo wood composite is 2300 USD/m³, approximately half of the cost of GFRP (Sun et al, 2008).



Figure 1 Advanced bamboo/wood composites for wind turbine blades

2.1.2 Bamboo scrimber manufactured with small-diameter bamboo species

Bamboo is strong in parallel grain but is rather weak in transverse direction. However, the shortcoming of weak transverse strength makes bamboo especially suitable for the production of bamboo scrimber, a new bamboo composite produced with similar processing technique of wood scrimber (Yu, 2011). In the production of bamboo scrimber, bamboo is firstly separated into a loose mat of interconnected strands by passing through a custom-built crusher. The loose mat was then immersed in PF resin and air-dried. The dried mat strands are then put together and pressed into panels or lumbers (Figure 2). In China, bamboo scrimber has been commercialized and developed very quickly in the past 5 year. However, bamboo scrimber normally uses Moso bamboo as raw materials. In order to use bamboo species with thin culm wall, the experts from Wood Industry Institute, Chinese Academy of Forestry (CAF) developed special equipment which combined “separating bamboo into strand” with “resin application” in one step (Zhang et al, 2011). This equipment minimized the negative effect of the layers of bamboo green and bamboo yellow on the internal bonding of the

composites. Furthermore, a special forming device was developed to minimize the variation of density profile across the panels. This technique has been transferred to several plants and realized pilot production.



Figure 2 Bamboo scrimber (left: panels; right: lumber)

2.1.3 Bamboo based structural lumber for construction purpose

Bamboo was mainly used with the form of round culms in construction. It is generally used for the low-cost housing, not for the modern houses. The experts from ICBR and CAF used laminated bamboo strips lumber as basic materials to produce various structural components with different shape and size for a house, such as beams, poles, roofs, through the process of lengthening and thickening with adhesives for outdoor application (Jiang et al, 2006). The bamboo mat plywood was used as the covering and supporting materials of a wall. The developed bamboo based structural components and walls were used in a teaching building of a primary school located at Ping Bian county of Yunnan province of China (Figure 3), which might be the first modern building with bamboo based composites as main construction materials.



Figure 3 Bamboo structural ladders used as the roofs of teaching building

2.2 Bamboo fibers based materials

2.2.1 Extraction of natural fibers from bamboo for textiles production

In China, the technique of using bamboo to produce bamboo viscose fibers for textiles production has been developed and successfully commercialized. The bamboo viscose fibers actually belong to the category of regenerated cellulose fibers. There are many difficulties in producing real natural bamboo fibers that can be used for the production of fine textiles. Recently, the scientists from ICBR found ways to extract natural bamboo fiber bundles from Ci bamboo, a kind of sympodial bamboo mainly grown in Sichuan province of China (*Neosinocalamus affinis*(Rendle) Keng f). The textile fibers produced with this technique not only remain the characters of natural bamboo fibers, but also own some extent of spinnability. Some special equipments and key textile process for bamboo textile fibers were also developed. Some pilot textile products, for example cushion, mat and jeans, were manufactured with these fibers (Figure 4). Moreover, two relevant products standards have been prepared and issued.



Figure 4 Some textile products made with natural bamboo fibers

2.2.2 Fire-retardant bamboo fiber based insulation materials with super-low density for building walls

Recently, the experts from Fujian Agriculture and Forestry University in China successfully developed fire-retardant bamboo fiber based insulation materials with super-low density by jointly using techniques of bamboo fiber modification and polymer forming. The developed material is characterized with super low density ($20\sim 50\text{kg/m}^3$), low thermal conductivity ($0.024\sim 0.037\text{ W/MK}$), high sound adsorption coefficient ($0.61\sim 0.83$), high fire-retardant performance (B1 grade) and high resistance to fungus attack. These properties make it very suitable to be used as the sound and heat insulation materials for the walls of high high-rise buildings that have high requirements for fire safety.

2.2.3 High-yield mechanical and chemical pulping of bamboo

Bamboo is the ideal raw materials for paper making due to its high aspect ratio of fibers. In 2008, the production of bamboo pulping in China was estimated to be 1050 thousand tons valued at 0.89 billion U.D dollar. Recently, the scientists from Institute of Chemical Industry of Forest Products, CAF improved the traditional process of chemical and mechanical pulping and applied it to bamboo. The results indicated the pulping yield reached as high as 82%, 30% higher than that of traditional pulping process. Meanwhile, the chemical consumption reduced by more than 65% and the tensile strength of pulping sheet reached 27 N.m/g. This technique has been successfully extended to a large paper and pulping factory in China.

2.3 Environmental-friendly protection of bamboo

Bamboo is easier to decay and grow mold than wood during outdoor service due to its higher content of sugar and starch, which act as feed for fungi or insects. Although the existing wood preservatives might be also valid for bamboo, most of them are not environmental friendly. There is great need to develop green or low poisonous agents for protecting bamboo from decay and mold growth.

2.3.1 Environmental-friendly natural preservative for bamboo

It has been found the extractives of many natural plants own high antifungal and insecticidal activity. The scientist from ICBR and Central South Forestry Science and Technology University found the resistance to mold and termite of bamboo strips can be greatly improved after soaking, boiling and steaming at certain temperature for a while, in the aqueous solution of a mixture of Camphor leaves, Camphor roots, and Cedar pine needles. Compared with traditional chemical treatment, this approach gives no poisonous effects on human being, consumes much less energy and has a low treatment cost of about 15-22 U.D dollar/m³. The treated bamboo's mildew proof level reached the highest level of British Standard and its anti-termite reached the higher level of ASTM standard. This technique has been extended to a bamboo floorings factory.

2.3.2 Environmental-friendly bamboo wood composite floorings for outdoor application

It is much more difficult to treat bamboo with preservatives than wood. The experts from ICBR improved the penetration and distribution of copper-rich preservatives in bamboo by using the process of intermittent vacuum and pressurization immersion. They further used the treated bamboo and wood materials to produce bamboo/wood composite floorings for outdoor application. The test results show the preservative performances reach the requirements of A grade (strong decay resistance) of China national standards.

2.3.3 Surface functionalization of bamboo with nanostructured ZnO

Imparting excellent preservative performances to bamboo is the key to expand the applications of this extraordinary non-wood forest resource. The scientists from ICBR reported on the formation of ZnO nanostructured networks films on the surface of bamboo via a simple two-step process. This process consists of generation of ZnO seeds on the bamboo surface followed by a solution treatment to promote crystal growth. The results indicate the approach can simultaneously furnish bamboo with excellent photostability, antifungal and antibacterial performances (Yu et al, 2011).

2.4 Bamboo houses construction techniques

Bamboo house is mainly made of bamboo, although other materials such as wood, adobe, mortar or vegetable fibers may also be used. Bamboo houses are traditionally regarded as the "poor man's houses", or a temporary solution for dwelling, it extensively exists in the tropical area of the world. Some countries in South America, such as Colombia, Ecuador and Peru have long history and plenty of experience of using bamboo for housing. The traditional bamboo houses directly uses round bamboo culms as the main structural elements of the house. However, the natural variability of bamboo culms makes the standardization of traditional bamboo houses nearly impossible. In China, several institutes are conducting studies on the modification of traditional bamboo houses and using bamboo based composites as the main construction materials of the houses.

2.4.1 Prefabricated modular Bamboo houses for temporary accommodation

Prefabricated modular house is a very important component and the development direction of modern construction. It is characterized with modularization, standardization, easy transportation, fast assembly and disassembly and so forth. The experts from ICBR and CAF developed a full set of techniques for the construction of prefabricated modular bamboo houses for temporary accommodation. This modular houses use standardized light frame of steel as supporting elements, bamboo-mat plywood as the covering materials of house walls and roofs. All the walls and roof coverings are prefabricated in the factory and assembled on site. Some important performances, such as houses air quality, heat insulation and sound insulation were evaluated. The results indicate that all the above performances meet the requirements of relevant standards for permanent civil buildings of China. In 2008, ICBR built and donated 70 sets of prefabricated modular bamboo houses for the survivals of Sichuan earthquake hit areas (Figure 5).



Figure 5 Prefabricated bamboo modular houses built for the victims of Sichuan earthquake hit areas in China

2.4.2 Pre-fabricated bamboo culms house integrated with solar energy technology

The scientists from ICBR and Hunan University developed a novel bamboo culms house integrated with collection technology of solar energy. They not only designed high efficient truss completely consisting of round bamboo culms, but also use round bamboo culms as the supporting elements of walls. In the house, round bamboo culms are used both for structural elements of the house and the conduction channels for heat collected by solar energy plates. The collected thermal energy can be stored in a special energy storage flooring module that can controllably release the heat when needed. This technique not only made full use of bamboo resources, but also significantly decreased the consumption of energy during the service life of a house.

2.5. Chemical utilization of bamboo resources

Bamboo contains many components with physiological activities. The chemical components of bamboo and its biological activities and biometric functions come to people's attention. The studies on chemical components and biological activity, bamboo vinegar and bamboo tar have been carried out extensively in China. Some new products have been developed for food additives, biopesticide/medicine, food additives and chemical industry etc.

2.5.1 Research on the chemical constituents of bamboo leaves

The scientists from ICBR have conducted systematic studies on the chemical constituents of *Bambusa pervariabilis* McClure bamboo leaves. 30 compounds were isolated from the bamboo, which belong to flavonoids, coumarin, lignans, Anthraquinones, Xanthenes, Sterols and so on. They were identified as: 5,7,3',4'-tetrahydroxy-6-C- β -L-arabinosylflavonoside, 5,7,4'-trihydroxy-6-C- β -D-glucopyranosyl flavonoside, 5,7,3',4'-tetrahydroxy-6-C- β -D-glucopyranosyl flavonoside, 5,7,3',4'-tetrahydroxy-3-O- α -L-rhamnosyl flavonoside, 5,7,3',4'-tetrahydroxy-8-C- β -D-glucopyranosyl flavonoside, 5,7,3',4'-tetrahydroxy-5-O- β -D-glucopyranosyl flavonoside, 5,7,3',4'-tetrahydroxy-7-O- β -D-glucopyranosyl flavonoside, 5,4'-dihydroxy-7-O- β -D-glucopyranosyl flavonoside, 2,3,5,7-trihydroxy-xanthone, physcion, 5,7,3',4'-tetrahydroxy flavonoside, Sitosterol, Bamboo lignan I, Bamboo lignan II, Bamboo lignan III, 5,7,4'-trihydroxy-8-C- β -D-glucose- α -L-rhamnosyl flavonoside, 5,4'-dihydroxy-3',5'-dimethoxy-7-O- β -D-apiose-(1 \rightarrow 2)]- β -D-glucopyranosyl flavonoside, 5,7,4'-trihydroxy-7-O- β -D-glucopyranosyl flavonoside, Triperpenoids, 7,2'-dihydroxy-3',4'-dimethylisoflavan-7-O- β -D-glucopyranoside, 7,8-Dihydroxy-3-(3-hydroxy-4-oxo-4H-pyran-2-yl)-2H-chromen-2-one, 2'-hydroxy-4-O- β -D-glucopyranosyl-4'-O- β -D-apiosyl hydroxychalcone, 6-methoxy-7-O- β -D-glucopyranosyl-chromen, 5,7,3',4'-tetrahydroxy-3-O- β -L-glucopyranosyl flavonoside, 7-hydroxy-4-O- β -D-glucopyranosyl flavanone, 5,7,4'-trihydroxy-flavonoside, 3,5,7,3',4'-polyhydroxy flavonoside, 7-hydroxy-6-methoxy-chromen, 5,4'-trihydroxy-7-O- β -D-apiose-(1 \rightarrow 2)]- β -D-glucopyranosylflavonoside. 5,4'-dihydroxy-3',5'-dimethoxy-7-O- β -D-apiose-(1 \rightarrow 2)]- β -D-glucopyranosyl flavonoside, Bamboo Lignan compounds I, II and 7,8-Dihydroxy-3-(3-hydroxy-4-oxo-4H-pyran-2-yl)-2H-chromen-2-one were discovered new compounds for the first time. 10 species were first isolated from bamboos found, including the flavanol, flavonone, chalcone.

2.5.2 Bamboo leaf flavonoid and its utilization

Flavonoids occur widely in bamboo species, content for an average of about 2%. Research shows that bamboo leaf flavone has strong antioxidant activity. It has a high application potential and can be used as antioxidants, acrylamide inhibitor in heat processing food, anti-free radical, anti-aging, hypolipidemic and so on. The flavonoids distribution in bamboo leaf of main bamboo species were analyzed in China. The bamboo species which have higher content of flavonoids in leaf were screened out. The technology for bamboo leaf flavonoid extraction and preparation was established, and the bamboo leaf flavonoid products have been produced and have dozens of related enterprises in China.

2.5.3 Study and utilization of biopesticide originating from bamboo leaves

Extracts of bamboo leaves have good antimicrobial activities on agri-microorganisms and food microorganisms. They could effectively inhibit the growth and propagation of microorganisms during food storage. The inhibited microbe include *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum* etc.

Extracts of bamboo leaves also have certain contact toxicity, antifeeding and repellent effect on insects. Study indicated that extracts of *Pl. juxianensis*, *Br. albostratum*, *Ph. platyglossa* and *Pl. amarus* had potent toxicity against the larvae of *Culex pipiens pallens* at the concentration of 0.1g/L. The median lethal concentration of 24h was 30.65 mg/L, 53.94 mg/L, 41.21 mg/L, 54.49 mg/L, respectively. The field experiment of 10% extracts of *Pl. amarus* showed that the effect of 7d after treatment on *Aphis gossypii* was 63.8% at the dosage of 1500mL/ha. It was equal to the commercial insecticides.

2.5.4 Study on the natural bamboo food additive

The antibacterial and growth promoting substance was extracted from bamboo processing waste to use as natural food additive. Application results showed that the average slaughter weight of the table poultry which were fed by bamboo natural food additives raised by 2.2% and the whole death rate reduced by about 0.4%. The incidence of mastitis of dairy cattle in experimental group felled by 1.1% and the average daily milk production increased by 1.9%. Random sampling inspection showed that antibiotic residues were not detected in chicken and milk samples of experimental group.

2.5.5 Study on the preparation and utilization technology of bamboo vinegar

Bamboo vinegar is the by-product of bamboo charcoal production. Currently, it is developed as soil conditioner, biological pesticide synergist and fruit and vegetable preservative and so on for agricultural production. According to incomplete statistics, there are approximately 150 enterprises which product bamboo charcoal in China, about 200,000 tons bamboo charcoal and 40,000 tons bamboo vinegar are produced every year.

Researchers in ICBR developed bamboo vinegar insecticide and fungicide, bamboo vinegar fruit and vegetable preservative and other products successfully by screening with efficient additives, after overcoming the difficulties of mixing bamboo vinegar with chemical pesticides as well as its stronger acidity. According to the research results, the used amount of chemical pesticides is reduced by more than 50%. Consequently, the risk of pesticide residues of agricultural products is significantly reduced.

2.5.6 Synthesis technology of bamboo tar based high-temperature resistant resin

Polycyclic multi-core aromatic hydrocarbon resin was synthesized by bamboo tar as raw material and xylenediol as crosslinking agent. Its β resin content was up to 57.8%, which was higher than the polycyclic aromatic hydrocarbon resin that was synthesized by coal and petroleum pitch. B-stage polycyclic aromatic hydrocarbon resin can solidify to C-stage resin, which had good heat resistance. Compared to commonly used phenolic and epoxy resin, bamboo tar based high-temperature resistant resin had good heat resistant, and its raw materials can be renewable. It can be used in the pipe plugging of petrochemical industry and other fields.

3. Prospect

It is foreseeable that in China's 12th Five Year Plan (2015-2020) and even a longer period Chinese government will continue to increase its support for the scientific research on bamboo. Therefore, China's bamboo industry will witness further robust development. What's more, China's scientists on bamboo will further focus on areas like breeding technology of bamboo with high disease-resistance and quality, sustainable cultivation and management of bamboo forest, bamboo bio-technology, high value-added processing and utilization of bamboo, and chemical utilization of bamboo resources according to the technological requirements of bamboo industry. Their work will provide strong technical support for the sustainable development and industrial upgrading of bamboo industry.

References

- Huang, X.D, Jiang, Z.H, Sun, Z.J. 2007. The status and trend of wind turbine blades development. *Solar Energy* (In Chinese), 4, 37-38.
- Liese W (1987) Research on bamboo. *Wood. Sci. Technol* 21: 189-209.
- Jiang, Z.H; Sun, Z.J; Ren, H.Q. 2006. Application of advanced bio-composites in wind blades. *Acta Materiae Compositae Sinica* (In Chinese), 23(3), 127-129
- Jiang, Z.H; Wang, Z; Chang, L; Gao, L; Cheng, X.H. 2006. Manufacture technology of the bamboo building wall. *Journal of Beijing Forestry University* (In Chinese), 28(6), 155-158
- Jiang, Z.H. 2007. *Bamboo and rattan in the world*. China Forestry Publishing House, Beijing, China.

- State Forestry Administration of China (SFA). 2009. China forestry yearbook (In Chinese). China Forestry Publishing House, Beijing, China
- Sun, Z.J; Cheng, Q; Jiang, Z.H. Mechanical Property of Bamboo/Epoxy Composites. *Aeronautical Manufacturing Technology (In Chinese)*, 6, 89-91
- Yu, W.J. 2011. Development of bamboo fiber based composites. *China Wood Industry (In Chinese)*, 25(1), 6-10.
- Yu, Y; Jiang, Z.H; Tian, G.L; Wang, H.K. 2011. Improving Photostability and Antifungal Performance of Bamboo with Nanostructured ZnO. *Wood and Fiber Science*, 43(3), 1-12
- Zhang, Y.H; Meng, F.D; Yu, W.J. 2011. Crushing Effectiveness on Properties of Crushed Bamboo-Mat Composites. *China Wood Industry (In Chinese)*, 25(5), 1-4

Susceptibility of Bamboo species in India to the attack of powder post beetle, *Dinoderus minutus* and prophylactic and curative measures for their management

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Abstract

Bamboo wood is gaining more of popularity in India in diverse applications and serving to reduce the irrational use of scarce timber sources of the country. Currently many bamboo species are in the market being sold for purposes like making handicrafts, furniture, and structural material and also for bamboo composites. Freshly felled bamboo is prone to the attack of ghoon borers, mainly *Dinoderus minutus* Fab. (Coleoptera: Bostrychidae). The attack leads to the powdering and loss of strength of the bamboo culms. The products made from infected bamboo continue to propagate the life stages of the beetle. The valuable products become useless in no time when it contracts the beetle menace. Treatments by various methods like spraying or surface application using toxic insecticides may yield desired control, but are not preferred due to the indoor usage of products in contact with human habitation and also due to environmental concerns. The durability of species like *Bambusa pallida*, *B. balcooa*, *Dendrocalamus strictus* and *D. stocksii* against beetle attack were tested by adult and larval inoculation technique. Many botanicals including plant extracts (of *Cleistanthus collinus*, *Propolis juliflora* and Neem and Pongam formulation) and oils (Eucalyptus oil, Camphor oil, Clove oil, Cedar oil, Castor oil, Turpentine oil, Honge oil, Olive oil, Neem oil and Peppermint oil) were tested as prophylactic treatments and exposed to adult beetles and larvae to test their efficacy to ward off or kill the stages of the pest. Bamboo pieces were treated with 4% bark and leaf extract of *C. collinus* and 6% of *C. collinus* complex and *P. juliflora* complex. The treatments were very effective and there was no powder formation even after six months. The curing of attack is highly difficult as the larvae dwell inside the culms with least exposure to outside. In this context, the fumigants like Phosphine can be useful to penetrate the culms and kill the existing infestations. Phosphine gas in various dosages (0.5, 1.0, 1.5 mg/L) was tested against adult borers and larvae by in vitro testing. While the control specimens were 100% alive, none of the adults and larvae could survive after 96 hour exposure to all the dosages of phosphine. The results revealed the utility of phosphine fumigation for arresting the attack of borers in bamboo handicrafts and other artifacts.

Keywords

Bamboo, *Dinoderus minutus*, durability, curative measures, botanicals, fumigation, management

Introduction

Bamboos in India have a wide range of distribution forming an under storey in several forest types and the tropical moist deciduous forests of North and South India, the deciduous and semi-evergreen regions of North Eastern India are the home of bamboos (Tewari, 1992). The forest area over which bamboos occur in India, on a conservative estimate, is 9.57 million hectares and it is about 12.8 percent of the total forest area of the country. Only a few species like *Bambusa arundinacea*, *B. tulda*, *Dendrocalamus hamiltonii*, *D. strictus* are commercially utilised for pulp and paper though other species are used for cottage/rural industries. Bamboo being the fastest growing plant, efforts by various state departments and private growers are underway to raise bamboo plantations in a mass scale. Many industrial set ups have been established to promote bamboo craft. The powder post beetle, *Dinoderus minutus* (Coleoptera: Bostrychidae) powder the bamboo and poses immense threat to the harvested bamboo and also the products made out of them leading to colossal loss to the industries.

Stebbing (1910) was perhaps the first authority to report on the protection of bamboos from the attack of bamboo culm borers. The quantity of starch, the species and variety of bamboo, the age of the culm and the physical properties of the bamboo are the factors influencing the attack and control of the powder post beetles. The seasonal variations of starch content of wild *Bambusa arundinacea* and its relation to beetle borer *Dinoderus minutus* and *Minthea rugicollis* infestation was carried out in Kerala State (Joseph, 1958). The life history of *Lyctus brunneus* (Steph.) was studied and laboratory experiments were made on a possible inexpensive method of minimizing damage caused by the pest on bamboo soaked in water for 7-8 months (Kallapur 1968). During storage for up to 12 months, about 20-25% damage of culms has been reported in India (Varma and Bahadur 1980). Mathew and Nair (1990) have reported 12 species of insects attacking stored reeds and bamboos in Kerala. RajaMuthukrishnan *et al.* (2009) listed 65 species of insects occurring on bamboos under storage.

The results on the laboratory and field experiments on the protection of Bamboos (*Bambus nutans* and *Dendrocalamus strictus*) in storage against ghoon beetles, *Dinoderus* spp. using BHC and DDT in kerosene oil were reported by Roonwal *et al.* (1959). Clump curing of Bamboo (*Dendrocalamus strictus*) was found ineffective in protecting this bamboo species from powder post beetles (Mathur 1961). The relationship between starch content and susceptibility to *Dinoderus minutus* in the bamboo reed, *Ochanlandra travancorica* indicated that there was no correlation between the numbers of progeny produced by *Dinoderus minutus* with the starch content of the culms. However, the culm age and starch content might influence beetle reproduction (Damodaran *et al.* 1986). Protection of bamboo and bamboo products by proper seasoning and preservation methods has been investigated by many researchers (Puroshotham 1963; Verma and Pant 1981; Sharma *et al.* 1987; Singh and Tewari 1980 and Satishkumar *et al.* 1985). The present paper deals with the susceptibility of Bamboo species in India to the attack of powder post beetle, *Dinoderus minutus* and the prophylactic and curative measures for their management.

Materials and methods

Culture of *Dinoderus minutus*

The different species of bamboos collected from plantations/ bamboo depots were cut into small pieces (5 cms – 30 cms), dried to 20-30% moisture content and provided to adult *Dinoderus* released into mesh boxes/ glass bottles/ cages. Also tapioca chips cut into 5mm thickness and dried in sun shade were used for rearing the beetles. The dried chips were kept inside culture bottles and adult

Dinoderus minutus were released on them. The culture was maintained at laboratory conditions to get sufficient stages of the insect pest for experiments.

Studies on Management methods

Bambusa pallida, *B. balcooa*, *Dendrocalamus strictus*, *D. stocksii* and *D. asper* were used for this study. All bamboo culms were cut and separated into bottom, middle and top pieces. Two different types of treatments were given. Untreated pieces served as control.

a. Dip treatment

The chemical/ extract was taken in plastic vessels and different sizes were dipped for 24hrs and were taken out and kept for drying.

b. Pressure treatment

Bamboo pieces measuring 5cm from different positions were used for beer test. The culms of bamboo were treated by pressure impregnation (15 minutes vacuum followed by 50 lbs./sq.inch air pressure for 30 minutes).

c. Larval inoculation method

On the cut ends of the specimens (5 cm in length) 5 holes of 2-3mm diameter and 5mm depth were made and with the help of fine brush, early stage larvae of *Dinoderus minutus* were inoculated. Monthly observation was made. Powder obtained was collected and weighed. The observations were continued for 6 months. Number of adults emerged from each treatment was recorded.

d. Adult release method

5 cm long bamboo stakes from different parts of the bamboo culms (Bottom, middle and top portion) were treated and kept inside aerated mesh boxes. 10 adult *D. minutus* were liberated on the stakes in each box. Monthly observation upto 6months was made on powder formation. Powder obtained was collected and weighed. Number of adults emerged from each treatment was recorded.

e. Efficacy of botanicals/ oils for control of bamboo borer, *Dinoderus minutus*

Preliminary study was conducted to know the efficacy of oils to protect bamboo from borers. For this study, five different types of bamboos, *Bambusa bambos*, *B.pallida*, *B. balcooa*, *Dendrocalamus strictus* and *D. stocksii* were used. Pieces from bottom, middle and top of the culms were made. Ten different types of oils, Eucalyptus oil, Camphor oil, Clove oil, Cedar oil, Castor oil, Turpentine oil, Honge oil, Olive oil, Neem oil and Peppermint oil were used as such. With the help of paint brush, oils were applied on the bamboo pieces and kept for drying. Larvae (5 nos) were inoculated in each piece and kept inside the aerated box for observation. Each treatment was replicated four times. Similarly the tests were conducted by releasing adults on treated bamboos. Untreated bamboos served as control. *B. pallida* *B. balcooa*, *Dendrocalamus strictus*, *D. stocksii* were treated with 1%, 2% and 4% bark and leaf extract of *Cleistanthus collinus*, 6% of *C. collinus* complex (1: 3: 4 - extract, Copper and chromium) and 6% *Prosopis juliflora* extract (1: 3: 4 - extract, Copper and chromium) complex were treated on *B.pallida* and *B. balcooa*. *D. asper* *D. stocksii*, *D. strictus* and *B. nutans* were treated with a formulation of Neem and Pongam oil (neem seed oil 10% + Pongam seed oil 10%, Solvent 80 %)

f. Fumigation

Phosphine gas was generated by placing a pellet of Aluminium phosphide tablet in a gas burette placed in a solution of 5% sulphuric acid in a 1L beaker. For dosing, the required volume of gas was drawn from the gas burette using a gas-tight syringe, and injected in to desiccators through the self sealing septum fitted in the desiccators which were used as test chambers for exposing the life stages of insects. Cloth bag containing mixed-age culture contents were placed inside 2.85L desiccators. Three test concentrations *viz.*, 0.05, 1.0 and 1.5mg/l of phosphine were tested by injecting into desiccators.

Results and discussion

Durability of bamboo against borer attack

Natural durability

Natural durability of four species, *Bambusa pallida*, *B. balcooa*, *Dendrocalamus strictus* and *D. stocksii* against the attack of borer, *Dinoderus minutus* were tested in the laboratory by adult release and larval inoculation methods (Table 1 and 2). All the bamboos were susceptible to borer attack. Higher the powder, indication is lesser is the durability. The results revealed that *D. stocksii* and *D. strictus* were more susceptible followed by *B. balcooa* and *B. pallida*. The top portion was more susceptible to attack. This may be due to the higher starch content in the growing part of culm. The mean number of progeny emerged from each of the batch of bamboo within 6months was 32.5, 43.0, 44.5 and 45.5 for *B.balcooa*, *B .pallida*, *D. stocksii* and *D. strictus* respectively.

Table 1. Natural durability test against borers (adult release)

Bamboo species	Bottom	Middle	Top	Mean
<i>B. balcooa</i>	6.0750	5.5500	7.9500	6.5250 a
<i>B.pallida</i>	6.2000	5.3250	8.2500	6.5917 a
<i>D. stocksii</i>	7.4500	6.9750	9.1250	7.8500 b
<i>D. strictus</i>	7.5750	7.0500	8.9250	7.8500 b
Grand mean	6.8250 b	6.2250 a	8.5625 c	

ANOVA

SED CD (0.05) CD (0.01)

b 0.14815 0.30142 0.40496 **

p 0.12830 0.26104 0.35070 **

bp 0.25660 0.52207 0.70140 NS

Table 2. Natural durability test against borers (larval release)

Bamboo species	Bottom	Middle	Top	Mean
<i>B. balcooa</i>	6.6500	6.1000	9.1250	7.2917 b
<i>B.pallida</i>	6.4750	5.8750	8.1000	6.8167 a
<i>D. stocksii</i>	8.2750	7.1500	9.4750	8.3000 d
<i>D. strictus</i>	7.5000	6.8000	8.9500	7.7500 c
Grand mean	7.2250 b	6.4812 a	8.9125 c	

ANOVA

SED CD(0.05) CD(0.01)

b 0.17543 0.35692 0.47952 **

p 0.15193 0.30910 0.41528 **

bp 0.30385 0.61820 0.83055 NS

Durability of treated bamboos

Water extracts of *Cleistanthus collinus* 1, 2 and 4 % of leaf/ bark were used to test their efficacy to control borers . For this study, Bottom, middle and top parts of mature culm (*Bambusa balcooa*, *B. pallida*, *Dendrocalamus stocksii* and *D. strictus*) were used. As the lower doses were not effective, 6 % of *C. collinus* complex and *Prosopis juliflora* complex were tested against *B. balcooa* and *B. pallida* . For this study both larvae and adults were used.

The result revealed that the treatment with 1% and 2% bark extracts of *C.collinus* was not effective in controlling the borers, though the powder formation was lesser than control. Both pressure and dip treatments were on par with each other. There was highly significant variation with respect to Bamboo and position. The larval tests revealed that *D. stocksii*, was more susceptible to larvae followed by *D. strictus*, *B.pallida* and *B. balcooa*. The susceptibility of different portions varied in different treatments. Bottom portion was more susceptible followed by middle and top portion in 1% treatment. There was highly significant variation with respect to Bamboo and position.

Though 2% extract also could not fully stop powder formation, there was less powder as compared to 1% treatment and control. Pressure treatment controlled forming powder followed by dip treatment. The result revealed that *D. stocksii* was more susceptible to larvae followed by *D. strictus*, *B. balcooa* and *B.pallida*. Both treatments were on par with each other. There was highly significant variation with respect to Bamboo.

Table 3 Adult release assay -Efficacy of 1% bark extract of *C. collinus* against powder formation in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>Bambusa balcooa</i>	3.4250	3.3750	3.1250	2.9250	2.7250	2.8250	3.0667 d
<i>B.pallida</i>	3.3750	2.4000	2.6250	2.3750	2.5000	2.3750	2.6083 c
<i>D. stocksii</i>	2.7750	2.2500	2.7250	2.4750	2.4250	1.7000	2.3917 b
<i>D. strictus</i>	2.6250	2.5000	1.1750	2.7000	1.4750	2.4750	2.1583 a
Mean - Method	3.0500	2.6313	2.4125	2.6188	2.2813	2.3438	
Bamboo species	Bottom	Middle	Top	Dip method	Pressure method		
<i>B. balcooa</i>	3.4000	3.0250	2.7750	3.0917	3.0417		
<i>B.pallida</i>	2.8875	2.5000	2.4375	2.8333	2.3833		
<i>D. stocksii</i>	2.5125	2.6000	2.0625	2.6417	2.1417		
<i>D. strictus</i>	2.5625	1.9375	1.9750	1.7583	2.5583		
Grand mean	2.8406 c	2.5156 b	2.3125 a	2.5813	2.5313		

ANOVA:

SED CD(0.05) CD(0.01)

b 0.10195 0.20339 0.27011 **

p 0.08829 0.17614 0.23392 **
 m 0.07209 0.14382 0.19100 NS
 bp 0.17658 0.35229 0.46785 NS
 pm 0.12486 0.24910 0.33082 **
 bm 0.14418 0.28764 0.38199 **
 bpm 0.24973 0.49821 0.66163 **

Table 4. Larva release assay- Efficacy of 1% bark extract of *C. collinus* on powder formation in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	1.6000	1.6750	1.6500	1.5750	0.7750	1.2000	1.4125 a
<i>B. pallida</i>	1.7250	1.5500	1.5500	1.3750	1.6750	1.1250	1.5000 a
<i>D. stocksii</i>	1.8000	1.6250	1.7250	1.9000	1.6750	1.9750	1.7833 b
<i>D. strictus</i>	1.6250	1.7750	1.6500	1.7250	1.8000	1.7500	1.7208 b
Mean for method	1.6875	1.6563	1.6438	1.6438	1.4813	1.5125	

Mean powder formation from different positions/ treatments

Bamboo species	Bottom	Middle	Top	Dip method	Pressure method
<i>B. balcooa</i>	1.6375	1.6125	0.9875	1.3417	1.4833
<i>B. pallida</i>	1.6375	1.4625	1.4000	1.6500	1.3500
<i>D. stocksii</i>	1.7125	1.8125	1.8250	1.7333	1.8333
<i>D. strictus</i>	1.7000	1.6875	1.7750	1.6917	1.7500
grand mean	1.6719 b	1.6438 b	1.4969 a	1.6042	1.6042

ANOVA

SED CD(0.05) CD(0.01)

b 0.06038 0.12047 0.15998 **
 p 0.05229 0.10433 0.13855 **
 m 0.04270 0.08518 0.11313 NS
 bp 0.10459 0.20866 0.27710 **
 pm 0.07396 0.14754 0.19594 NS
 bm 0.08540 0.17037 0.22625 **
 bpm 0.14791 0.29508 0.39188 *

Table 5. Adult release assay- Efficacy of 2% bark extract of *C. collinus* on powder formation in four bamboo species in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	1.7000	1.7500	1.6000	1.9500	1.2500	1.7250	1.6625 a
<i>B.pallida</i>	2.0000	1.3250	1.8000	1.4750	1.5750	1.1500	1.5542 a
<i>D. stocksii</i>	2.1250	2.0500	2.3000	2.3000	2.2500	2.2750	2.2167 b
<i>D. strictus</i>	2.6250	2.4500	2.9000	2.7000	3.2750	2.4750	2.7375 c
Mean for method	2.1125	1.8938	2.1500	2.1063	2.0875	1.9063	
Bamboo species	Bottom	Middle	Top	Dip method	Pressure method		
<i>B. balcooa</i>	1.7250	1.7750	1.4875	1.5167	1.8083		
<i>B.pallida</i>	1.6625	1.6375	1.3625	1.7917	1.3167		
<i>D. stocksii</i>	2.0875	2.3000	2.2625	2.2250	2.2083		
<i>D. strictus</i>	2.5375	2.8000	2.8750	2.9333	2.5417		
Grand mean	2.0031	2.1281	1.9969	2.1167	1.9688		

SED CD(0.05) CD(0.01)

b 0.08384 0.16725 0.22212 **

p 0.07260 0.14485 0.19236 NS

m 0.05928 0.11827 0.15706 *

bp 0.14521 0.28969 0.38472 *

pm 0.10268 0.20484 0.27204 NS

bm 0.11856 0.23653 0.31412 **

bpm 0.20536 0.40969 0.54408 NS

Table 6. Larva release assay- Efficacy 2% bark extract of *C. collinus* on powder formation in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	0.9750	1.0750	1.1250	1.0750	1.0250	0.8750	1.0250 b
<i>B. pallida</i>	0.9500	0.4500	0.9000	0.9000	1.1000	0.8250	0.8542 a
<i>D. stocksii</i>	1.0750	1.1500	1.0500	1.1250	0.8500	1.2000	1.0750 b
<i>D. strictus</i>	1.0250	1.1750	0.9250	1.0500	1.0500	1.1000	1.0542 b
Grand mean for method	1.0063	0.9625	1.0000	1.0375	1.0063	1.0000	

Bamboo species	Bottom	Middle	Top	Dip method	Pressure method
<i>B. balcooa</i>	1.0250	1.1000	0.9500	1.0417	1.0083
<i>B. pallida</i>	0.7000	0.9000	0.9625	0.9833	0.7250
<i>D. stocksii</i>	1.1125	1.0875	1.0250	0.9917	1.1583
<i>D. strictus</i>	1.1000	0.9875	1.0750	1.0000	1.1083
Grand mean	0.9844	1.0188	1.0031	1.0042	1.0000

ANOVA :

SED CD (0.05) CD (0.01)

b 0.05827 0.11625 0.15438 **

p 0.05046 0.10067 0.13369 NS

m 0.04120 0.08220 0.10916 NS

bp 0.10092 0.20134 0.26739 NS

pm 0.07136 0.14237 0.18907 NS

bm 0.08240 0.16440 0.21832 **

bpm 0.14273 0.28474 0.37815 NS

Studies were conducted with 1 and 2% leaf extracts of *C. collinus* using adult release and larval release techniques

Table 7. Adult release assay – Efficacy of 1% leaf extract of *C. collinus* on powder formation in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) of bamboo culm –

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	3.7000	2.7750	3.4500	3.1000	3.4000	3.6500	3.3458 c
<i>B. pallida</i>	3.7000	2.6250	2.7500	2.5500	3.2250	3.7500	3.1000 b
<i>D. stocksii</i>	3.1000	2.5500	2.9750	2.7250	2.7500	3.3500	2.9083 a
<i>D. strictus</i>	2.8250	2.6500	3.3750	2.9750	3.7000	3.3500	3.1458 b
Grand mean for method	3.3313	2.6500	3.1375	2.8375	3.2688	3.5250	

Bamboo species	Bottom	Middle	Top	Dip method	Pressure method
<i>B. balcooa</i>	3.2375	3.2750	3.5250	3.5167	3.1750
<i>B. pallida</i>	3.1625	2.6500	3.4875	3.2250	2.9750
<i>D. stocksii</i>	2.8250	2.8500	3.0500	2.9417	2.8750
<i>D. strictus</i>	2.7375	3.1750	3.5250	3.3000	2.9917
Grand mean	2.9906 a	2.9875 a	3.3969 b	3.2458	3.0042

ANOVA:

SED CD(0.05) CD(0.01)

b 0.08163 0.16285 0.21627 **

p 0.07069 0.14104 0.18730 **

m 0.05772 0.11516 0.15293 **

bp 0.14139 0.28207 0.37460 **

pm 0.09998 0.19945 0.26488 **

bm 0.11544 0.23031 0.30586 NS

bpm 0.19995 0.39891 0.52976 **

Table 8. Larva release assay- Efficacy of 1% leaf extract of *C. collinus* on powder formation in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	1.7250	1.7000	2.0250	1.9500	2.5250	2.4250	2.0583 a
<i>B. pallida</i>	2.1750	1.7250	1.9000	1.7500	2.4750	2.0500	2.0125 a
<i>D. stocksii</i>	2.2250	1.9000	2.0250	2.1000	2.4750	2.3500	2.1792 b
<i>D. strictus</i>	1.9500	2.1000	1.8500	1.9250	2.2500	2.1000	2.0292 a
Grand mean for method	2.0188	1.8563	1.9500	1.9313	2.4313	2.2313	

Bamboo species	Bottom	Middle	Top	Dip method	Pressure method
<i>B. balcooa</i>	1.7125	1.9875	2.4750	2.0917	2.0250
<i>B. pallida</i>	1.9500	1.8250	2.2625	2.1833	1.8417
<i>D. stocksii</i>	2.0625	2.0625	2.4125	2.2417	2.1167
<i>D. strictus</i>	2.0250	1.8875	2.1750	2.0167	2.0417
Grand mean	1.9375 a	1.9406 a	2.3313 b	2.1333	2.0063

ANOVA

SED CD(0.05) CD(0.01)

b 0.05834 0.11639 0.15456 *

p 0.05052 0.10079 0.13386 **

m 0.04125 0.08230 0.10929 **

bp 0.10105 0.20159 0.26771 **

pm 0.07145 0.14254 0.18930 NS

bm 0.08250 0.16459 0.21859 *

bpm 0.14290 0.28509 0.37860 NS

Table 9. Adult release assay- Efficacy of 2%leaf extract of *C. collinus* on powder formation in four bamboo species. The powder formation in grams for dip(1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	2.2500	2.3750	2.2000	2.0000	2.1250	0.7250	1.9458 b
<i>B.pallida</i>	2.0500	2.0250	2.0500	1.7750	2.2000	2.0750	2.0292 b
<i>D. stocksii</i>	1.8500	1.6250	1.9250	1.6750	1.7500	1.7750	1.7667 a
<i>D. strictus</i>	2.3500	2.0750	2.8000	2.5000	2.2750	2.5500	2.4250 c
Grand mean for method	2.1250	2.0250	2.2437	1.9875	2.0875	1.7813	

<i>Bamboo species</i>	Bottom	Middle	Top	Dip method	Pressure method
<i>B. balcooa</i>	2.3125	2.1000	1.4250	2.1917	1.7000
<i>B.pallida</i>	2.0375	1.9125	2.1375	2.1000	1.9583
<i>D. stocksii</i>	1.7375	1.8000	1.7625	1.8417	1.6917
<i>D. strictus</i>	2.2125	2.6500	2.4125	2.4750	2.3750
Grand mean	2.0750 b	2.1156 b	1.9344 a	2.1521	1.9313

ANOVA

SED CD(0.05) CD(0.01)

b 0.07341 0.14646 0.19450 **

p 0.06358 0.12684 0.16844 *

m 0.05191 0.10356 0.13753 **

bp 0.12716 0.25367 0.33689 **

pm 0.08991 0.17937 0.23822 NS

bm 0.10382 0.20712 0.27507 *

bpm 0.17982 0.35875 0.47643 **

Table 10. Larva release assay- Efficacy of 2% leaf extract of *C. collinus* on powder formation in four bamboo species. The powder formation in grams for dip (1) and pressure treatment (2) on three positions of (bottom, middle and top) bamboo culm

Bamboo species	Bottom		Middle		Top		Grand mean
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
<i>B. balcooa</i>	1.7000	1.5000	1.4750	1.4000	1.5500	1.4750	1.5167
<i>B. pallida</i>	1.4000	1.4750	1.6250	1.3750	1.7250	1.6250	1.5375
<i>D. stocksii</i>	1.7750	4.2250	1.7750	1.7250	1.6750	1.6500	2.1375
<i>D. strictus</i>	1.3750	1.5250	1.5750	1.7500	1.5500	1.5750	1.5583
Mean for method	1.5625	2.1813	1.6125	1.5625	1.6250	1.5813	

Bamboo species	Bottom	Middle	Top	Dip method	Pressure method
<i>B. balcooa</i>	1.6000	1.4375	1.5125	1.5750	1.4583
<i>B. pallida</i>	1.4375	1.5000	1.6750	1.5833	1.4917
<i>D. stocksii</i>	3.0000	1.7500	1.6625	1.7417	2.5333
<i>D. strictus</i>	1.4500	1.6625	1.5625	1.5000	1.6167
Grand mean	1.8719	1.5875	1.6031	1.6000	1.7750

ANOVA

SED CD(0.05) CD(0.01)

b 0.29393 0.58638 0.77873 NS

p 0.25455 0.50782 0.67440 NS

m 0.20784 0.41463 0.55065 NS

bp 0.50910 1.01564 1.34881 NS

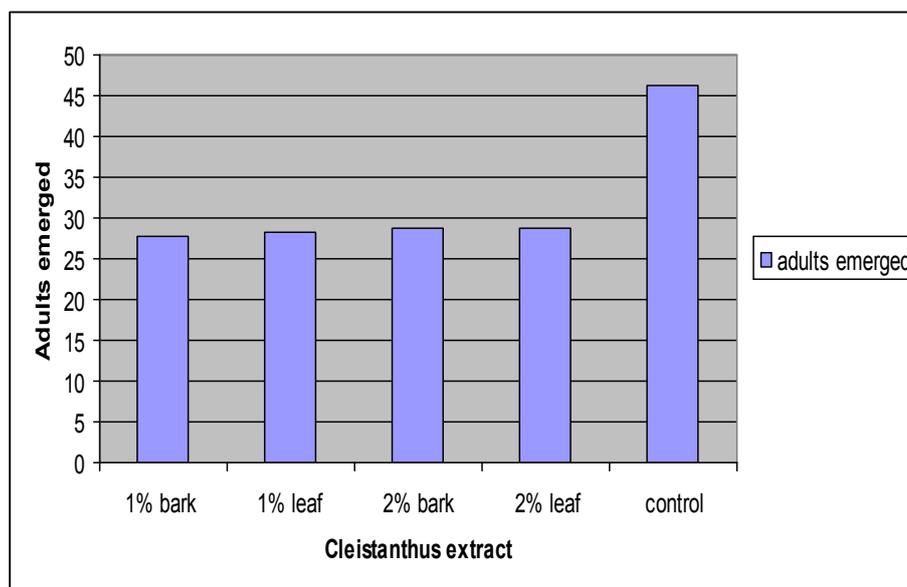
pm 0.35999 0.71817 0.95375 NS

bm 0.41568 0.82927 1.10130 NS

bpm 0.71997 1.43634 1.90750 NS

ANOVA B= Bamboo, P= position, M= Method.

The results of the treatments of 1 and 2% leaf extract of *C. collinus* revealed that they are not effective in stopping the *Dinoderus* attack. Pressure treatment was comparatively better than dip treatment in all the cases. The powder formation was slightly reduced in 2% treatment. There were variations in species susceptibility, though mostly *D. stocksii* was more susceptible to attack. There was highly significant variation with respect to Bamboo, method and position. Bamboo pieces were treated with 4% bark and leaf extract of *C. collinus* and 6% of *C. collinus* complex and *P. juliflora* complex. The treatments were very effective and there was no powder formation even after six months.



The study showed that the extracts (leaf and bark) at 1 and 2% were not effective in protecting the different species of bamboos from borer attack. However the powder formation was lesser than in control and the adult emerged from the treated batches were also lesser than in the control. But higher concentrations like 4 and 6% were very effective in protecting the bamboos from borer attack.

Efficacy of Neem based formulations

D. asper, *D. stocksii*, *D. strictus* and *Bambusa nutans* was cut into bottom, middle and top. All the pieces treated with a formulation of Neem and Pongam formulation (neem seed oil 10% + Pongam seed oil 10%, Solvent 80 %). Both pressure and dip treatment were given. After treatment both adult and larvae were liberated. Adult and larva liberated bamboo pieces kept inside aerated mesh box for observation. There was no powder formation.

Efficacy of oils for control of bamboo borer

Dried bamboos are severely attacked by *Dinoderus* spp. To control *Dinoderus* borer different types of oils were used without diluting and not making different concentrations. For this study both larva and adult *Dinoderus minutus* was used. The result revealed that all the oils were useful in controlling formation of powder upto one year. Garden (1945) showed that the mixtures of creosote and fuel oil (1: 1) or rape oil (1: 4) had completely protected entire culms for two and half years and prevented cracking; split culms were lightly attacked after one year.

Phosphine fumigation for control of bamboo borer

To protect bamboo from the borer attack, phosphine gas at different dosages (0.5, 1.0, and 1.5 mg/L) was tested against adult borers and their immature stages by *in vitro* testing. Post-fumigation studies indicated that all the three dosages of phosphine caused 100% kill of all life stages of *D. minutus*.

Natural durability of four species, *Bambusa pallida*, *B. balcooa*, *Dendrocalamus strictus* and *D. stocksii* against the attack of borer, *Dinoderus minutus* were tested in the laboratory by adult release and larval inoculation methods. All the bamboos were susceptible to borer attack. *D. stocksii* and *D. strictus* were more susceptible followed by *B. balcooa* and *B. pallida*. The top portion was more susceptible to attack. Starch in the culms of *Bambusa bambos* (L.) Voss and its influence on borer damage was reported by Bhat *et al.* 2005. The treatments with 4% bark and leaf extract of *Cleistanthus. collinus* and 6% of *C. collinus* complex and *P. juliflora* complex were very effective against borers and there was no powder formation even after six months. Fumigation studies with Phosphine indicated that all the three dosages of phosphine caused 100% kill of all life stages of *D. minutus*.

Various workers studied controlling *Dinoderus* spp. (*Dinoderus brevis*, *D. minutus* and *D. ocellaris*) by using synthetic pyrethroid. Thapa *et al.* (1992) reported that synthetic pyrethroid, fenvalerate even at higher concentration of 0.4% in diesel oil was ineffective for providing protection to bamboo culms against ghoon borers, *Dinoderus* spp. Cypermethrin at 0.4% in diesel oil and endosulfan at 1.5%, with small quantity of sticker triton, proved fairly effective by providing good protection for 4-5 months until the rains during July- August. Phosphine fumigation method is mostly studied with various grain beetles. Howe (1973) in his studies with eggs of rice weevil, *Sitophilus oryzae* (L.) observed that the mortality of eggs after fumigation for 1-5 days with phosphine at 0.1 to 0.4 mg/l was identical irrespective of the period of exposure. Rajendran (2000) in his study on *Tribolium castaneum* Herbst. (Stored grains beetle) observed that hatching was delayed and lower when 0±1-day-old eggs of the flour beetle were exposed to 30±50 ppm of phosphine for 72±120 h causing 70% or more mortality. The present study clearly indicated that botanicals from *C. collinus* and *P. juliflora* and oils were very effective against borers as a prophylactic measure and phosphine fumigation is efficient in eradicating the existing infestation.

References

- Bhat, K.V., Varma, R.V., Raju Paduvil, Pandalai, R.C. and Santhoshkumar, R. 2005. Distribution of starch in the culms of *Bambusa bambos* (L.) Voss and its influence on borer damage Bamboo Science and Culture: The Journal of the American Bamboo Society 19(1): 1-4.
- experiments on protection of bamboos in storage against ghoon beetles. *Dinoderus* spp. (Coleoptera:Bostrychidae) - *Indian For. Bull.* (NS) (Ent.) No.216: 2+ 1-32 +2pls.
- Howe, R.W., 1973. The susceptibility of the immature and adult stages of *Sitophilus granarius* to phosphine. *Journal of Stored Products Research* 8, 241±262.
- insecticides for the protection of bamboos in storage against ghoon borers, *Dinoderus*
- K. V. Joseph 1958. Preliminary studies on the seasonal variation in starch content of bamboos in Kerala State and its relation to beetle borer infestation. *J. Bombay Nat.Hist. Soc.* 55, 221 – 227.
- Kallapur, V.L 1968 Biology of the powder post beetle *Lyctus brunneus* (Steph.) and possible measures to control its damage. *Journal of Animal Morphology and Physiology India* 15 (1/2): 177-182.
- Mathew, G. and Nair, K.S.S. 1990. Storage Pests of bamboo in Kerala. **In**: Bamboo: Current Research (Eds. Rarnanuja Rao, I.V., Gnanaharan, R. and Sastry, C.B). Proc. Intl Bamboo Workshop, Cochin India.14-18 November, 1988. Kerala Forest Research Institute.Kerala. India. International Development Research Center, Ottawa, Canada. 212 - 214 pp.
- Mathur, R.N. 1961. Effect of clump-curing of bamboos and susceptibility to powder-post beetle attack: 11p.
- Purushotham, A. 1963. Utilization of bamboos. *J. Timb. Dev. & Press. Assoc.* (India) 9 (2): 1-18.
- Rajamuthukrishnana, Remadevi, O.K. and Sundararaj. R. 2009. A Checklist of insects infesting commercially available bamboos under storage condition. *The Indian Journal of Academy of wood science*, (N.S), Vol.6 (1and 2) : 45-54.
- Rajendran (2000). Inhibition of hatching of *Tribolium castaneum* by phosphine *Journal of Stored Products Research* 36 : 101-106.
- Roonwal, M.L., Chatterjee, P.N. and Thapa, R.S. 1959. Results of laboratory and field
- Satish Kumar, K.K. Kalra and P.B. Dobriyal. 1985. Protection of pulp bamboo in outside storage. *Jour. Timb. Dev. Assoc.* (India), 31:5-12.
- Sharma, S.N, A.K. Ananthanarayana, R.P. Bhaskar and T. Chandrashekar. 1987. Effect of pre-freezing on circumferential shrinkage of *Bambusa gigantea*. *Jour. Timb. Dev. Assoc.* (India), 33:26-32.
- Singh, B. and M.C. Tiwari. 1980. Studies on the treatment of green bamboos by stepping and sap-displacement methods. *J. Ind. Acad. Wood Sci.* II (I): 21-27.
- sp. *Journal of Indian Academy of Wood Science* 23(1); 39-47.
- Stebbing E.P 1910. A note on the preservation on bamboo from the attack of the beetle or shoot borer. *Indian For.*, (Pamphlet), No. 15: 18 pp.
- T. K. Dhamodaran, George Mathew, R.Gnanaharan and K. S. S. Nair 1986. Relationship between starch content and susceptibility to insect borer in the bamboo reed *Ochlandratravancorica*. *Entomon*11, 215 – 218.
- Tewari D.N. 1992. A Monograph on Bamboo. Published in India by R.P Singh Gahlot for International book distributors Dehra Dun. 273-292.
- Thapa, R.S.; Singh P.; Bhanadari, R.S. (1992). Prophylactic efficacy of various
- Verma, J.C and Bahadur 1980. Country report and status of bamboos in India. *Indian Forest Records*, 6: 28pp.. New Delhi, India. 200 pp.
- Verma, J.C. and M.M. Pant. 1981. Production and utilization of bamboos. *Indian Forester*, 107: 465-468.

Session 3. Ornamentals

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The Bamboo Garden

Ned Jaquith

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Bamboo Garden is a bamboo nursery in a typical northwest conifer forest near North Plains, Oregon. Our nursery is on 8 hectares about 30 Km west of Portland in the foothills of the Oregon Coast range. Our elevation at 100 meters is rather low, and though we are only about 75 Km from the Pacific Ocean, we can get rather cold. Our record is about -12 degrees Celsius and it is sometimes very snowy. Some winters, notably this one just finishing in 2012, heavy wet snow has broken a large number of culms, especially in our *Ph. vivax* groves.

My obsession with bamboo probably began as the result of living for 10 years across the alley from a grove of *Bambusa oldhamii* in Southern California. When I moved to Oregon in 1976 I brought a piece with me. Since *odhamii* was a subtropical bamboo, and because of my lack of bamboo cultivation knowledge it did not survive. But in 1980 I went to a plant sale for the Huntington Garden, in California where I met Richard Haubrich, President of the newly formed American Bamboo Society. I bought a dozen different bamboos and I was hooked, I joined the Bamboo Society, and for several years drove 1500 Km to meetings in San Diego.

For several years I was a hobby grower with plants in our city yard. In 1984 I rented a decrepit old greenhouse on 1 hectare in nearby Milwaukie Oregon. After 10 years at that rented facility, we bought our present nursery. It was 8 hectares of second growth forest which we logged to make way for our bamboo nursery. We recently bought the 2 hectares adjoining our nursery to the north, which gives us a total of 10 hectares.

We have 6 Greenhouses at Bamboo Garden totaling about 2400 m² for propagation and winter protection. With about 330 different bamboos we occasionally have bamboos in flower, which allows us to grow seedlings of many different bamboos. We are always watching our seedlings to look for useful new traits. We are also growing some bamboos of Bamboo Select tissue culture plants which are good for uniformity, whereas we value the seedlings for their diversity.

Shortly after joining the ABS Richard Haubrich put me in contact with Mike Bell in Cornwall, and Max Riedelsheimer in Munich Germany with whom I exchanged plants and seeds. In 1984 I went to my first bamboo conference outside of the western US in Mayaguez, Puerto Rico. There I met a number of *Bambuseros* of international stature. A Few were Tom Stoderstrom, Wolfgang Eberts, Deiter Orenberger, and Yves Crouzet, Oscar Hildago, Jorge Moran. There were many others, including Roger Stover who got lost in the jungle, when we went to search for McClure's *amabilis* grove in the mountains. He was carried out on a makeshift stretcher the next afternoon by the civil guard.

Our nursery now days is mostly run by Noah Bell who has been with us since 2001. Our web site is very important in the success of our nursery. Noah does most of the photography and writing on it. His bamboo photos and articles have been published by the ABS, the German Bamboo Society, (EBS), Paul Whitakers book: *Practical Bamboos*, and of course the catalog of Bamboo Garden.

We grow and sell hardy clumping, and timber bamboos in large sizes up to 12 + meters tall. Our market is the United States, and Canadian provinces on both the Pacific and Atlantic coasts.

At Bamboo Garden we do presentations for Garden clubs, host bamboo society and other Garden club visits. We have hosted Bamboo Society campouts and presentations. We support bamboo artists, even

having one, Charissa Brock, who designed our catalog, working at the nursery. She also teaches classes for those who wish to work with bamboo for crafts. Another valuable artist of international fame that is a friend of Bamboo Garden is Jiro Yonezawa. He was born and trained in basket making in Japan and moved to Oregon where he has lived and raised a family.

Our location in the foothills of the Oregon Coast Mountain Range is a very pleasant country setting. We have two ponds and a stream on the property that attract waterfowl and other birds. We often see deer, and occasionally elk and beaver. We also have problems with varmints such as gophers, moles and voles. Their tunneling and gnawing of bamboo roots and rhizomes combined with snow and wind can be very problematic for us.

Most of our bamboo sales are for landscaping, both residential and commercial. For me personally, the Lan Su Chinese Garden in Portland is the most important landscaping project our bamboo has been included in. I was on the Board of Directors before it was built and all 17 species of bamboo in the garden were donated by us. Our largest job was supplying 3 semi trucks full of bamboo for the Asia Trail exhibit at the Smithsonian National Zoological Park in Washington, DC .

Originally I just wanted to have a beautiful bamboo garden, which we are still developing; but due to our web page, some successful advertising, and attention to customer care, we are forced to sell some of our bamboos. Now sales both retail and wholesale require digging and propagation from most of our groves. Our main Moso grove is the only one that is sacrosanct. It is our special display, right by the office. It is large and tough, and has little damage from heavy snow. It is edged by a traditional bamboo fence made by Jiro, that graces its edge.

The garden has developed into a very nice place to visit. Nice enough, in fact , that we have had film crews and photographers come to shoot advertisements, commercials, and even a couple of documentary's. Including the Oregon Field Guide show: Trees and Pollution, where Andrea Melnychenko and Noah Bell discussed isoprene, a potentially polluting organic volatile emission of some plants. Which includes some bamboo. The video can be seen at www.opb.org

Creating Bamboo Garden has been one of the great joys of my life. People have taught me about bamboo, and I have had the pleasure of teaching others also. Many have helped us, Richard Haubrich, Kioshi Yoshida, Susanne Lucas, James Clever, Mike Bell, and from back when we used to write, I used to correspond with Max Riedelsheimer. There have been employees who found their passion for bamboo at Bamboo Garden then moved on to pursue their own vision. Our employees have been an absolute essential part of our success. Several have been with us 10 or 15 years. Without them there would be no Bamboo Garden. Visitors have had the opportunity to come and see the vast diversity bamboo has to offer and have walked away with a better understanding of this remarkable plant.

An Introduction to Bambuparque - A unique nursery and artist refuge in Portugal

Helder Carvalho

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To tell the story about Bambuparque I must go back around 40 years, where everything started in “La Bamboueraie of Prafrance”, in Anduze, South France.

Yves Crouzet, passionate about bamboo, had some difficulties to maintain the tropical bamboo collection that he had collected from around the world. Every winter they were suffering from the frost, so he imagined building greenhouses 15meter tall in order to accommodate them, but the cost of this project was prohibitive. The other solution was to find a better place somewhere else, where winters are not so cold, like in Southern Europe or Northern Africa, in order to transfer and maintain the tropical bamboo collection.

He found this in southern Portugal; he acquired “Herdade das Fontes,” a 100 hectares property where the climate is suited for tropical bamboo species. In order to settle the collection for a long time, it needed to be independent of the expense to maintain the plants under glass and a place with space to increase the collection. The nursery, named Bambuparque, was created in 1990. Because of the large area available, quite good growing conditions and the great demand for bamboo plants, Bambuparque developed itself faster and larger than he ever first imagined.

Nowadays, Bambuparque has as its main activities:

- The most important: production of more than 100 different tropical and temperate bamboo species for ornamental uses. Conscious of protecting the environment, we are certified by MPS (International organisation for sustainable implementations in Agriculture).
- The production and selection of bamboo poles for decoration and construction.
- The production and selection of bamboo foliage to feed the Pandas of the Zoo in Madrid, Spain.
- Technical support in landscaping projects.

Actually, in “Herdade das Fontes” we produce more than 1 million potted bamboos that we export to all Europe, Middle East and Africa

We all know that bamboo is an important part of Asian culture. The importance it takes gradually over thirty years in the West does not leave us indifferent. If Thomas Jefferson was right in saying: "The most useful service we can render a culture is to add a new plant to its agriculture," we feel sure that bamboo will soon become part of our western culture. This is why we decided to support this exciting integration.

Objectives

And how do we do that??

If we give the chance to everybody to discover bamboo, to meet bamboo and to appreciate and enjoy bamboo, and at the same time, we stimulate creativity, integrate bamboo into our landscaping, in our architecture, and in our everyday lives.

The place, in this case “Herdade das Fontes” in Portugal, valued for the several micro-climates, rich in water and sun, near the ocean, and more than that, it is a place with a lot of potential for inspiration.

- > The nursery with around 50 hectares and more than 100 bamboo species.
- > The increasing bamboo collection that it contains.
- > Forty years of knowledge and experience.
- > The diverse literature available for examination.
- > The network of associations, landscapers, urbanists, etc...

Line of action

The creation of a Bambuparque’s satellite company – ArtistEco.
Settled in the same place complementing the aim of Bambuparque,
ArtistEco’s prime goal is to implement bamboo in the twenty-first century’s dynamic with a particular thoughtfulness for Nature and Environment.

We are doing this through:

- Creation of awareness about the abilities of bamboo utilization,
- Welcoming artists and imaginative people and giving them the opportunity to be inspired by bamboo,
- Association of bamboo as an option to multiple daily concerns,
- and offering courses about bamboo cultivation, bamboo construction, etc.

In combination with ArtistEco, Bambuparque is not only a bamboo nursery, it is a place of encounter and exchange, a place of anticipation, resolutely turned towards the future while remaining strongly linked to Nature, values, and traditions that have stood the test of time.

www.bambuparque.com

ArtistEco Bambuparque has a Facebook page.

Ornamental Bamboos from Brazilian South East Atlantic Forest

Antonio Fernando C. Tombolato, Thiago Machado Greco and Moisés Medeiros Pinto

Abstract

The Atlantic Forest biome holds the highest diversity of bamboo species of the New World. However, the majority of these species is still unknown to the general public, mainly regarding their potential uses as ornamental plants. Among these species stand out those belonging to the genus *Chusquea*, *Olyra*, *Parodiolyra*, *Merostachys*, *Colantheia*, *Raddia* and *Pariana*, especially *Chusquea tenella* Nees, *Olyra glaberrima* Raddi, *Olyra latifolia* L., *Parodiolyra micranta* (Kunth) Davidse & Zuloaga, because the beauty of their shoots pattern and their flowering characteristics. Morphological and taxonomic studies involving these plants have significantly developed in recent years. Nevertheless, research about the adaptation of these species to growing conditions represent the main challenge to promote them as ornamental plants in Brazil and worldwide.

Resumo

O bioma Mata Atlântica abriga a maior diversidade de espécies de bambu do chamado Novo Mundo. Grande parte dessas espécies ainda é desconhecida do grande público, principalmente com relação ao potencial aproveitamento das mesmas como plantas ornamentais. Dentre essas espécies destacam-se as pertencentes aos gêneros *Chusquea*, *Olyra*, *Parodiolyra*, *Merostachys*, *Colantheia*, *Raddia* e *Pariana*, especialmente *Chusquea tenella* Nees, *Olyra glaberrima* Raddi, *Olyra latifolia* L., *Parodiolyra micranta* (Kunth) Davidse & Zuloaga, pela beleza das brotações e características de suas florações. Estudos morfológicos e taxonômicos envolvendo essas plantas evoluíram muito nos últimos anos, entretanto, pesquisas sobre a adaptação dessas espécies a condições de cultivo representam o principal desafio atual para que venham a ser difundidas como plantas ornamentais no Brasil e no mundo.

Ornamental bamboos in China

Hongchao Tan

Background

Professor Hongchao Tan was born in 1963 in Yunnan province of China. After graduation from Southwest Forestry University in 1985, he took his first job as assistant lecturer in the Afforestation Research Lab of SWFU, lecturing on Silviculture, Soil and Water Maintenance, Overview of Forestry and Technologies on Rapid Propagation and Productive Plantation of Bamboo. Since 1995, he has been lecturing in Yunnan Normal University on Cultivation and Processing of Bamboo, and Science of Ornamental Trees. In 1999, he obtained his master degree on silviculture from YNU. By far he has published 130 academic papers and 6 monographs.

Professor Hongchao Tan is also one of the evaluation specialists designated by the Science and Technology Department of Yunnan Province and Ministry of Environment Protection for bamboo, pulp and paper making projects. He has conducted over 100 bamboo projects concerning feasibility studies of bamboo projects, investigation and planning of bamboo resources, bamboo propagation, landscaping and plantation. He has experience of nursing bamboo seedlings on 6 million square meters of land and planting bamboo over 10 million square meters.

Due to his great contribution to bamboo industry, Hongchao Tan was awarded Exceptional Associate Professor in 1996 and Professor in 1998. With sound theory and rich experience on bamboo, Hongchao Tan is now devoted to promote planting and application of bamboo all over the world so that more and more people will understand and benefit from BAMBOO.

Presentation: Bamboo seeds and landscaping

1- flowers of bamboo

- a. Features of bamboo flowering
- b. Time of flowering
- c. Methodologies of bamboo flowering
- d. Boost bamboo flowering
- e. Enhance ripening rate

2- seeds of bamboo

- a. Morphology of bamboo fruits
- b. Storage and viability of bamboo fruits/seeds

3- propagation from seeds

- a. Sowing time
- b. Treating seeds
- c. Sowing methods
- d. Nurturing seedlings

e. Landscaping from seedlings

Session 4. Blue Economy

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The bamboo as a sustainable earthquake-resistant building material

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Abstract

In this lecture I want to share the rewarding experiences that I have had since I have been part of the ZERI Foundation since 1999 and in the 1st International Youth Congress with the Concept of Zero Emissions and the 5th World Congress carried out in the "*Eje Cafetero*", Colombia.

Objectives:

Introduce the bamboo (*guadua*) as a self-sustainable building material and earthquake-resistant, through some specific examples and teach the concepts used in these projects, in a presentation full of images and graphics.

Show with some architectural works how the human being manages in a good manner his creativity and applies it to the old and new techniques that adapt and respect the environment, and thus develops a habitat where all the basic needs are solved.

Demonstrate in the specific case of the bamboo (*guadua*) that if we take advantage of the local biodiversity and the resources in a responsible way we can mitigate the risks of natural disasters.

To begin I will present all the process of the building of the ZERI pavilion at Hanover, Germany, designed by the Colombian architect Simón Vélez. In this process I was present from the shipping and quality control of all the natural materials, structural tests, the construction process and finally for the inauguration of the pavilion, then during the experience during of several months of the EXPO, which generated a great expectation about this kind of constructions, natural materials and the other projects inspired by the ZERI initiative.

The pavilion was built with the best of nature, combined with the technology and creativity of the human being, applying concepts of sustainability and building techniques that led it to be one of the most prominent and admired in Hanover; therefore, it represented conclusively the theme of the EXPO: "Nature, humanity and technology". The ZERI pavilion is also a symbol of: biodiversity, synergy, creativity, new economy, tolerance, XXI century architecture, faith and hope, perseverance, sustainability, youth and perhaps many more.

In addition to the structural, earthquake-resistant and aesthetic qualities of bamboo (*guadua*) it is a species that fixes 40 times more carbon dioxide than wood, this kind of construction could be funded by the CO2 emissions rights for those who pollute the environment, thus supporting the most needy people and financing social housing.

After this experience I had the opportunity to design and build two houses in which I applied the knowledge I gained through this process and I resumed concepts (solar heating, natural ventilation, etc.) applied in other projects as in the case of *Gaviotas* in eastern Colombia, where Paolo Lugari

developed a self-sufficient hospital in the middle of nowhere

In these two houses the ZERI philosophy is perfectly reflected, because it developed the concepts such as self-sufficiency in water and energy, and respect and adaptation to the environment, all managed as a system within an experimental farm. In these cases, I combined native techniques like the “*bahareque*” with the latest techniques developed by the architect Simon Velez, where the mixture of natural materials with cement and steel create an earthquake-resistant structure capable of supporting buildings of the size of the ZERI pavilion. Here we see the importance of the use of local materials, which can help us to be prepared in a case of a natural disaster.

In the case of Colombia in 1999 there was an earthquake in the coffee region where many structures were severely affected and the houses built with bamboo were the less damaged, besides these constructions are lightweight that minimize the impacts. In this sense we must recover the local materials and the typical construction systems of each region, and thus take advantage of our ancestors’ wisdom to overcome natural disasters, and also we must maintain the intention to innovate with new techniques.

Keywords

guadua, bamboo, architecture, design, sustainable, pavilion

Zeri Pavilion for EXPO 2000

Symbols of the ZERI Pavilion

The ZERI Pavilion is a rich symbol offering a message, which goes beyond the mere building itself. The ZERI Pavilion offers a number of symbols to the world (Figure 1).

Biodiversity

It includes a plant, a flower and two types of grasses, which come from the same Andean ecosystem, with natural coloring from insects and preservation agents extracted from the same bamboo, which preserves itself against insects and molds (Figure 2).

Synergy

The best of nature is combined with the most creative forms of humanity, i.e. steel and cement. The performance of bamboo is dramatically enhanced with the technique of making joints with cement and iron (Figure 3).

Creativity

There are one hundred million people without a home. ZERI uses waste (used bottles) and weeds (bamboo, aliso and arboloco are considered weeds) for cheap, functional and beautiful housing as summarized in the book "Grow Your Own House" which is based on the experience gained in this pavilion (Figure 4).

New Economy

The present economy is good, but not good enough. The world needs a better production and consumption system, we need many more jobs. The ZERI Pavilion includes new building materials, grown and harvested sustainably, it demonstrates a preservation system eliminating toxic chemicals, and as such it creates new work and income. (Figure 5)

Tolerance

The ZERI Pavilion does not have an entrance nor an exit, it symbolizes the open mind, where all paths are welcome, from wherever they come, but having the same desire, to do more and better to respond to people's needs around the world (Figure 6).

21st Century Architecture

The ZERI Pavilion presents 7 new building techniques and 2 new construction materials approved by the German authorities, it offers a building method offering people a house which dances along with the movements of the Earth, and it is cheap (Figure 7).

Belief and Hope

The Pavilion was built without previous experience, without a clear budget, without a guarantee that the final permits would actually be obtained, though everyone who collaborated believed that it would be possible, and gathered all the energy needed to make it happen. And it did happen! (Figure 8).

Perseverance

The ZERI Foundation proposed to build the pavilion twice, once in Colombia in order to undertake the stringent stability tests by German professors, which were passed, to then build it at the World Expo. Never in history has anyone built twice any construction to be able to be present at an Expo. The cost of tests and approvals is higher than the building costs (Figure 9).

Sustainability

This building sequestered as much carbon dioxide as was needed to make it. Bamboo and *arboloco* used in ZERI housing, and fixed 40 times more carbon dioxide than timber; this building system could actually be financed with the CO₂ emission rights that the rich are offering the poor. Those who contaminate too much can now pay for social housing (Figure 10).

Youthfulness

The first ever event held at the ZERI Pavilion was a congress gathering 2.000 young people from around the world who saw in this building an opportunity to contribute to a better world. At the ZERI

Pavilion, over 100 volunteers welcomed everyone in nearly 40 different languages. It is an inspiration for all (Figure 11).

ZERI

There is no better symbol for the work ZERI wants to achieve, “use all waste and weed to generate food and housing”. This pavilion demonstrates that it is possible, and that it is cheap, therefore becoming a symbol for the poorest of the poor who now can take pride in their natural building materials. It is the same for our programs “beer bakes bread”, “cement factory goes organic”, “water hyacinth fights AIDS” (Figure 12).

EXPO

The ZERI Pavilion could very well become the symbol of the World EXPO. It is the only one considered a masterpiece by academics, which lead to the issuance of a diploma to all the workers. But more important, it is the only Pavilion, which introduces 7 new structural building systems, and 2 new building materials that were totally unknown to Germans. It is probably the best case of the theme “Nature-Humanity-Technology” (Figure 13).

Design

The pavilion was designed in a way that pushed the limits of materials and technologies, and knowledge gained immediately benefiting the less fortunate in social housing.

Facts

Form: Ten sided polygon inscribed in a circle (diameter=40m)

Area: Site 2.150m² – Foundations 684 m² – Mezzanine 458 m² – Roof 1.306 m²

Height: Total 14.40 m – Mezzanine 4.50 m

Weight: *Guadua*, *Aliso*, *Arboloco* and *Chusque* 100 Ton – Steel and Iron 10 Ton – Concrete 75 Ton Total 500 Ton

Roof slope: 33.3% = 17°

Columns: 40 *aliso* columns (20 interior – 20 exterior), 40 *guadua* columns on the second floor (20 interior – 20 exterior) Columns slope: 20% = 79°

Access to the mezzanine: Two spiral staircases from steel and bamboo.

Overhang length: 7.00 m

Materials and Tools

Guadua (Figure 14)

Family: *Gramineae*

Species: *Guadua angustifolia*, Kunth

Geographical distribution: Grows in the north of South America. Grows naturally in Colombia, Panama, Venezuela, Ecuador and Peru.

Ecology: Grows in fertile, rich and humid grounds at altitudes between 400 and 2000 msnm.

Maximum size of tree trunk: Height 25 m.

Diameter: 10-15 cm.

Environment: The compost of *guadua* leaves protect the earth and its extensive root system secures the existence of water.

Utilization in the pavilion: Beams, structure of the double flooring, internal columns, "flutes" (extensions of the columns), support of the roof, crowns and rings.

Aliso (Figure 15)

Family: *Betulaceae*

Species: *Alnus acuminata*, Humboldt, Bonpland and Kunth.

Geographical distribution: Grows in South America in countries like Bolivia, Chile, Ecuador, Peru

and Venezuela.

Ecology: Grows at altitudes between 2100 and 3000 msnm. Prefers humid grounds.

Maximum size of tree trunk: Height 35 m.

Diameter: 75 cm.

Mechanical qualities: (*Galanta 1953*)

- Specific weight: 0.325 to 0.461 kg/dm³
- Hardness according to Brinell 4.7 kg/cm² (tender)
- Resistance to traction 108 kg/cm²
- Resistance to the parallel compression of the fibre 357 kg/cm²
- Resistance to the perpendicular compression of the fibre 68 kg/cm²
- Cutting resistance 96 kg/cm²
- Bending resistance 504 kg/cm²

Utilization in the pavilion: Main Columns

Arboloco (Figure 16)

Family: Asteraceae

Species: *Montanoa quadrangularis*, Schultz Bip. In K.Koch

Geographical distribution: The Andean Zone of Colombia and Venezuela .

Ecology: Grows at altitudes between 1500 and 2500 msnm.

Maximum size of tree trunk: Height 20 m.

Diameter: 50 cm.

Mechanical qualities: (*Galanta 1953*)

- Specific weight: 0.68 kg/dm³
- Hardness according to Brinell 860.25 kg/cm²
- Resistance to traction 500 to 1500 kg/cm²
- Resistance to the parallel compression of the fibre 405 kg/cm²
- Resistance to the perpendicular compression of the fibre 131.71 kg/cm²
- Cutting resistance 111.27 kg/cm²
- Bending resistance 903 kg/cm²

Utilization in the pavilion: Beams in the double flooring.

Chusque (Figure 17)

Family: Poaceae Gramineae

Species: *Chusquea serrulata*, Pilger

Geographical distribution: Grows in high barren plateaus in the Central and Oriental mountain range of the Andes.

Environment: *Chusquea* secures riverbanks and protects rivers from evaporation, due to the shade it provides. *Chusquea* also has an esthetical value in gardens.

Utilization in the pavilion: Woven into the double flooring.

Others

Concrete: Roof, Footings, *Guadua* chambers and mezzanine. (Figure 18A, 18B, 18C, 18D)

Iron Washers: Produced by *SICOLSA "Siderurgica Colombiana S.A."* in Manizales - Colombia

Small washer: (Figure 19A)

Quantity: 2224

Weight: 0.14 Kg
 Diameter: 6cm
 Hole diameter: 2cm
 Shape: Curve
 Where? Along the *alisos* to fix them

Medium washer: (Figure 19B)
 Quantity: 918
 Weight: 0.66 Kg
 Diameter: 12cm
 Hole diameter: 2cm
 Shape: Flat
 Where? At the end of the roots and *Pie de amigos*

Big Washer: (Figure 19C)
 Quantity: 80
 Weight: 3.95 Kg
 Diameter: 20cm
 Hole diameter: 3cm
 Shape: Cup
 Where? Between the *alisos* and concrete footings

Technical Analysis of ductile casting

Pieces casted in Ductile Iron based in the standards ASTM A 536, Grade 65-45-12:

65 - Tensile strength min.: 65000 psi

45 - Yield strength min.: 45000 psi

12 - Elongation in 2 in. or 50 mm

Chemical Composition

% Carbon (C)	3.80% - 4.00%
% Silicio (Si)	2.40% - 3.00%
% Manganese (Mn)	0.20% - 0.35%
% Phosphorus (P)	<0.30%
% Magnesium (Mg)	<0.30% - 0.08%
% Other elements	<0.08%

Metallographic Structure

% Nodulizacion	>80%
Nodulos/mm ²	>150
% Ferrita	>60
% Perlita	<30

Screw, nuts and washers (Figure 20A, 20B)

Screws

80 of 1" x 1m

1.060 of ½" x 3m

192 of ½" x 1m

195 of 5/8" x 3 m
 87 of 3/4" x 3m
 160 of 5/16" x 1m

Nuts

80 of 1"
 650 of 3/4"
 2.150 of 5/8"
 23.455 of 1/2"
 1.700 of 5/16"

Washers

20.135 of 1/2"
 1.700 of 5/16"

Metallic Straps: Manual bending process. Used to attach the *alisos*, to give stability to the columns (Figure 21A, 21B, 21C, 21D).

Expanded Metal: To support and reinforce the layer of concrete in the roof (Figure 22).

Wire: Chicken wire in the mezzanine, to protect the *chusque* during the concrete work, and wire ties to secure the intersection of reinforcement bars (Figure 23A, 23B).

Recycled Bottles: The function is to contain the concrete, which is injected to the guadua and give it the form. All the bottles used are from own consumption waste. Glass bottles of water and champagne and plastic bottles (Figure 24A, 24B, 24C).

Macana: The *macanas* can be made from several palms, but those that were used in the pavilion are from the *Chontaduro* or *Chonta* palm (*Ceroxylon andicola*). They are a traditionally used in Colombia. Delivered by Gabriel Germán Londoño. (Figure 25A, 25B)

Diameter: 3 cm

Quantity: 1500

Bongosi: Support for the guadua roots in the aliso columns. Imported from Africa. (Figure 26)

Bend		25N/mm ²
Tension		15 N/mm ²
Pressure		20 N/mm ²
Cutter, cutting strength		2 N/mm ²
Tensile strength βzII		180 N/mm ²
Compressive strength βDII		95 N/mm ²
Flexural strength βB		180 N/mm ²
Shear strength Ta		14 N/mm ²
Bulk density	N	1,06 g/cm ³
Modulus of elasticity		
ET	1/S11	2060 N/mm ²
EL	1/S22	17000 N/mm ²
ER	1/S33	3230 N/mm ²

Aska Board: Product from Teiheiyō Cement (Japan), Made in Indonesia. Composition: 50% cement and 50% bamboo fiber. Measure 910x1820x9 mm. Quantity 1420 slates (Figure 27A, 27B).

Roofing felt: Waterproofing. Copper and bituminous felt. Hot application (Figure 28A, 28B).

Tools: Drills, hammers (industrial and handmade), concrete mixer, handsaw, frame handsaw, mortise chisel to make the “fish mouth”, plumb line, plane, ropes to lift tools and materials, belts to tighten the *guadua* during the process of the joints (Figure 29A, 29B, 29C, 29D, 29E, 29F, 29G, 29H, 29I, 29J, 29K).

Technologies

Support for pillars: By inclining the columns added support is achieved, making the pavilion stable and adding to its indifference to earthquakes (Figure 30).

Fiber and cement: The combination of bamboo fiber and cement is an innovation that can replace the asbestos in cement with natural fibers. This technology is using in the making of the roof of the pavilion (Figure 31).

Cement and *guadua*: Cement filled into the *cañutos*, the open chambers of the *guadua*, serve as reinforcement at the supportive points and also secures the iron fittings (Figure 32).

Guadua roots: This supportive construction uses the solid *guadua* roots to strengthen the structural system of the pavilion (Figure 33A, 33B).

Pillars: Pillars made out of concrete protect the wood from humidity coming from earth (Figure 34).

Smoked *guadua*: Immunization through the smoking of the *guadua* is a productive and sustainable alternative to chemicals used today. Speed of immunization is radically decreased, as is pollution (Figure 35A, 35B).

Overhang: The length of the eaves protects the wood structure from water (Figure 36).

Social Concept

Social Housing: The ZERI pavilion is like traveling to the moon. It pushes the construction techniques to the limits. Thanks to this experience, and thanks to new technologies recently acquired in Japan, a low cost house of less than 25 million Colombian pesos, which is about US\$10.000, can be constructed. The house is beautiful, functional and insensitive to the earthquakes and cheap. It has 65 square meters with a balcony, distributed over two floors (Figure 37A, 37B, 37C, 37D, 37E, 37F, 37G, 37H).

Environmental: Grasses like bamboo are the world's most renewable source of building material, growing 13 cm per day. Bamboo is harvested at 4-5 years of age and because it is a grass, it grows again immediately. Trees take minimum seven years to harvest time, and never grow again. ZERI scientists adapted a Japanese method of preserving bamboo with its own chemicals (Figure 38).

Simón Vélez developed new building techniques to create both, the ZERI pavilion and affordable homes. A 500 m² plot of bamboo yields the necessary amount for one house each year. This 65 m² house can be built for US\$10.000 in Colombia.

People

From idea to the construction, who has been involved?

Idea

Gunter Pauli, Founder and director of ZERI foundation, Belgium (Figure 39A).

Simon Velez, Architect, Designer of the Pavilion, Colombia (Figure 39B).

Paolo Lugari, Founder and director of “*Las Gaviotas*”, Colombia (Figure 39C).

Mario Calderon Rivera, President of *Camara de Comercio de Manizales* in 1999, Colombia (Figure 39D).

Carlos Bernal Quintero, Director of ZERI Latin America, Colombia (Figure 39E).

Construction

Sabine Bode, Architect, Project coordination, Germany (Figure 40A).

Volker Wehrmann, Architect, Site direction, Germany (Figure 40B).

Carolina Salazar Ocampo, Architect, Site supervision, Colombia (Figure 40C).

Pablo Atehortua, Foreman, Colombia (Figure 40D).

20 tradesmen and 20 laborers, Colombia (Figure 40E)

Supervising and approval

Wolfgang Schulz, Ministerium für Frauen, Arbeit und Soziales, Germany (Figure 41A).

Dip.-Ing. Josef Lindemann, Structural Analysis, Germany (Figure 41B).

Prof. Dr.-Ing. Klaus Steffens, Hochschule Bremen – Institut für Experimentelle Statik (IFES), Germany (Figure 41C).

Hans-Dieter Zeissner, EXPO 2000 Hanover GmbH, Germany (Figure 41D).

Hilmar Zander, Germany

Dr. Eng. Simon Aicher, Forschungs- und Materialprüfanstalt Baden-Württemberg (FMPA), Germany (Figure 41D).

Photography

Luis Guillermo Camargo, Colombia (Figure 42).

Cooks

Rosa Emilia Atehortua, Colombia (Figure 43A).

Ruby Esperanza Franco, Colombia (Figure 43B).

Reports and permits

Experimental evaluation of the load bearing properties of the pavilion, by Prof. Dr.-Eng. Klaus Steffens from the Experimental Statics Institute at the University of Bremen, Germany

Professor Klaus Steffens (director since 1980 of the Institute of Experimental Statics of the University of Bremen) has realized experimental evaluations of load bearing and safety for the reconstruction of the Reichstag building in Berlin, among others.

Cantilever-roof

Experimental trial burden: $F=6.5\text{kN}$

Consisted of determining the load bearing capacity of the cantilevers (a 7.30 meters overhang). (Figure 44).

This was done by hanging a weight of more than 650 kilograms in the middle of the third of their spans. A deformation of 7 millimeters was observed, which the structure recovered when it was freed of the burden.

Ceiling of gallery

Experimental trial burden: $F=4.0\text{ kN/m}^2$

Ceiling level 1: net weight + 2.0 kN/m^2 traffic load, including safety edge (Figure 45).

To test the capacity of the upper floor, this structure as loaded down with 55 gallon barrels, which were uniformly spread over the surface and filled with water until they reached a load of 400 kilograms per square meter. When the deformation of the upper floor under this burden was measured, it came to 5 millimeters, which were recovered when the weight was removed. It is important to note that the estimated deformation for this test was expected to reach 25 millimeters, which means that the result was a fifth of the estimate.

Frame

Experimental trial burden: $F=235\text{ kN}$

Horizontal bracing: net weight + 2.0 kN/m^2 traffic load + wind load, both without safety factors (Figure 46).

The third test involved a simulation of wind stresses and consisted of pulling the structure in a horizontal direction. This was done by placing one cable in the middle part and another in the upper part of each one of the pediments of the pavilion and the subjecting each cable to a horizontal load of five tons. The result obtained was a horizontal displacement of one centimeter.

After carrying out these tests in Manizales, Professor Steffens issued a technical assessment that helped to support the application for the construction permit that was granted for the pavilion in the Hannover Expo-2000 Fair. This study was complemented by a structural calculation carried out by Professor Joseph Lindemann, an estimate that was based, in part, on the results of traction, compression and flexion tests done by him in Germany. Thus *guadua* passed all the tests and was officially authorized for architectural use in one of the countries with the strictest construction codes in the world (Figure 47).

Letter from Klaus Steffens to Josef Lindeman after the structural tests in Colombia:

Engineer

J. Lindemann,

Lange Lambe 19, 3015Hannover, Fax 0049 511 196 66

Dear Mr. Lindemann:

I enclose the original results of the tests done to the cantilever roofs and the galleries. The deformations are surprisingly minimal and totally reversible without slow flow, even in the case of a continuous load.

In general, the building gives the impression of great solidity. There is no doubt that the pavilion will have no problems in Hannover, if it is done with the same quality. The execution of the manual work here is higher than the German standard. It seems to have the quality of fine carpentry!

Tomorrow we will do the horizontal test. Afterwards there will be a celebration! My presence here was necessary. There might not have been any progress this week without the general coordination of the tests that I carried out.

I am going to recommend, without hesitation, a rapid issuing of the partial construction permit, independently of Stuttgart, so that we are not vulnerable to setbacks due to lack of time.

Best wishes,

Klaus Steffen

Manizales 11-04-1999

Report by Dipl. -Ing. Josef Lindemann – Structural Analysis

Materials Evaluation in laboratory by Dr. Simon Aicher from the Forschungs- und Materialprüfanstalt Baden-Württemberg (FMPA) at the University of Stuttgart, Germany. (Figure 48A, 48B, 48C, 48D)

Construction

Preliminary stages

Before the construction process, there were some stages developed in Colombia. The previous stages were very important in order to obtain the German permits (Figure 49).

Selection and cut of materials

Guadua: Donated by Sr. Gabriel German Londoño Gutierrez from his farm "*San Jorge*" located in Pereira - Colombia (extreme coordinates latitude N 4° 45' - 4° 50' longitude W 75° 40' - 75° 55').

Zona Cafetera 1250 meters above sea level - 1900 mm of annual rainfall and 24° C average temperature. Cut in decreasing moon 3.500 pieces of *guadua* (9 m long) and 240 *guadua* roots (Figure 50A, 50B).

Aliso: Donated by *Aguas de Manizales S.A. E.S.P.* from its farm "Río Blanco" located in Manizales - Colombia. *Zona Cafetera* 2150 meters above sea level - 17° C average temperature. Diego Uribe was in charge of cutting the 200 *aliso* logs (Figure 51).

Arboloco: Some of the logs were donated by *Aguas de Manizales S.A. E.S.P.* from its farm "Río Blanco". The others were bought from *Maderas y Celulosa S.A.* in Manizales. *Zona Cafetera* 2150 meters above sea level - 17° C average temperature. Diego Uribe was in charge of cutting the 80 *arboloco* logs. 160 half pieces (Figure 52A, 52B).

Chusque: Donated by the *Comite de Cafeteros de Caldas* from its farm "Pedro Uribe Mejía" located in Manizales - Colombia. *Zona Cafetera* 2150 meters above sea level - 17° C average temperature. 8000 pieces of *chusque* (3m long) carried by mules (Figure 53A, 53B).

Quality Control

The German authorities request a quality control of *aliso* logs according to *DIN 4074*. The *guadua* quality control was made according to a standard created by Colombian experts and German engineers, especially for this construction. Quality control was not necessary for *arboloco* and *chusque*.

Aliso Quality Control: quality control was performed to every single log. Pablo Atehortua, responsible for the construction of the ZERI pavilion and the architect Simon Velez, revised all the logs. They affirmed that the quality of the *aliso* was excellent and even better than the logs used for the pavilion constructed in Manizales. The *alisos* were also checked and approved by Luis Miguel Alvarez, agronomy professor of Caldas University. After hearing the points of view of these people, the Quality Control requested by the German engineer Josef Lindemann, according to the *DIN 4074* standards, was made by Pamela Salazar (Industrial Designer) and Carolina Salazar (Architect). The diameters of the logs should be from 18 to 25 cm (Figure 54A, 54B).

This form was filled for every single log with 4 different tests. The logs should be in Class I or II according to the *DIN 4074* standard (Figure 55).

Guadua Quality Control (Figure 56)

Class I

Top: cross-sectional area $A > 40 \text{ cm}^2$ and $\phi \geq 10 \text{ cm}$ (e.g. $\phi 10$, $t=15 \text{ mm}$)

Base: cross-sectional area $A \geq 55 \text{ cm}^2$ (e.g. $\phi 14$, $t=15 \text{ mm}$ or $\phi 12$, $t=20 \text{ mm}$)

Middle: cross-sectional area $A \sim 47 \text{ cm}^2$ ($\phi 12$, $t=15 \text{ mm}$) and $\phi \geq 12 \text{ cm}$

Class II

Top: cross-sectional area $A > 30 \text{ cm}^2$ and $\phi \geq 10 \text{ cm}$ (e.g. $\phi 10$, $t=11 \text{ mm}$)

Base: cross-sectional area $A \geq 40 \text{ cm}^2$ (e.g. $\phi 12$, $t=12 \text{ mm}$)

Middle: cross-sectional area $A \geq 35 \text{ cm}^2$ and $\phi \geq 11 \text{ cm}$ ($\phi 11$, $t=11 \text{ mm}$)

Class III

The *guaduas* that do not match Class I and II, are not good for construction.

Immunitization

Every single *guadua* was immunized with smoke technique; this process involves treating the bamboo with its own chemicals, to protect it from insects and fungus. This technique was used in Japan, and now is being taken up to replace the toxic chemicals. The *guadua* used in the ZERI pavilion was immunized in two ovens in Colombia, most of them in Armenia, by Antonio Giraldo (Figure 57A, 57B) and the others in Pereira by Gabriel German Londoño (Figure 58A, 58B, 58C), both in Colombia.

Load and unload materials

Manizales: Two containers with *Alisos*, and one with *Arboloco* and *Chusque*.

Pereira: Ten containers with *Guadua*, *Guadua* roots and *Macanas*.

Most of the containers departed from Cartagena Port (Atlantic Ocean) and the others from Buenaventura Port (Pacific Ocean) in Colombia. They all arrived in Hamburgo Port in Germany, and then the containers were transported by trucks to Hannover. The transportation between Colombian and German ports took approximately 24 days. Panalpina was the company in charge of the transportation (Figure 59A, 59B, 59C, 59D, 59E).

Construction stages and timeline

Colombia: eight months to build the pavilion (Figure 60)

Germany: three months and two weeks (Figure 61)

Stage 1: The excavations and foundations were done by German workers (Figure 61).

Stage 2: Scaffolding – setting up elevation marks – installation of *guadua* rings – preparation of *aliso* (Figure 62A, 62B).

Stage 3: Installation of columns and *guadua* support rings – reinforcement of roof. (Figure 63A, 63B).

Stage 4: Construction of tuss, beams and diagonal support (Figure 64).

Stage 5: Reinforcement of floor by weaving together *arboloco*, *chusque*, iron and concrete (Figure 65A, 65B).

Stage 6: Finishing (Figure 66).

Construction techniques

Filling the *cañutos* (internal chambers of bamboo) with a mix of cement, sand and water (Figure 67A, 67B).

Fish mouth: Handmade technique to fit a bamboo with another (Figure 68A, 68B, 68C, 68D, 68E).

Concrete round bases made by hand (Figure 69A, 69B, 69C, 69D, 69E, 69F).

Bottles (Figure 70A, 70B, 70C, 70D, 70E, 70F).

Details

Finishing

Bamboo parket (Figure 71)

Staircase (Figure 72)

Railings (Figure 73)

Cochinilla (*Dactylopius coccus*) phytophagous insect parasite that lives as a guest of the tuna belongs to the family *Dactylopidae*. The *cochinilla* insect is mainly used for the extraction of the dye compound of two substances known as carmine and carminic acid (Figure 74A, 74B, 74C, 74D).

Sketches and drawings

The prototype of ZERI pavilion in Manizales was made with the original sketches of Architect Simon Velez.

To get the construction approval in Germany we had to make the complete drawings with all the structure details. Then the drawings were reformed and approved by, Dipl. Eng. Josef Lindemann and German authorities.

Simon Velez sketches (Figure 75A, 75B, 75C).

Approved drawings (Figure 76A, 76B, 76C, 76D, 76E, 76F, 76G, 76H, 76I, 76J, 76K, 76L).

Expo 2000 (Figure 77)

Press

Some German media who wrote about the ZERI pavilion:

Neue Osnabruecker Zeitung (Figure 78A)

(Figure 78B)

Kurier am Sonntag (Figure 78C)

Die Welt (Figure 78D)

Aus aller Welt (Figure 78E)

Wiesbadener Tabblatt, Die Stadtzeitung, (Figure 78F)

Pictures

Bus stop (Figure 79A, 79B, 79C)

Daily life (Figure 79D, 79E, 79F)

Football game (Figure 79G, 79H, 79I)

Winter (Figure 79J, 79K, 79L, 79M)

Surroundings (Figure 79N, 79O, 79P)

Kiosk (Figure 79Q, 79R)

Pavilion (Figure 79S, 79T, 79U)

Miñoca I

Experimental farm in the *Eje Cafetero*, Manizales, Colombia

With the development of an experimental farm in the coffee zone, ZERI *Eje Cafetero* wanted to show various technologies, processes and systems, which can contribute to sustainable development of the coffee zone. These technologies meet the basic needs of the environment, such as water, food, health, housing, energy, labor and education (Figure 80).

Location

Vereda la Trinidad, Manizales, Caldas, Colombia (Figure 81A, 81B, 81C, 81D).

Concepts applied

To start this project a first house of small dimensions was built (October 2003 - January 2004), which was designed taking into account the determinants of place and ecological principles (Figure 82A, 82B).

Dry sanitary and gray water treatment: Observing the lack of drinking water, the managing of black waters discharging without treatment in the basins, or using septic tanks with chlorine, it was considered that the long term most important challenge is the provision of high quality drinking water. Black waters are a source of bacteria and viruses, which are a threat for the health. The black water treatment at the farms requires and expensive infrastructure, chemistry and an elaborated maintenance (Figure 83).

But the greater usage of water is in the toilets, which require up to 20 liters of water per discharge. The human body has developed itself for 10 million years in a digestive system, that separates the liquid residues (dry urine in potassium) and solids (excrements), but the first thing we do is mixing both with drinking water?

Under the management of one of the scientific of ZERI Global Network, a dry toilet was designed, which allows the continuity in the separation of dry and liquid solids of our body, as our intestine and kidneys had provided before and to assure its use under the more strict sanitary rules (Figure 84).

The urine contains 80% of the nutrients leftover of our food, after a year, this mineralize itself and is free of hormones and antibiotics, in this condition, it is an excellent fertilizer for plants. The urine is collected in a tank next to the house and is used with water en portions from 1 to 8 (1 portion of urine and 8 of water) to irrigate the soil around the plants (Figure 85).

The excrements are full of bacteria and viruses, but in a dry environment these are eliminated naturally; after a one-year maturation process, the are mixed with the result of the composting of other organics wastes (as the kitchen wastes) and earthworms, to obtain excellent compost for the plants (Figure 86).

The architect Anders Nyquist designed this dry toilet with scientific support from Mats Wolgast of the Medicine Faculty in the University Uppsala in Sweden. The efficiency of this system was proved when after 18 months of its installation, an odor was never felt. The ingenuity of this toilet lies in its functionality, its sanitary hygiene and its low cost saving around 100 liters of drinking water per day per family (Figure 87).

For the gray water treatment, the most important is the choice of the products that are going to be used

at home, such as detergents, shampoo and soaps. All sorts of hardly biodegradable chemicals as benzene, perfumes and synthetic dyes and optical polishers and parabens are prohibited within the system. Natural products (preferably from ayurvedic source) do not impose a special treatment, neither expensive filtering system.

The gray water from the kitchen and the bathroom (there are no black waters) is canalized in an open system of red clay, which facilitates the water aeration before going into a filter constructed at ground level (of one cubic meter) with levels of stone going from big to small from the bottom to the top and sand at the bottom. The red clays channel is surrounded by lemongrass plants (*Cymbopogon citrates*) to avoid mosquito invasion and furthermore the sun ensures that the UV rays sterilize the channel each day (Figure 88).

Rainwater: The farm *La Miñoca* provides itself from rainwater, that are collected from the roofs in storage tanks, these waters pass through a filtering system allowing to eliminate the impurities with a size larger than 20 microns. To obtain drinking water, this rainwater is passed through a ceramic filter that eliminates impurities greater than one micron. With the constant rain of the zone, the rainwater is enough for irrigation, food preparation and toilet (Figure 89A, 89B, 90A, 90B, 90C).

Ventilation of the termites: The Sweden scientific Bengt Warne observed in the 50s, the capacity of the termites to keep stable the temperature (26°C) and humidity (61%) within their colonies; the phenomenon necessary to farm fungus in their nest was described in the book "BIOMIMICRY" of the American scientific Janine Benyus. Nowadays the air conditioning technique of the termites is the basis of the ZERI fable "The fan of the Zebra", which teaches children the principles to control humidity and temperature in the house. These principles were applied in the constructions of the experimental farm *La Miñoca* (Figure 91A, 91B).

Termites ensure the hot air out, creating a vacuum, which is compensated by the inflow of air through underground tunnels, cooling the hot air that by lowering the temperature has to leave some of its moisture, allowing the entrance of cool dry air (Figure 92A, 92B, 92C).

Construction with smoked bamboo: The engineer Antonio Giraldo (from Armenia, Quindío), learned from Marcelo Villegas the essential principles in the smoked bamboo process, a Japanese natural treatment based on 500 years of experience. The Architect Simón Vélez, Marcelo Villegas and Olga Lucia Londoño, were invited by the ZERI Global Network to make a technical visit to Japan with the support of the "Natural Life Foundation" of Japan, to know the ovens for smoking wood (including bamboo). The ovens use wood waste for production of charcoal and use the smoke of this process to treat fresh and green bamboo. It was demonstrated in Japan that the mixture of the smoke generated from wood with the juice from the bamboo plant protects bamboo against insects and fungi attacks. Antonio developed an experimental furnace and after 6 months he built a larger oven with capacity to treat 200 pieces of bamboo of 9 meters long at the same time.

Scientific investigations have confirmed the efficiency of this treatment, which eliminates harmful gases in the production of charcoal while immunizing the bamboo (against insects and fungi) without the need to use toxic industrial chemicals that pollute the house and are harmful to health.

On balance, ZERI Coffee Zone not only recommended, but also shows the efficiency of this treatment of smoked bamboo, using it in all its constructions.

In the oven that used to be in Armenia, Colombia can fit 900 bamboos 9 meters long, with diameters between 10-12 cms. According to all tests performed, it has been concluded that the cisco coffee is best for using with this system.

Process of the bamboo:

It must be between 4 and 5 years

It must be cut on waning in the 3 nights of more darkness

In the dawn 10:00 pm - 6:00 am

It should be cut between the first and second joint to prevent fungal attack and moisture. And facilitate regeneration and grow of new ones.

Leave vinegar in the bamboo field for 2 weeks (so that the sap goes down and prevent pest attack)

Choose good quality bamboo

Take out from the bamboo field and dry vertically for 2 weeks

Immunize: put in the oven for 2 weeks at 50°C

Cleanness

Design and construction

First level area: 43.63 m²

Warehouse and bathroom area: 34 m²

Total Area: 77.63 m²

Height: 5.90 m

Ceiling: At two waters

Foundation: Reinforced concrete

Warehouse and bathroom walls: Confined brick in concrete structure

First level walls: Bamboo, vein mesh and plaster with mortar

Covering structure: bamboo

Cover: Clay tile

Painting: Cal (white) and earth mixed with water and Acronal (terracotta)

Construction Stages

October 30, 2003: Foundations (Figure 94)

November 8, 2003: Foundations and lower plant walls (Figure 95)

November 20, 2003: Guadua Structure (Figure 96)

November 30, 2003: Roof structure, vein mesh and rework the walls and ceiling (Figure 97)

December 12, 2003: Wood Doors and windows, handrail (Figure 98A, 98B)

December 17, 2003: Ventilation systems and external finishing (Figure 99A, 99B)

December 27, 2003: Excavation for gray water channels, stairs and ceiling (Figure 100A, 100B)

January 1, 2004: Walls finishing and electric installations (Figure 101A, 101B)

January 7, 2004: Latest finishing (Figure 102A, 102B)

Drawings

(Figure 103A, 103B, 103C, 103D, 103E, 103F)

Miñoca II

Experimental farm in the *Eje Cafetero*, Manizales, Colombia

This project is based on a high quality housing, no frills, designed with ecological concepts and innovative technologies. All design meets the basic needs raised by the ZERI Foundation (Zero Emissions Research & Initiatives), applying concepts of conservation, adaptation and environmental friendliness (Figure 104).

Architectural design concepts

Sun and warm protection in a natural way

Climate and sun: Large overhangs on the covers: 1.70 - 2.70m. Double walls in places where the sun is stronger. Through these techniques a microclimate inside is created naturally (Figure 106).

Utilization of sights of the site

In direction to Manizales (Figure 107A), Nevado del Ruiz (Figure 107B) and Northern Caldas (Figure 107C).

Sun as an energy source: On the roof is placed a system of solar panels to heat the water supplied by Gaviotas, Colombia (Figure 108).

Winds: Natural ventilation system within the house (Figure 109).

Self-sufficiency in water: Rainwaters collection for consumption. Through the high ceilings, water is collected in storage tanks located in different parts of the house. Gray water is treated to avoid an impact on the environment, in the same way as in the small house (Figure 110).

Clay tile channels

Tanks for rainwater collection

Filters and water conductors.

Use of renewable materials and resources in the región

Guadua (*Guadua angustifolia* Kunth): Used in the bahareque walls and throughout the house structure (Figure 111A, 111B, 111C).

Cañabrava Family *Poaceae* (*Gynerium sagittatum*): used in the ceilings (Figure 112A, 112B, 112C).

Palma Macana or *Chonta* (*Iriarteia deltoidea*): Used in the handrails, finishing in interior stairs and doors (Figure 113A, 113B, 113C).

Soil of the site: Used as part of the mix for the base of ceilings and to paint and walls (Figure 114A, 114B).

Fique: Used on roofs as basis for soil mix (Figure 115A, 115B, 115C)

Name: *Fique*

Kindom: Vegetable

Gender: *Furcraea*

Family: Agavacense

Class: Angioesparmae

Subclass: Monocotyledonae

It is a Colombian native stalk grown in the Andes mountain range, especially in the departments of Antioquia, Boyaca, Cauca, Nariño and Santander.

Specialized manpower in bamboo construction techniques (Figure 116).

Quality of life and health (Figure 117): Materials that do not affect the health of people. In the construction of the house, no chemicals are used, pvc, asbestos, etc. Designing comfortable spaces large and healthy, to achieve a cozy atmosphere.

Implementation of natural systems that respect and adapt to the environment

Dry toilet: Two dry toilets will be installed in the house (Figure 118A, 118B, 118C).

Systems for gray waters (Figure 119).

Ventilation system for storeroom (Figure 120A, 120B, 120C, 120D).

Design with organic forms: Imitating and adapting to nature. In nature there are no right angles (Figure 121).

Implementation of technologies for construction in bamboo

Design that works self-sufficiently considering the Basic Needs such as water, energy, communications, etc.

Construction techniques

Cyclopean concrete foundation (Figure 122).

Foundation for bamboo protection (Figure 123).

Bamboo structure, reinforced with steel and concrete (Figure 124).

Daub walls with double covering: to generate isolation (Figure 125A, 125B, 125C, 125D).

The spaces are of great height and have openings in the top of the walls to facilitate the exit of hot air (Figure 126).

Immunitization with bamboo smoking system, without chemicals (Figure 127).

Double Roof: It consists of a double ceiling for air circulation, preventing that this passes hot inside the house (Figure 128A, 128B, 128C).

Overhangs and balconies: they protect the walls from sun and rain (Figure 129).

Doors and windows: Using doors and windows in wood and *macana*, with expansion to maintain a constant circulation of air inside the house (Figure 130).

Walls inclination for better stability (Figure 131).

Construction

Preliminary stages

February 2006: location and layout, excavations and foundation (Figure 132A, 132B, 132C).

March and April 2006: Foundations, stem walls and columns of bamboo (Figure 133A, 133B).

Guadua Structure

April 2006: Columns, structures of the intermediate level and daub walls (Figure 134A, 134B, 134C).

May 2006: Covering structure and stem walls (Figure 135A, 135B, 135C).

July 2006: Covering (*cañabrava*, *fique*, mix and tile), bamboo structure and stem walls (Figure 136A, 136B, 136C).

August 2006: Double ceiling in *cañabrava* (Figure 137A, 137B).

September 22, 2006: Volume of access and inner painting (Figure 138A, 138B)

September 27 of 2006: Dining room walls and outer painting (Figure 139A, 139B)

October 2006: Painting and finishing (Figure 140A, 140B).

Drawings

Ground plan (Figure 141A)

Access level (Figure 141B)

Level -1.87 and level +2.55 (Figure 141C)

Section (Figure 141D)

West elevation (Figure 141E)

East elevation (Figure 141F)

People

Engineer, Gonzalo Salazar, Advisor (Figure 142A)

Architect Javier Gutierrez, Constructor (Figure 142B)

Designer Pamela Salazar, ZERI Eje Cafetero Sub-director (Figure 142C)

Salvo and Fulvio, Politecnico of Torino, practice students (Figure 142D)

Gunter Pauli, ZERI Foundation and Achitect Carolina Salazar, House Designer (Figure 142E)

Guadua Architecture

Guadua Toll, between Manizales and Chinchina, Colombia (Figure 143A, 143B, 143C)

Bus stop, Autopista del Cafe, Colombia (Figure 144A, 144B)

Bridge Jenny Garzón in Bogota, Simon Velez (Figure 145A, 145B, 145C)

Bibliography

- Hidalgo, Oscar, Manual de Construcción con Bambú – Construcción Rural, Estudios Técnicos Colombianos Editores, Universidad Nacional de Colombia, Colombia
- Janine M. Benyus, 1997, Biomimicry: Innovation Inspired by Nature, William Morrow Paperbacks, USA.
- Kries, Mateo; Dethier, Jean; Steffens, Klaus; The Vitra Design Museum, 2002, Grow your own house Simón Vélez and the bamboo architecture, Vitra Design Museum, Germany.
- Pauli, Gunter, 2009, Gunter's Fables, Energy, Fundacion Hogares Juveniles Campesinos, Colombia, pp 128
- Villegas, Marcelo, 1989, Bambusa Guadua, Villegas Editores, Colombia
- Villegas, Marcelo, 2003, Guadua Arquitectura y Diseño, Villegas Editores, Colombia

Captions for figures

(Figures are provided separately)

- Figure 1: Image of the pavilion and a fungus
- Figure 2: Nature
- Figure 3: *Chusque*, chicken wire and concrete
- Figure 4: Social Housing, Simón Vélez design, Quindio Colombia
- Figure 5: Immunization oven, Armenia, Colombia
- Figure 6: ZERI pavilion inauguration, Hannover, Germany
- Figure 7: ZERI pavilion view, Manizales, Colombia
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- Figure 9: Josef Lindemann and Klaus Steffens
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- Figure 18B: Concrete footings
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- Figure 18D: Mezzanine
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- Figure 19B: Medium washers
- Figure 19C: Big washers
- Figure 20A: Nuts and washers
- Figure 20B: Screw
- Figure 21A: Bending process of metallic straps
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- Figure 23A: Chicken wire on the mezzanine
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- Figure 24A: Glass bottles

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 Figure 27A: *Aska* board tiles
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 Figure 28A: Roofing felt
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 Figure 29A: Drill
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 Figure 29E: Frame handsaw
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Bamboo Urban Density Housing

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Abstract

Mega city housing needs will require a higher urban density, which typically has meant concrete structures. However, multi-story, urban housing is today entirely feasible with bamboo based load bearing components, which could to a large extent replace reinforced concrete and steel beams. Most issues associated with bamboo construction are resolved today. Biological decay can be prevented by low toxic treatment, splitting and cracking can be avoided by pressure distributing connectors, dimensional standards can be achieved with new production techniques. The “poor-man’s-timber” stigma can be overcome with new architectural designs.

Urban growth scenarios

- Urbanization is accelerating even more with the impact of climate change. The consequences are already detected in terms of exacerbated rural conditions with extreme floods, landslides and droughts. Over time raised sea water levels and saline farming land will further push these migration trends.
- With escalating demand the cost of urban land increases dramatically and housing is forced to make use of inappropriate land such as steep hills with landslide hazards, swamps or other unhealthy environments.
- Conventional bungalow style housing schemes as promoted by banks, or huge squatter settlements and other *one level development* create an unprecedented *urban sprawl*. Mega-cities grow like cancer without the necessary facilities. Transport systems and urban infrastructure networks, totally insufficient today, will further deteriorate. Private solutions such as four wheel drives, personal generators and pumps can help only a few who can afford them but at a high environmental cost for everybody.

Innovative architecture needed

- Urban planning is needed more than ever for a reasonable housing density with functional utilities and services. Synergy effects could then be achieved when systems and networks are coordinated such as when waste problems are turned into energy resources, sewers to recycled water etc. Public transport can be organized for efficiency and cleaner environment.
- However higher urban density usually implies multi-level structures which typically means steel beams and reinforced concrete. These building materials are extremely energy consuming, CO₂ heavy and subject to rocketing prices as the demand for raw materials increases.

Hence innovative architecture using renewable materials is also needed. One resilient option is bamboo based design.

Bamboo

Bamboo is fast growing and can provide durable building materials in 3 year cycles if managed, harvested and treated properly. It does not compete with agriculture; it can grow on hill sides, around fish ponds and rice fields, along walk ways thereby also providing shadow. In landscaping it can be used for erosion mitigation and soil preservation. Another environmental advantage is the high CO₂ sequestration capacity, the carbon sink.

Bamboo promotes rural and local economies, offers livelihoods for unskilled people in the whole supply chain from cultivation to processed building materials. In addition other commodities can be produced such as furniture, food, fodder, pharmaceuticals, textiles....

Most issues associated with bamboo construction are resolved today, as can be seen in Colombia and Brazil. Decay by insects and fungi can be prevented with low toxic treatment based on boron derivatives. Curing and drying methods similar to what is used in the timber industry can prevent cracking and splitting. Most importantly, a conscious building design will then protect against weather impact such as driving rains, moisture and UV radiation from the sun.

Multistory housing requires dependable and earth quake safe column-beam connections. Combined with a few key connector components, bamboo can provide remarkable protection under seismic stress.

Prefab construction of bamboo building components in small workshops would allow for quality control, dimensional standards and facilitate adequate assembly on the construction site. In Sweden multistory structures in wood up to 8 floors are now becoming competitive and could inspire to similar concepts based on bamboo. Many issues are in common such as insects and fungi attacks, fire protection, sound insulation, weather impact...etc.

Innovative architecture and new construction methods can offer the urban style most people expect when leaving the rural poverty. Flush and planar walls, floor tiles in kitchen and modern bath rooms, AC thermal insulation; all of it is possible to combine with bamboo loadbearing structures.

Multi story, urban permanent housing is today entirely feasible with bamboo based components which could to a large extent replace reinforced concrete and steel beams.

Keywords

permanent housing, urban density planning, new architectural bamboo design

The Rhizome Approach: Towards holistically sustainable bamboo design

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Abstract

Bamboo dovetails into the growing demand for sustainable products and lifestyle options. It is a highly renewable timber replacement material which does not cause deforestation and preserves the environment. The eco-friendly potential and image of bamboo has already been tapped through several cutting edge products designed to be produced from industrially processed bamboo. These products successfully leverage bamboo's ecological and economic potential towards sustainability. However, they do not leverage bamboo's potential to create livelihood opportunities for both the urban and rural poor, and therefore do not positively impact the social and cultural tenets of sustainability.

This paper therefore focuses on the role of design as an enabler to achieve holistic sustainability across the bamboo production to consumption system, and presents the Rhizome Approach towards this. The approach is designed to facilitate the design of products which take into consideration the social and cultural tenets of sustainability, alongside the ecological and economic tenets. The approach was trialed through a collaborative workshop in India and the findings of this intervention are also presented in this paper.

Keywords

Sustainability, bamboo, design, craft, livelihood, holistic

1. Introduction

Design decisions and specifications have a significant impact on sustainability due to their economic, environmental, social (White et al 2008) and cultural spin-offs. Over 70% of the costs incurred over the product life cycle (Waage 2005), including product development, material production and processing, fabrication, distribution, use, and end-of-life handling (Waage 2005; White et al 2008) are determined by design decisions. Many product life cycle impacts that need to “cleaned up” could be eliminated or minimized by envisaging and addressing them at the conception and design stage (Maxwell et al 2003). This back-casting and visualization exercise is possible because design is a “problem-solving activity lodged between art and science (Greenhalgh 1997)”, which has at its core the design process, based on research, analysis and synthesis. These tools allow designers to create diverse and distant scenarios - including sustainability related scenarios- and innovate accordingly.

Yet, despite these facts establishing a strong case for sustainable design, a commonly accepted road-map or methodology to actualize the potential of design in addressing sustainability and sustainable-development is not yet in place. This is essentially because the science of sustainability is still a nascent discipline, with an emerging and growing body of scholarship. Various constructs of sustainability were studied to arrive at the construal of sustainability that would form the integral framework and reference point for this research. The understanding of the emerging discipline and science of sustainability; and recognition of specific tenets that influence it has expanded from the ecological context during industrialization, to include the social and economical tenets of the Triple Bottom line (Elkington 1997), and the cultural tenet of the Four pillars model (Hawkes 2001) post industrialization. This research defines sustainability as ‘A continual process of actualizing “the possibility that humans and other life will flourish on the Earth forever” (Ehrenfeld 2008) by maintaining the balance between diverse tenets including ecological, cultural, social and economic conditions.’

While there has been progress in addressing the ecological tenet of sustainability through tools such as the Life Cycle Analysis (LCA), Eco-compass etc., and through strategies such as Ecodesign, Design for environment (Dfe) and Closed-loop Design, there is little integration of holistic sustainability criteria, especially a systems-based view of sustainability, in mainstream design processes (Waage 2005). Interestingly, the fields of design and sustainability have a huge resonance: both are constantly evolving in meaning and understanding (White et al 2008). The creative, intuitive, and fluid nature of design positions it to solve complex and dynamic sustainability problems, through unorthodox means.

This paper reports on action- research that investigates the role and potential of design in addressing the social and cultural tenets of sustainability alongside its ecological and economical tenets, in the domain of bamboo.

Bamboo is a highly renewable timber-replacement material which restores degraded lands, prevents soil erosion and helps mitigate water pollution (Reubens 2010a, 2010b, 2010c). The tremendous interest in bamboo as a sustainable material has led to its application in a plethora of designed ranging from the Asus Bamboo Eco book computer and the I Pod Bamboo Shuffle case, to bamboo textiles, housing, furniture and lifestyle accessories, medical products and cosmetics. Workshops such as “Bamboo Boards and Beyond” facilitated by M.P. Ranjan in 2002, and “Dutch Design Meets Bamboo” (van der Lugt 2007) have successfully explored bamboo’s potential for commercially viable, innovative applications through a “technology-push” (van der Lugt,2007) approach, where bamboo was used in an industrially processed form.

While the resulting products, concepts and explorations, like the several applications described earlier, have contributed to the appreciation of bamboo as a commercially viable i.e. economically sustainable, and renewable i.e. ecologically sustainable material; they do not address the social and cultural tenets of sustainability as effectively. This is because actualizing products produced through industrial, technology-intensive protocols push traditional bamboo-working communities lower in the value addition chain: from being involved in all the processes from growing to final assembly, their role becomes limited to growing, managing, harvesting, transporting and at the most primary processing of bamboo (Reubens 2010a, 2010b, 2010c).

Bamboo has a tremendous potential to positively impact the social and cultural tenets of sustainability given that it is easily available to poor communities in Asia, Africa and Latin America in their natural environment, often, even in their homesteads. Bamboo's linear fibers allow it to be easily processed using simple tools including by marginalized groups such as women. Bamboo-based enterprises require lower capital, raw-material and machinery investments than other micro, small and medium enterprises. Crafting bamboo is part of traditional non-industrialized production, social and cultural systems (Reubens 2010c, 2010d).

Designs that allow traditional bamboo-working communities greater participation in the production to consumption chain can address the issue of sustainability in a holistic manner, while simultaneously actualizing bamboo's potential to allow for economically viable, culturally sensitive, socially equitable, and eco-friendly production (Reubens 2010c, 2010d).

2. Barriers to holistic sustainability in bamboo product design

As discussed above, the thrust area for the development of designed bamboo products is bamboo processed through new industrial technologies: technologies which exploit and bring to fore previously unknown possibilities of bamboo as a material. Reconstituting the natural bamboo culm through 'technology-push' approaches, allows for standardization of bamboo's dimensions and properties. However, treating bamboo industrially can turn it into an ecologically unsustainable 'monstrous hybrid' (C2C framework *in* Shedroff 2009): it is difficult to recycle or dispose bamboo bonded in a resinous matrix.

Maxwell et al (2003) identified barriers to sustainable innovation in product and service development which are very relevant to the bamboo sector. These are outlined below:

Lack of approaching sustainability holistically by simultaneously addressing the social, economic and cultural tenets alongside the ecological tenet

Inability to mainstream sustainability concerns in the business system, thereby not facilitating the sharing of sustainability related experiences and concerns across the business itself
Lack of integrating sustainability criteria, (social, economic, ecological, and cultural) at a strategic corporate level alongside traditional criteria such as market, quality, technology etc. and thereby also in the design brief

Focus on "cleaning-up" product end-of-life environmental impacts rather than addressing sustainability holistically at the concept generation or design stage

Lack of focus on achieving sustainability across product supply and value chains from the Original Equipment Manufacturer upwards and downwards

In addition to these, the action research on which this paper reports, indicates that a lack of awareness

of non-traditional, non-industrial production to consumption systems and value-chains is another major barrier to sustainable innovation in product and service development in the bamboo sector. The word “industrial designer” implies a strong connect to the process of industrialization: an industrial designer by definition is therefore a designer trained to work along the industrial principles of division of labour and assembly line production (Rees 1997). Rees argues that “designers design for the industry, rather than for the consumers of the products of the industry. It is a way of thinking about the world which, by implication, denies the social, cultural and economic significance of consumption” (Rees 1997).

In industrialized set-ups the designer’s role is based underlying principle of division of labor, but in non-industrial set-ups a more systemic overview is necessary. Designers need to be oriented to integrated scenarios or provided with a framework or guidelines regarding how non-industrial set-ups function. Without these, they will veer towards the familiar conventional industrialized “technology push” approach; which at best addresses the ecological sustainability, especially as this is becoming unavoidable given the current policy and regulation environment.

3. The Rhizome Approach

The Rhizome Approach was developed during this action research. It has been applied to facilitate several collaborations between craft and design, including through a collaborative multi-institution, 14 day workshop which began on the 20th of January 2011; at the Design Innovation and Craft Resource Centre (DICRC) at the Centre of Environmental Planning and Technology University (CEPT), Ahmedabad in India. The 24 design participants included professionals; fresh graduates and post graduates and students from the Faculty of Design, CEPT University, Ahmedabad and the Indian Institute of Crafts and Design (IICD), Jaipur. The 24 Craft participants from Waghai were Kotwalia bamboo-working trainees linked to the Tapini Bamboo Development Center (TBDC) and the Eklavya Foundation in Ahmedabad.

The Rhizome Approach aims to address the barriers to sustainable bamboo product development identified earlier. The approach consists of a 7 point system which is represented in Table 1.

Table 1: The Rhizome approach

Step	Barrier	Aim	Mechanism
1	Lack of knowledge about sustainability	Inform designers about sustainability, its identified tenets, and the inter linkages between them	Book titled “Bamboo in Sustainable Contemporary Design” which discusses the linkages between bamboo, sustainability and design
2	Lack of holistic oversight of production to consumption and value chain	Sensitize designers to the systemic production to consumption and value-chain picture	Exposure visits to traditional bamboo-working communities, community enterprises, industrial enterprises and the other stakeholders in the PCS chain
3	Lack of including sustainability at a strategic level in the overall	Provide direction on the larger goal and its blue-	Sharing and explaining the Rhizome framework for an

	approach	print that the organization is aiming/aspiring for	overall picture towards which all departments will work jointly.
4	Lack of including sustainability criteria alongside traditional criteria in the design brief	Articulate sustainability criteria in the design brief so that it can be addressed early on at the design concept stage	Clear brief to “design a commercially-viable bamboo product, using local production capacities, that leverages indigenous knowledge systems.”. Provision of a “sustainability check-list” to clarify the criteria desired in the product.
5	Lack of collaborative design process	Provide inputs from different disciplines so that the design process is collaborative and different concerns are represented and addressed	Constant linkage and interaction with representatives from the PCS and VC including experts in the fields of sustainability, production, marketing, community, finance etc.
6	Lack of tool to measure holistic sustainability against indicators	Increase designers accountability to consciously, and seriously factor sustainability into the design, and to provide an opportunity for evaluation against the same indicators outlined at the concept stage	Using the “sustainability check-list” as the indicators, perform a 360 degree evaluation of the design, which includes self evaluation by the designer, and cross- validation of results by a sustainability expert and a community representative
7	Lack of keeping design team in the end loop of product actualization	Continuing the collaborative design process by keeping design team in the loop until the final product actualization stage, thereby retaining the overall perspective of the product sustainability	Involving design team in all changes required from the perspective of the product actualization, until all issues, including production systems, costing etc. are resolved.

Step 1: Providing knowledge about sustainability: the book

This step addresses the fact that in order to design sustainable products, designers have to be knowledgeable about the concept of sustainability as a systemic construct which rests on interconnected ecological, economic, social and cultural tenets. While sustainable design needs to consider these tenets both singly and systemically during the design process, most industrial designers lack expertise and knowledge in this area.

The first step of the Rhizome Approach therefore aims to bridge the knowledge gap on sustainability

in general and the interlinkages between bamboo, sustainability, craft and design which are the domain of this research specifically; by providing designers with information through focused presentations, supplemented by reading material.

During the workshop, both design and craft participants were exposed to the concepts underpinning sustainability and sustainable development, through an interactive power-point presentation. Following this, Rebecca Reubens presented her book titled 'Bamboo in Sustainable Contemporary Design', which encapsulates her experiences while at the International Network for Bamboo and Rattan (INBAR), where she worked as part of an expert interdisciplinary team in the area of bamboo based development. The book is aimed at designers and the layout is therefore very visual. Sharing the book with designers facilitated their access pertinent and basic information on sustainability, bamboo and sustainable design.

The book maps concepts such as sustainability, sustainable development, a sustainability timeline, and bamboo vis-a-vis sustainable design thus enabling meaningful learning. It includes sections on bamboo as a material resource i.e. the morphology of bamboo, bamboo usage and traditions, the natural characteristics of a bamboo culm and their design implications, and species-wise resource planning considerations. Different processing set-ups and the facilitating, enabling and challenging factors pertinent to each set-up are discussed. Production and material information, such as joinery for different applications, and traditional to industrial techniques and potential combination materials are covered.

Step 2: Holistic Oversight of Production to Consumption System: Exposure Visits

Step 2 addresses the fact that designers, like the other actors in the industrial production to consumption chain have lost oversight of the systemic picture due to task specialization and division of labor. This loss of the systems perspective makes it difficult to approach sustainability in a holistic manner: designers look at addressing immediate issues (such as the fragile ecological situation) rather than exploring holistic, integrated and sustainable solutions. Step 2 supplements the didactic learning inputs in Step 1, through exposure-visit based experiential learning inputs. This is done through a first-hand exposure to how the various different, yet interlinked, actors of the value chain together contribute to sustainability or unsustainability through the production to consumption process. The aim is to create and use dynamic experiences as a kinesthetic learning tool, to sensitize designers to the systemic production to consumption and value chain picture.

This was done by an intensive visit to Waghai, a town in the Dang region of South Gujarat where the participants visited different scales and types of bamboo and timber production set-ups to understand the dynamics of each on ground level. On the first day, two groups of participants visited nearby Kotwalia villages along with facilitators, to experience how this community works. The participants interacted with the craftspeople, and saw the lives, homes, culture and tradition of this community first hand. Several of the participants tried their hand at bamboo working and realized that while the craft practice appeared to be deceptively simple, it actually required very high skill.

Following this, the participants visited Vanil Udhyog, an integrated woodworking unit established by the State Forest Department Corporation in Vansda. The unit infrastructure includes a saw mill, seasoning plants, wood processing plant, and joinery and assembly workshop. Experiencing industrial scale timber operations allowed the participants to make a comparison between the industrialized timber, and labor intensive bamboo production to consumption systems.

The final visit of the day was to Waghai botanical Gardens, where the participants saw different species of bamboo and understood the morphology of bamboo discussed during the presentation in Step 1.

On the second day, the participants visited the TBDC-Eklavya training and production center where they experienced the how a traditional craft can evolve into a craft industry set up, through skill and capacity building. The participants interacted with the Kotwalia trainees, and also saw how the use of power tools, production streamlining, and product and systems design could enhance productivity and recontextualize traditional craft practice, while still maintaining the ethos of holistic sustainability embedded in craft.

Sharing of experiences through informal and formal discussions, and interacting with stakeholders and actors in different set-ups; allowed the designers to internalize the potential for realizing sustainability through a paradigm shift in the production set-up, including production volume, livelihood opportunities, preservation of the social and cultural nucleus, and the use of materials.

Step 3: Including sustainability at a strategic level: the Rhizome Framework

A holistic strategy to achieve sustainability and reduce unsustainability is not often part of organizational mandates: designers therefore lack both an immediate reference point and the backdrop of the larger organizational scheme.

Step 3 focuses on sharing the Rhizome Framework with the participants to provide an overarching strategy towards holistic sustainability in the bamboo sector, through design. The Framework is a proposition towards a model which offers different design directions, to harness local and craft-based production possibilities. Bamboo-craft is a vital force in communicating and substantiating the culture and tradition of bamboo-working communities. Simultaneously, bamboo's huge commercial potential can be exploited to help contribute to large scale employment of these indigent communities, who do not have much capital, but are rich in indigenous knowledge and have a strong skill and resource base. Therefore, the Rhizome framework seeks to use indigenous knowledge as a design input during the innovation process. The indigenous knowledge is viewed by the designer in the context of the sustainability, and factored into innovation in collaboration with the craftsman. This collaboration between the two maximizes the skill and knowledge each of them brings to the innovation process.

The Rhizome Framework was presented to both design and craft participants, and discussed in detail. Following this, a series of group brainstorming exercises were conducted in order to involve both design and craft participants in the strategy and rationale of the Framework. An exercise on whether craft is relevant was followed by an exercise on understanding the systems impact of each direction the Framework proposed vis a vis the tenets of sustainability. Both design and craft participants brainstormed on the product possibilities for each direction, which they represented through words and visuals. The outputs of all the exercises were presented through interactive sessions.

Step 4: Including sustainability in the design brief: the Sustainability Checklist

In the absence of a clear brief which clearly articulates the desired sustainability criteria, the onus of incorporating sustainability into the design brief is on the designer: this is difficult, considering that sustainability has not been part of the expertise of traditional design function. Step 4 therefore focuses on providing a clear brief. The participants in the workshop were clearly briefed 'to design a commercially-viable (economically sustainable), product made from mature, sustainably-harvested bamboo (ecologically sustainable), using local production capacities (socially sustainable), that leverages indigenous knowledge systems (culturally sustainable).'

In addition to the brief, a Sustainability Checklist developed by Rebecca Reubens as part of her PhD research, was shared with the participants. The checklist supplements the rules of thumb developed in the Design for Sustainability D4S-DE Manual, with inputs from the Global Reporting Initiative (GRI) Guidelines (2000) and Hawkes 2001 publication 'The Fourth Pillar of Sustainability'.

The Sustainability Checklist illustrates the generic product production to consumption system and the

sustainable design parameters relevant at each stage to enable the innovator to understand the interlinkages between the tenets and the production to consumption better. The tenets of sustainability strongly influenced by each parameter are indicated, along with the potential of craft practice to address and be fortified by these parameters. By understanding the systemic perspective through the deconstructed parameters, the collaborative craft-design object and be strategized to be culturally, ecologically, socially, economically or holistically sustainable. The checklist makes the innovator aware of the potential and desired criteria that can make a product more holistically sustainable and also serves as an indicator of sustainability factors achieved, once the product is developed.

Step 5: Collaborative Design Process: Dialogue and Technical Backstopping

There is a need to bridge diverse actors within the organization to facilitate transitioning from a pipeline design sequence to an integrative design process. Step 5 of the Rhizome Approach facilitates developing systems, methodologies, platforms and frameworks that allow for communication and collaborative decision making and participatory design by encouraging and actively facilitating a constant linkage and interaction between the actors, facilitators and enablers of the value chain. Designers are ideally placed to facilitate this process since they are good at intuitively uncovering needs as well as interpreting and communicating abstract of 'soft' information.

Three exercises were designed and facilitated during the workshop in order to facilitate collaborative design. These ice-breaking exercises were conducted in order to help the designer-craftsperson team find a working comfort level which would make it easier to communicate and collaborate. The first exercise was structured along a game called 'find your partner' and helped create a playful and conducive atmosphere. The second exercise required each team member to find out 3 'secrets' about their partner: the process led to discussions and confidence and interaction building. Following this each participant was asked to draw their hands in order to facilitate psychological profiling by the expert facilitators. Some of the pertinent observations were shared with the group, which helped team members have a better insight on the psyche and working style of their partners and themselves.

During the innovation process, besides the constant collaboration between designer and craftsperson, the facilitators provided expert inputs from the craft, bamboo, sustainability, and interior spaces perspective. This was supplemented by morning talks from different experts in different parts of the production to consumption system, so that different concerns were represented, and could be addressed during innovation.

Step 6: Measuring Sustainability: evaluation against the Sustainability Checklist

The rationale of Step 6 is to increase the accountability of designers to deliberately and strategically, factor holistic sustainability into innovation by presenting with an evaluation of the sustainability achieved against the same indicators outlined at the concept.

The Sustainability Checklist was introduced in step 4 as a tool to help designers be aware of the indicators of the social, economic, ecological and cultural tenets of sustainability. In step 6, the designed product is evaluated against the checklist by the designer, a sustainability expert and a community representative. These three sets of data allow for investigator triangulation as a method of cross-validating the data from multiple sources to identify regularities and discrepancies between the data sets. The result yields an indicative 'sustainability-quotient' of the product: this can be used as a reference for further development and also figured into the marketing strategy.

Each of the products developed during the workshop were evaluated against the checklist by the designers, Sonal Mehta a community expert, and Prof. Kireet Patel who has a huge body of experience in the areas of craft, design and the systems perspective. The evaluation was interactive,

so the designers understood the reasoning of the evaluators for further reference.

The findings from the evaluation allowed the designers to reconsider certain aspects of their design, to achieve better holistic sustainability during the final product actualization phase.

Step 7: Final Product actualization

In the traditional pipeline design sequence, the production, costing and marketing revisions often happen between the time product is realized and is marketed. By this time, the product design function is essentially disbanded and changes in the product are often made without the information or agreement of the innovator/innovation team. As a result, nobody has the bird's eye view of the product and the cascading effect of the changes - including vis-à-vis sustainability.

Step 7 therefore involves incorporating the necessary tweaking and changes arising as a result of step 6, and the additional feedback from the actors across the production to consumption chain; in a continued collaborative manner. The design team is therefore in the loop along with the other design collaborators, until the final actualization of the product.

The prototypes created during the workshop were analyzed in detail by production and marketing experts. Changes were suggested in order to streamline production, and make the products more appealing and cost-effective. Simultaneously, the designers envisaged some changes as a result of the feedback they received during Step 6. Some of the changes required by the designers, production experts, and marketing experts meant radical restructuring of the product's form, construction and joinery. All of these changes were examined collaboratively, and the relevant changes were incorporated in the product design, with the consent of and in agreement with original design team. Thus, the design team was involved even after the workshop in developing the final prototype.

4. Discussion and Results

The development of the Rhizome Approach, and workshop to trial it, were part of an action research process; and so monitored and documented carefully, both audio-visually, and through text. Some of the key findings are outlined below:

4.1. Increase in knowledge

48% of the participants were more familiar with concepts relating to sustainability, after the workshop. 100% of the participants were more familiar with concepts relating to sustainable development following the workshop.

4.2. Exposure visits

100% of the participants who visited the Kotwalia community answered that they were better able to understand the production to consumption value chain more clearly and thoroughly, than before the visit. 100% were also felt that there are differences between industrial and non-industrial or craft set ups, in terms of production, design requirements and potentials; and that the exposure visit was helped them understand the difference.

4.3. The Rhizome Approach

70% of design participants felt that the three directions developed through the Rhizome framework i.e. Prosumer, Expressive and Glocal, are relevant directions for craft; while 26% were not sure.

43% of participants rated the exercise on mapping the systems effect of their direction as number 1 in helping them work jointly with their craft partner, towards a strategic goal; while 17% rated the relevance of craft exercise as number 1. 17% rated the designer's brainstorming about the potential

directions as number 1 and 4% rated the craftspeople's brainstorming as number 1.

The designer group brainstorming exercise was very much helpful to 65% of design participants, and somewhat helpful to 30% of the design participants; in seeing new product possibilities which they would not have considered alone.

The craftspeople group brainstorming exercise was very much helpful to 26% of design participants, and somewhat helpful to 43% of the participants; in seeing new product possibilities which they would not have considered alone.

43% of the design participants found the outcome of the crafts people's brainstorming session much more creative than they expected and 17% found it much more in touch with the market than they expected.

70% of design participants found the exercise on mapping the systems effect of their direction very helpful in seeing the larger picture at the strategic level; 26% found it somewhat helpful.

4.4. The Sustainability Checklist

65% of design participants found the Sustainability Checklist very helpful in understanding the different sustainability concerns and factors at each stage of the product life cycle; 30% found it somewhat helpful.

43% of design participants were informed about a lot of new factors relating to sustainability through the Checklist; 52% were informed about a few new factors.

61% of participants used the Checklist somewhat during the innovation process, and 9% used it a lot. 31% of participants barely used the Checklist during the innovation process.

The 22% of the participants would have used the checklist more if they had more time to design; 9% if it using the Checklist was made compulsory by the client. 13% would have used the checklist more if it were better looking graphically; and 17% if each point was better explained through a booklet.

52% felt that a small booklet explaining each factor of the Checklist would be very helpful in understanding the checklist better; 43% felt it would be somewhat helpful.

43% of participants are very likely to use the Checklist when practising sustainable design in the future; and 48% are somewhat likely.

4.5. Collaborative Design Process

81% of participants felt that their final product would have been very different without the collaborative process created by different inputs. Of these 48% felt it would have been very different while 43% felt it would have been somewhat different.

82% of the participants found the ice breaking exercises helpful in facilitating each design-craft team work towards one strategic goal. Of these 43% found the exercises very helpful, while 39% found it somewhat helpful.

17% of the participants were very surprised, and 61% were somewhat surprised by the 3 things they found out about their craftsperson team member. 17% of the participants felt that their craftsperson team member was much more similar to them than they expected; 48% felt they were somewhat similar.

65% of participants felt the input sessions from different speakers helped expand their design concerns to the larger picture a lot; 30% felt it helped somewhat.

4.6. Evaluation against the sustainability checklist

52% of design participants felt their design could be much improved after self-evaluation against the Sustainability Checklist; 48% felt it could be somewhat improved. 67% of design participants found the external evaluation very helpful in rethinking their design with regards to sustainability.

4.7. Final Product actualization

91% of participants wanted to make changes in their prototypes before final product actualization, but only 14% of participants were clear on exactly what changes they wanted.

100% of the participants were not ok with passing the prototype on to experts who would make changes without consulting them. 62% of participants wanted to make changes in their existing prototype, but wanted technical and other inputs before final product actualization.

4 Conclusion

This paper has focussed on two main aspects: Understanding how the relevance of craft as an input into sustainable design, and proposing the seven point Rhizome Approach, which facilitates designers figuring in holistic sustainability through collaborative design-craft innovation. Though the paper discusses these aspects in the context of bamboo craft, this research is relevant to the design of products using renewable materials in labor intensive i.e. developing countries in general.

It is very important that designers view the concept of sustainability in a systemic manner, so as not to simply capitalize on the market opportunity that the trend of “green design” presents, but to go beyond this to develop products that are strategized within systems of integrated social, economic, ecological and cultural sustainability. The organization’s commitment, overview and investment in technical backstopping and dialogue across the organization is a huge step towards ensuring that product designers and integrated design teams are aligned to innovate on sustainable products, and equipped to do so.

A pilot workshop following the Rhizome Approach was trialed in India in January 2011 and the findings for the same have been discussed in this paper. Following the workshop, the Rhizome Approach was shared with a focus group of the Sustainable Product Innovation (SPIN) project in Vietnam and their detailed feedback was collected. This is in the process of being analyzed.

It is hoped that the products and findings arrived at as a result of this action-research process will contribute to the existing scholarship on formulating a road-map for designers to design more sustainably using craft-based production to consumption systems.

References

- Ehrenfeld, J. R. (2008) *Sustainability by Design: A Subversive Strategy for Transforming Our Consumer Culture*. New Haven, Yale University Press.
- Elkington, J. (1997) *Cannibals with Forks: The Triple Bottom Line of 21st Century Business*. Oxford, Capstone Publishing.
- Greenhalgh, P. (1997) The history of craft. In: Dormer, Peter (ed.) *The Culture of Craft*. Manchester, Manchester University Press. Pp. 20-52.
- Hawkes, J (2001) *The Fourth Pillar of Sustainability: Culture's Essential Role in Public Planning*. Melbourne, Cultural Development Network (Vic.) in association with Common Ground Publishing.
- Maxwell, D.; Sheate, W. & van der Vorst, R. (2003) "Sustainable Innovation in Product and Service Development", paper presented at *Towards Sustainable Product Design 8*, Stockholm, 27-28 October.
- Rees, H (1997), 'Patterns of making: thinking and making in industrial design', in Peter Dormer (ed.), *The Culture of Craft*, Manchester: Manchester University Press, pp. 116–136.
- Reubens, R. (2010a), *Bamboo Bridge: Sustainability in Indigenous Product Design through Bamboo*, <http://www.indigodesignnetwork.org/?p=637>. Accessed 6 May 2010.
- Reubens, R. (2010b) *Diagnostic Study Report for Development of Bamboo Craft Cluster at Vyara, Songadh, Utchal and Valod Blocks of Tapi District*. National Bank for Agricultural and Rural Development.
- Reubens, R. (2010c) *Bamboo in Sustainable Contemporary Design*. International Network for Bamboo and Rattan. Beijing .
- Reubens, R. (2010c), 'Bamboo canopy: Creating new reference-points for the craft of the Kotwalia community in India through sustainability', *Journal of Craft Research* 1, pp. 11–38.
- Shedroff, N. (2009) *Design is the Problem: The Future of Design Must be Sustainable*. New York, Rosenfeld Media.
- Van der Lugt, P. (2007) *Dutch Design Meets Bamboo*. Eindhoven, (Z)oo Producties.
- Waage, S. A. (2005) Re-considering product design: a practical "road-map" for integration of sustainability issues. *Journal of Cleaner Production*, 15 (2007), 638-649.
- Walker, S. (1998) Experiments in sustainable product design. *The Journal of Sustainable Product Design*, 7 (1998), 41-50.
- White, C.; Stewart, E.; Howes, T. & Adams, B. (2008) *Aligned for Sustainable Design: An A-B-C-D Approach to Making Better Products*. Business for Social Responsibility and Ideo.

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ADDENDUM

POSTER ABSTRACTS

Management of Bamboo Genebank at the Los Baños Experiment Station- Ecosystems Research and Development Bureau

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In its desire to establish a living collection of various taxa of endemic and exotic bamboos to serve as ready source of planting stocks, educational area to promote the importance in growing bamboo, seat for scientific study for bamboos, among others. The Ecosystems Research and Development Bureau through its Los Baños Experiment Station (LBES) established a genebank for bamboo that is also known as ERDB Bambusetum. The area lies within the midst of Makiling Forest Reserve, Los Baños, Laguna, Philippines.

The presence of the Bambusetum in the area clearly helps the surrounding environment in its land management, including provision for cover and aesthetic value.

The various management practices to maintain, sustain, and promote the genebank including the data storage and retrieval system are presented. The experience of LBES serves as a guide to those interested in establishing and maintaining bamboo genebank. The details of management practices are discussed in this paper.

Propagation of Common Bamboo Species at Los Baños Experiment Station- Ecosystems Research and Development Bureau

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The Los Baños Experiment Station (LBES) of the Ecosystems Research and Development Bureau (ERDB) has devoted many years and valuable resources to develop technology for propagating common bamboo species in the Philippines. Among the common bamboo species being propagated are Kawayang tinik (*Bambusa blumeana*), Buho (*Schizostachyum lumampao*), Kawayangkiling (*B. vulgaris*), and Bayog (*Bambusa sp.*). These species are selected because they are very popular among community folks for their economic uses. They are commonly associated with livelihood needs as sources of food in the form of edible shoots; for housing materials; and for furniture and handicraft items.

To help increase the areas planted to bamboos, the LBES-ERDB propagates the abovementioned species using the following vegetative propagation techniques: a) clump division- uses two or more stumps or culms with the attached rhizomes, b) offset as basal stem division-one or two stumps or culm portions with attached rhizomes, c) whole culm- the whole portion of the culm with or without an attached rhizome, and d) culm cutting- a portion or segment of a culm. Detailed procedure for each of the propagation techniques are discussed in this paper.

Conservation of Economically Important Bamboo: The Los Baños Experiment Station - Ecosystems Research and Development Bureau Experience (LBES-ERDB)

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The Bambusetum of the LBES-ERDB at the Mudspring, Makiling Forest Reserve (MFR), Los Baños, Laguna, a six-hectare area planted within the 48-hectare LBES-ERDB site, boasts of a rare collection of clumps of bamboos in the Philippines and exotic species. In fact, among the four Bambusetum in the entire country, the LBES-ERDB Bambusetum is the only one with majority of the species from other countries and four species indigenous to the Philippines.

This piece of work is part of the continuing research activity that focuses on the conservation of economically important bamboo species.

Bamboo species were collected for proper identification and documentation. After which, they were planted in the area then propagated and conserved.

Results showed that of the 40 bamboo species grown, majority were found to be economically important and ecologically significant.

Schizostachyum lumampao known locally as Buho, an indigenous species to the Philippines, are used for paper-making. On the other hand, *Bambusa blumeana* or Kawayang Tinik is sought after in the furniture industry. *D. latiflorus* Munro or Machiku, young shoots are a delicacy and fast becoming famous to first class hotels and restaurants. There is also the *Dendrocalamus asper* better known as Giant bamboo that can equal the material of Kawayang Tinik in the furniture industry.

Furthermore, bamboo species demand for landscaping can no longer be ignored. *Thyrsostachys siamensis* better known as Thailand bamboo are very much needed by landscapers. This is also true with *Melocana baccifera* (Muli bamboo), *Guadua angustifolia* (Iron bamboo), *Schizostachyum lima* (Anos) whose size, shape, and color make a difference in the world of landscaping. There are more ornamental bamboos which are economically important to many of the Filipino people.

The work was beefed-up by interviewing respondents from Luzon Island, Philippines. Interviewed were farmers, fisher folks, handicraft and furniture workers, landscapers, bamboo growers and businessmen.

Origin, Growth and Anatomy of the Rhizome of *Dendrocalamus strictus* Nees.

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Though rhizome system performs important functions, it is one of the least understood parts of the bamboo plant. Moreover, an understanding of the form of the rhizome system is prerequisite to understand the clump habit in any bamboo. The diverse manifestations of rhizome system afford challenging research openings for molecular, physiological, biochemical, anatomical, morphological and taxonomical studies. The present knowledge for anatomy of bamboo rhizome is narrow and rudimentary due to difficulty of precocious rhizome induction under *in vitro* condition and also rhizomes produced under natural conditions are considered as cumbersome, hard and bulky propagules for carrying out histological studies. Precocious rhizome induction was achieved from caryopses of *Dendrocalamus strictus* (Roxb.) Nees after three weeks of inoculation on MS basal medium supplemented with 5 μ M BAP + 25 μ M NAA + 0.1 μ M GA₃. Developmental pattern, growth and branching behaviour of rhizome were studied. Anatomical studies revealed the presence of axillary buds at each node and the origin of rhizome from the lowermost node of the primary shoot. Longitudinal section of the basal portion of eight day old seedling showed the development of meristematic tissues into primary thickening meristem of rhizome having distinct protoderm and promeristematic region. Differentiation of leaf primordia was observed after twelve days of culture. The deeply stained apical dome was covered with three to four layers of leaf primordia. Vascular bundles were scattered in the cortical region. The histochemical Periodic Acid-Schiff's test showed large accumulation of starch grains at the promeristematic region during rhizome initiation. The starch grains were sparsely distributed in the region where rhizome primordium differentiated whereas starch grains were abundantly present in the parenchymatous cells away from the primordium.

Authenticated reference of bamboo species occurring in Malaysia

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Malaysia is endowed with more than 50 species of bamboo, 25 of them are indigenous, while the rest are known exotics. All the bamboo species are grouped under 11 genera namely *Bambusa*, *Dendrocalamus*, *Gigantochloa*, *Chusquea*, *Dinochloa*, *Melocanna*, *Phyllostachys*, *Racemobambos*, *Schizostachyum*, *Thyrsostachys* and *Yushania*. Of the total species, only about 14 are commercially utilised while the rest are left idle in their habitat with lack of knowledge on their properties and potential usage. With bamboo is now seen as potential alternative to forest timber, collecting and documenting all the bamboo species and its properties is now of significant important. Effort has been initiated by establishing a bamboo authenticated reference centre at the Forest Research Institute Malaysia (FRIM) with samples collected within Malaysia. The reference centre would create a database on authenticated bamboo with complete information made available to scientists and bamboo related individuals. Initially, the study began with bamboo species collected within FRIM campus and this would later be extended to the whole of Malaysia as and when funds are made available. The bamboo would be scientifically identify based on existing plant taxonomy method and protocol and would later be cross-checked and verified with other established centre. However, continues improvement is being sought and made to expedite efficient collection of bamboo samples and documentation in compliance to established standards. The study would also include establishing the morphology and anatomical structure of the bamboo as well as the physical and mechanical properties of these authenticated samples. This study which began in Jun 2011, as to date has seven bamboo species with their complete database being documented. It is hope that this project would provide a complete portfolio of each bamboo species found in Malaysia and in long term would benefit the bamboo community and the bamboo manufacturing industry in Malaysia.

Keywords: Malaysian bamboo-Authenticated reference-Morphology-Properties-Taxonomy

Uptake of zinc and lead by *Phyllostachys humilis* – Prospects for phytoremediation ?

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Mankind has always been relying on biomass as a source of energy and non-food-related materials. It is therefore not so strange to see that biomass is one of the strongest pillars in the frame of the current transition of the Western economy towards a (more) sustainable society. However, with the food versus fuel debate looming over the future of biomass crops in Europe – for justified reasons –, scientists and entrepreneurs need to look for sources of biomass elsewhere than on classic farmland.

One of the options is to turn to polluted wastelands. Sadly enough, large areas in Europe still bear the marks of historical pollution (or worse, continuing contemporary pollution); however, with good management and proper handling of the harvests, these sites could be given an economic value as well as being cleaned in the process, through phytoremediation. Within this context, bamboo offers a lot of potential. It is one of the fastest growing crops on the planet, continuing its growth even under more extreme circumstances.

To assess the possibility for phytoremediation with bamboo, 60 plants of the species *Phyllostachys humilis* and *P. atrovaginata* were planted on a historically polluted terrain, belonging to a chemical company situated in the South-West of Flanders and the North of France. Of these two plants, only *P. humilis* seems to have withstood the winter period without damage.

Every three months, samples were taken from the different organs of these plants; extracts were made and the heavy metal content measured utilising atomic absorption spectroscopy according to Blust et al. (1988). Table 1 gives an overview of current results. Substantiated quantities of Zn and Pb were found to be taken up by the plants and transported to the aboveground parts. Cd could not be detected in the plant tissues, which is not so surprising, given the low doses of Cd in the soil at the test site (Table 2).

Comparison with earlier results on the uptake of heavy metals by bamboo (details in Potters et al. 2009) and the two main biomass producing crops in Europe, poplar (details in Laureysens et al. 2005) and willow (details in Vervaecke et al. 2003) shows: (1) that the pot test was quite suitable to give a quick idea of the heavy metal uptake capacity of the bamboos; (2) uptake of Zn was comparable between poplar and bamboo whereas willow leaves seem to perform better; (3) uptake of Pb was clearly higher than seen in poplar ; (4) Cd seems to be taken up more swiftly by poplar or willow than by bamboo. This indicates that bamboo is a useful crop to be grown on marginal soils, offering three distinct advantages: the production of bio-energy without having to compete with food and feed production, an economic revalorisation of polluted and less fertile fields and areas, and the possibility to slowly remove the heavy metal pollutants from the soil.

Table 1. Comparison of heavy metal uptake capacity between three major biomass crops

The range, given for bamboo heavy metal uptake, indicates the variability of the measurements on the field test, as well as between 5 different species and different ranges for the pot experiment (taken from Potters et al. 2009). Data on heavy metal uptake by willow and poplar were taken from Vervaeke et al. (2003) and Laureysens et al. (2005). ND: Not detected..

Bamboo (field, 6 months)	Leaves	Culms	Rhizomes
conc. Zn ($\mu\text{g/g}$)	49-100	25-140	50-290
conc. Pb ($\mu\text{g/g}$)	50-710	50-190	150-250
conc. Cd ($\mu\text{g/g}$)	ND	ND	ND
Bamboo (pot, 4 months)	Leaves	Culms	Rhizomes
conc. Zn ($\mu\text{g/g}$)	87-450	141-795	72-1900
conc. Pb ($\mu\text{g/g}$)	33-60	33-61	32-260
conc. Cd ($\mu\text{g/g}$)	8-27	7-27	5-50
Poplar (field)	Leaves	Stem	Roots
conc. Zn ($\mu\text{g/g}$)	362,5	146,1	243
conc. Pb ($\mu\text{g/g}$)	2,9	12,7	17,7
conc. Cd ($\mu\text{g/g}$)	4,3	3,6	3,2
Willow (field)	Leaves	Stem	Roots
conc. Zn ($\mu\text{g/g}$)	411-695	24-40	ND
conc. Cd ($\mu\text{g/g}$)	3,07-8,26	0,80-3,29	ND

Table 2. Heavy metal concentrations at the different test sites in table 1.

Data for the pot experiment were taken from Potters et al. (2009), for willow from Vervaeke et al. (2003) and for poplar from Laureysens et al. (2005).

Soil	poplar	willow	bamboo - field	bamboo - pot
conc. Zn ($\mu\text{g/g}$)	60-486	437.3	40-440	125-1000
conc. Pb ($\mu\text{g/g}$)	11-171	142.9	10-200	50-400
conc. Cd ($\mu\text{g/g}$)	0.05-1.60	3	0-3	1-8

Literature cited

- Blust, R.; Vanderlinden, A.; Verheyen, E.; Declair, W. 1988. Evaluation of microwave-heating digestion and graphite furnace atomic absorption spectrometry with continuum source background correction for the determination of iron, copper and cadmium in brine shrimp. *Journal of Analytical Atomic Spectrometry* 3, 387-393.
- Laureysens I, de Temmerman L, Hastir T, van Gysel M, Ceulemans R (2005) Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture. II. Vertical distribution and phytoextraction potential, *Environmental pollution*, 133, 541-551
- Potters G, Brems A, Valcke R, Dewil R, d' Haese L, Samson R, Gielis J (2009) Energy crops in Western Europe: is bamboo an acceptable alternative? 8th World Bamboo Congress proceedings: vol. 3 - S.I., , 2009, p. 22-34
- Vervaeke P, Luyssaert S, Mertens J, Meers E, Tack FMG, Lust N (2003) Phytoremediation prospects of willow stands on contaminated sediment: a field trial, *Environmental Pollution* 126, 275-282

Genetic diversity in populations of *Chusquea* (Bambusoideae, Poaceae) of the Venezuelan Andes

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Chusquea represents the most diverse genus of woody bamboos in neotropical montane ecosystems^{1,2,3}. In the Venezuelan Andes, *Chusquea purdieana*, *C. serrulata* and *C. spencei* represent the bamboos with the broadest distribution range. The first two species are woody climbers of the upper montane cloud forests, whilst the third is a shrub of the treeline-páramo ecotone². Although it might be particularly interesting to analyze distribution patterns in bamboos due to their clonal propagation, very few phylogeographic studies have been carried out so far, mostly in Asiatic species and recently in *Guadua angustifolia*^{4,5,6}; thus, the present research represents the first study of this nature in this genus. Genetic diversity was studied in these three species, using molecular markers that targeted ISSR, RAPD⁷ and SSR⁸ loci. The sampling was carried out along altitudinal gradients between 2000 and 3650 m a.s.l. in the Cordillera de Mérida. Fresh leaf samples were collected from genets separated 50 m at different altitudes in each locality. After DNA extraction and quality verification, markers were amplified separately, and PCR products were visualized in 1.5 % agarose gels⁹. Only clear and reproducible bands were tallied to generate binary matrixes that were analyzed with POPGEN32¹⁰ and ARLEQUIN¹¹. These three species present an elevated genetic diversity, both within and among populations, regardless life-form or habitat. This diversity was evident by the number of polymorphic loci, proportion of heterozygotes per population, and the absence of dominant genotypes in all of the populations sampled. Our findings suggest that none of these populations are of clonal origin.

References

- 1- Clark, L. G. 1997. Diversity, biogeography and evolution of *Chusquea*. In: *The Bamboos*. Chapman, G. P. (Ed.). Academic Press, London. p. 33-44.
- 2- Clark, L.G. and Ely, F. 2012. Lista de Géneros de bambúes leñosos (Poaceae: Bambusoideae: Arundinarieae, Bambuseae) de Venezuela. *Acta Botanica Venezuelica*. Accepted, in press.
- 3- Ely, F. 2009. Comportamiento ecofisiológico y diversidad genética de *Chusqueas* (Bambusoideae, Poaceae) de la Cordillera de Mérida. Doctoral dissertation. Facultad de Ciencias, Universidad de Los Andes. Mérida, Venezuela. pp. 211.
- 4- Marulanda, M. L.; Márquez, P.; Londoño, X. 2002. AFLP analysis of *Guadua angustifolia* (Poaceae: Bambusoideae) in Colombia with emphasis on the Coffee Region. *Bamboo Science and Culture*, vol. 16, 1, 32-42.
- 5- Muñoz, F. J. E. 2011. Diversidad genética, estructura poblacional y selección de clones superiores de *Guadua Angustifolia* Kunth en la eco-región cafetera de Colombia. Doctoral dissertation. Universidad Nacional de Colombia, Palmira, Colombia. pp 151.
- 6- Rugeles, P. A. 2001. Genotipificación mediante marcadores moleculares RAMs (Microsatélites amplificados al azar) y multiplicación de materiales superiores de *Guadua angustifolia* Kunth. Masters dissertation. Universidad Nacional de Colombia, Palmira, Colombia. pp. 117.
- 7- Lai, C.C.; Hsiao, J.S. 1997. Genetic variation of *Phyllostachys pubescens* (Bambusoideae, Poaceae) in Taiwan based on DNA polymorphisms. *Bot. Bull. Acad. Sin.* 38,145-152.

- 8- Nayak, S.; Rout, G.R.; Das, P. 2003. Evaluation of the genetic variability in bamboo using RAPD markers. *Plant soil and environment*, 49, 1, 24–28.
- 9- Sambrook, J.; Fritsch, E.F.; Maniatis, T. 1989. *Molecular Cloning: a Laboratory Manual*. 2nd edition. Cold Spring Harbour Laboratory Press, New York. pp. 320.
10. Yeh, F. C.; Yang, R.; Boyle, T. 1999. POPGENE Ver. 1.31. Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta and the Centre for International Forestry Research. Canada.
11. Excoffier, L.; Smouse, P. E.; Quattro, J. M. 1992. Analysis of molecular variance inferred by metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, 479-491.

Combining cryo-scanning electron microscopy, μ X-ray fluorescence spectroscopy and X-ray absorption spectroscopy to probe copper speciation in bamboo plants

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Water and organic wastes recycling is a critical environmental issue. In order to take up this scientific technologies and economic challenges, plantations for wastewater disposal are an interesting solution. Among various plants used by this technology (reed, willow), bamboo is particularly relevant. But some organic wastes contain high contents of copper (Cu) and data about Cu tolerance of bamboo is still lacking. Copper is an essential element for plant growth but, high levels of Cu can be phytotoxic. Copper uptake, localization and speciation were studied in hydroponically-grown *Phyllostachys fastuosa* plants at different concentrations of Cu (0.2, 1.5 and 100 μ M). The spatial distribution and speciation of Cu in tissues of the bamboos were investigated using cryo-scanning electron microscopy, coupled with an energy dispersive spectrometer (SEM-EDS), μ X-ray fluorescence spectroscopy (μ XRF) and X-ray absorption spectroscopy (XAS). Inhibitory effects of Cu on plant growth were observed only for the highest Cu concentration. Copper content in roots and shoots increased with increasing Cu concentration in the growth solution. Copper accumulation followed the order roots>stems>leaves. Analysis by SEM and μ XRF studies revealed the presence of Cu mainly in the epidermis of the root. A significant amount of Cu seems to be adsorbed and/or precipitated at the surface of the roots i.e. into apoplast material. This suggests that the root apoplast acts as a major barrier of Cu, which limits the symplastic pathway of Cu and the root-to-shoot translocation. Cu K Edge X-ray absorption near edge structure (XANES) showed that the Cu speciation depends on the part of the plant: in roots most Cu was in divalent form, whereas in stems and leaves Cu was in monovalent form. The reduction of Cu(II) to Cu(I) was only described once for creosote bush and may reveal mechanisms underlying the plant tolerance to high Cu concentrations.

Remediation performances of the Bambou-Assainissement[®] filter for a food industry effluent - A new promising phytoremediation technology for the waste waters management.

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PHYTOREM is a French company which has developed a phytoremediation based-technology using bamboos to remediate wastewater. This innovative water treatment named “Bambou-Assainissement[®]” was patented in 2002. Initially the Bambou-Assainissement[®] was designed for agricultural and domestic wastewater. In the frame of an eco-innovation European program (BRITER-WATER, 2009-2012), PHYTOREM has developed a pilot plant to remediate a high loaded food industry effluents (3,5 and 1,7 g/l for COD and BOD, *i.e.* Chemical and Biological Oxygen Demand respectively). In this phytoremediation device, bamboos are implanted in an impervious basin filled with several filtration materials. The remediation performances of this Bambou-Assainissement[®] filter for the COD, the BOD, the TSS (Total Suspended Solid), Nt and Pt (total Nitrogen and Phosphorous respectively), were weekly monitored from September 2010 to June 2011. During this period, the average hydraulic load was 34 m³/day with a maximum of 100 m³/day. The average remediation performances are 92, 94, 81 and 81 % for COD, BOD, TSS and Pt respectively, and 60 % for Nt. With a specific optimization procedure it was possible to improve the average treatment effectiveness up to 99, 98, 93 and 94 % for COD, BOD, TSS and Pt respectively. These encouraging results were obtained after less than one year of bamboo growth, and we expect even better remediation performances with the growth of bamboos in the incoming years. A longer time of monitoring is planned, and it will allow us to bring some insights on the bamboos contribution to the global treatment effectiveness. Besides remediating waste waters, the Bambou-Assainissement[®] can be also regarded as a production site of high-value biomass. Thus this green technology may constitute a new sustainable approach for the wastewater management.

Effect of high fertilization level on leptomorph and pachymorph bamboo species.

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In a context of waste water treatment by a phytoremediation technology using bamboos, the amount of nutrients in the effluents can be excessive. Thus, it is necessary to assess the effect of excessive nutrient applications on bamboo growth. In most studies dealing with fertilization on bamboo, the level of fertilization applied remains low as compared to the level which can be reached by some effluents. In addition, these studies are often realized with only one or two bamboos species simultaneously, rendering difficult the comparison of the fertilization effect on different bamboo species, since the experimental conditions differ. In order to select the most adapted bamboo species for the waste water treatment, a full randomized bloc experiment was conducted with seven leptomorph rhizome bamboos and ten pachymorph rhizome bamboos. The bamboos were put in pots (about 70 liters) and two fertilization levels: 45 and 228 g/pot per year for N, P and K were applied with a chemical fertilizer (20:20:20 NPK). At the end of the experiment, all the culms were measured (diameter and height) and weighted to establish allometric biomass equations. Samples of leaves and culms were collected for carbon and nitrogen analysis. The highest fertilization level did not induced any significant stress and whatever the fertilization level, the pachymorph bamboos produce more biomass than leptomorph bamboos; the average total aboveground biomass production was assessed to 29,3 and 10,5 t DM/ha respectively during this year of experiment. The biomass increases significantly for all the bamboo species with the higher fertilization level ($p < 0,05$). Obviously, the amount of carbon and nitrogen stored in the biomass follows the same trend as the biomass production; however, the increase of carbon and nitrogen contents between the two fertilization levels is five times higher for the leptomorph than the pachymorph bamboos.

Biodiversity and Resource Utilization of Bamboo species in Manipur, NE India

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Bamboo is usually found in many bio climatically defined forest types. The diversity of bamboo forests has affected a lot because of our exploitation, shifting cultivation, gregarious flowering and extensive forest fires. There has been a steady decline in the species diversity and the extent of bamboo resources due to the lack of proper regeneration after gregarious flowering. Bamboos species are distributed in all forest types found in state of Manipur. Out of 125 species found in India 55 species are recorded from the state of Manipur, NE India. In the present study almost all the 55 species found in Manipur are used for all purposes such as food, building materials, paper pulp resources, agricultural complements, fishing rods, weaving materials etc.

Key words: Bamboo, Biodiversity, Utilization of bamboo species, resource utilization.

A new inventory method for *Dendrocalamus hamiltonii*

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Lack of adequate resource assessment methods jeopardize sustainable harvesting of non-timber forest products (NTFPs). Community-based management of low cost, abundant NTFPs, such as bamboos requires simple, but accurate inventory methods, which can be implemented with limited funds and external support.

We developed a modified variable-radius angle count method, implemented with a simplified relascope to inventory culms of *Dendrocalamus hamiltonii*, a large, sympodial bamboo. The relascope consisted of a transparent ruler with an attached chain, used to hold the ruler at fixed distance from the observer's eye, with the ratio of ruler width to eye distance resulting in a basal area factor of 8. The ruler was held perpendicularly with one end fixed at eye height, thereby accounting for slope correction. Clumps appearing wider than the ruler were included in the sample. For each clump, we estimated the proportion of an imaginary line intersecting the clump at breast height appearing covered by bamboo culms, and counted the number of live culms. The proportion, together with clump basal area was used to calculate "covered basal area" for each clump. We modeled the number of culms per clump as a function of covered basal area using a power function, corrected by a multiplier, accounting for error resulting from back-transformation of the mean of logarithmic data. Differences in counted and modeled culm numbers per clump were not significant (t-test, $p=0.763$). Calculation of clump numbers per hectare followed regular procedures for evaluating angle counts. Additionally, clump numbers represented by each clump in the sample were multiplied by the modeled number of culms per clump to obtain the number of culms per hectare.

The new method allows quick estimation of bamboo culms and can be implemented with simple, locally available tools. The model can be parametrized for other sympodial bamboo species and site conditions.

Harvesting of *Borinda grossa* for current-year culm production

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Borinda grossa is the most important bamboo species for domestic use in temperate regions of Bhutan. Supply from natural stands is declining as a result of unsustainable harvesting. While having great economic value, *B. grossa* is a serious competitor to forest regeneration in the fir and hemlock forests it occurs in. Our study aimed to determine appropriate harvesting methods to achieve sustainable resource management for the production of current-year culms for weaving purposes, while encouraging tree regeneration. We sampled 16 plots located at three sites in east central Bhutan. We applied harvest treatments along a gradient of harvesting intensity: control (no harvest), retain old culms, retain young culms, and clearcut. Analyses were carried out utilizing general linear mixed models. Tree seedling recruitment increased and number of mature culms decreased with intensity of harvesting methods. Mean clump diameter increased over time when retaining old culms and decreased when retaining young culms and clearcutting, as compared to the control. Harvested mature culm yield increased with increasing intensity of harvesting treatments. Current-year culm recruitment declined over time, but did not between treatments. Harvested periodic current-year culm yield was greatest when retaining young culms, followed by retaining old culms and clearcutting treatments. Diameter of bamboo shoots declined with harvesting intensity of treatments. We propose application of the retain young or retain old treatments based on local priorities. Further testing of the combination of the two methods, resulting in a more balanced age distribution of culms within a clump is necessary.

Identification and Function Analysis of Flowering Related Genes from *Bambusa oldhamii*

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Abstract: Bamboo flowering is unpredictable, long-periodic, gregarious, and uncontrollable, and bamboo usually die *en masse* after flowering. The flowering mechanism in *Arabidopsis thaliana* is well established, but it remains unknown in bamboo species. In this study, the cDNA libraries of flowering and vegetative bamboo plants *in vitro* of *Bambusa oldhamii*, an economic bamboo species with delicious shoots, were established and sequenced, respectively. 4470 and 3878 expressed sequence tags (ESTs) from these two kinds of cDNA libraries were got, and function annotation and classification of these ESTs was done. Corresponding cDNA microarray was made with the PCR products of those ESTs as probes, then the gene expression in vegetative and flowering bamboo plants were analyzed by cDNA microarray technology. As a result, 227 upregulated genes and 30 downregulated genes in flowering plants were determined. These differential expressed genes were verified by semi quantitative RT-PCR, and full length cDNA sequences of 7 genes among them were got. When the full length cDNA of *BoMADS1* was overexpressed in *Arabidopsis* and rice, the transgenic *Arabidopsis* and rice exhibited early flowering. On the contrary, the *35S::BoYAB2* transferred *Arabidopsis* and rice produced the genotype of late flowering. Our data provide a useful basis for clarifying the flowering mechanism of bamboo plants.

Keywords: *Bambusa oldhamii*, EST, cDNA microarray, differential express genes, flowering mechanism.

References

- Lin, X.C.; Chow, T.Y.; Chen, H.H.; Liu, C.C.; Chou, S.J.; Huang, B.L.; Kuo, C.I.; Wen, C.K.; Huang, L.C.; Fang, W. 2010. Understanding bamboo flowering based on large-scale analysis of expressed sequence tags. *Genetics and Molecular Research*, 9(2): 1085-1093
- Tian, B.; Chen, Y.; Yan, Y., Li, D. 2005. Isolation and ectopic expression of a bamboo MADS-box gene. *Chinese Science Bulletin*. 50, 217-224.
- Tian, B.; Chen, Y.; Li, D.; Yan, Y. 2006. Cloning and characterization of a bamboo Leafy Hull Sterile1 homologous gene. *DNA Sequence*, 17, 143-151.

Impact of cold temperature on bamboo shoots regarding two species.

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Phyllostachys violascens and *Semiarundinaria fastuosa* shoots come to the soil under two conditions: day temperature should rise around 10°C and night frost should cease.

In april and may 2011, around the city of Reims, eastern France, the weather conditions were exceptionally fair : it didn't rain yet the soil remained humid ; daily temperature rose up to 20°C.

But 3 frosty nights in a row with a temperature of minus 5°C triggered the following alterations :

- *P. violascens* shoots under 2 m tall aborted.
- On *P. violascens* shoots over 7 m tall, which were lignifying, sun exposed branch buds up to 1.50m from the ground, would show dramatically shorter branch internodes.
- Culm top would remain unchanged.
- Hard frost upon culm buds of *Semiarundinaria fastuosa*, altered its culm: it curved, the base internodes got reduced by half. Branch buds grew with the shape of long branches.
- Culm straightness resumed at 1m tall.
- These altered internodes look like *Phyllostachys edulis* 'Heterocyclus's ones, an early bamboo that comes through the soil at the end of winter.

On the one hand let us recall the two following facts :

- Shoot growth lasts 2 months, by successive steps – first culm, then branch, then leaf.
- A sudden episode of cold temperature puts an end to growing process.

On the other hand we may draw the following conclusions :

- The least lignified the shoot is, the more dramatic the impact of cold is.
- Shoots that emerged during a noticeable warm period and that suffered from night frost, display a development decrease on growing internodes and the emergence of sun exposed latent buds. .
- The bamboo shoots growth program is still in progress due to its growth hormones. Internodes and buds are altered under the influence on the growth hormones of temperature variations, sun exposure and the relative humidity.

Notes :

Les mots de la botanique de Françoise Brice, Editions Actes Sud.

La régulation du développement de Ludovic Thebault, Document Internet.

ANATOMICAL PROPERTIES OF *Schizostachyum brachycladum* (BULUH LEMANG)

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Schizostachyum brachycladum or locally known as buluh leman is indigenous to Malaysia. The culms are commonly used for cooking glutinous rice called 'lemang'. It is also used for crafting basket and mats. Information on the properties and potential usage of this species is rather limited. So effort has been done to study the anatomical properties of *S.brachycladum* since this properties have been known to have significant effects on their durability, physical and strength properties. The anatomical properties study were include the determination of vascular bundle distribution, vascular bundle length and width, fibre length, width, thickness, lumen diameter and the anatomical structure of this species. Three culms of four year old *S.brachycladum* which used in this study was obtained from Forest Research Institute of Malaysia. From the result it shows that, the average value of vascular bundle distribution, vascular bundle length and width, fibre length, width, thickness, lumen diameter were 6 mm², 695 µm, 647 µm, 760 µm, 6.0 µm, 2.34 µm and 2.45 µm respectively. In terms of radial variation, the distribution of vascular bundle was decreased from outer to inner position of the culm. On the other hand, the vascular bundle length has the highest value in middle layer whilst the width was found to have higher value in the inner layer of the culm. Fibre length and lumen diameter give the highest result in the middle layer of the culm, whilst fibre diameter and wall thickness showed increased trend from outer towards the inner position. From the anatomical structure it shows that *S.brachycladum* was in the type II grouped.

Keywords: *Schizostachyum brachycladum*-Anatomical properties-Vascular bundle-Fibre-Radial variation

Impact of stress on European bamboos (from North of France up to Holland).

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It is a well known fact that various stresses may cause bamboo blossoming. However harsh unusual weather conditions might trigger other stress responses.

For instance, a repeated cold and dry north and east wind blowing for several months (from January to March 2010). The dryness of the air and of the soil provoked a degeneration of numerous shoots of *Phyllostachys violascens* sprouting in April and May. Actually, a few culms grew correctly. But among them some suffered from an unusual pattern of branches. Instead of the two or three regular branches of the genus *Phyllostachys* emerging from upper nodes, there were up to 10 short branches per node (probably secondary branches), displaying a phenotype unrelated to *Phyllostachys* at first glance.

- Second example. On the most *exposed* parts of the culms (*Phyllostachys violascens* and *vivax Aureocaulis*), if facing cold winds, normally green and yellow culms turn into black - This fact doesn't occur with bamboos protected from cold winds.

At last, a third example comes from the historic drought of spring 2011. Despite the drought, numerous shoots of *Phyllostachys viridiglaucescens* did normally sprout in April. Then, most of the new shoots degenerated and died. However, a few of them got soaked in the heavy rains of July. Unexpectedly, some degenerating shoots could recover, as they displayed new extension of culms with long internodes. This tends to demonstrate that the cycle of neurodegeneration -- probably triggered by apoptosis (a genetic cell death program), can be reversed, or reprogrammed, if not too late, in order to adapt to new positive weather conditions.

Intrinsic physical variability of three bamboo species from eastern Madagascar

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Bamboos are important in the lives of both rural and urban households in Madagascar. But there is a lack of information about wood qualities. The knowledge of the culm physical properties and their variability allows to propose the appropriate use of bamboo.

The aim of this study was to analyze the effects of those selected two variables: age (1 and 3 years old) and vertical location (top, middle and bottom) on physical properties of the bamboos in the Eastern region of Madagascar (Analanjirifo and Atsinanana). The study used the three most abundant species *Dendrocalamus giganteus*, *Dendrocalamus asper* and *Bambusa vulgaris constrictinoda*. Physical properties included in the analysis were the infradensity and the green moisture content.

Statistical analysis indicated that the age and vertical location had effect on physical properties. Moisture content at different vertical locations was different for all the three studied species. It increased from the bottom to the top for *Dendrocalamus giganteus*, while the middle is the driest part for *Dendrocalamus asper* and *Bambusa vulgaris constrictinoda*. *Dendrocalamus asper* had the highest moisture content in green condition. The infradensity was higher for the 3 year-old culms than for the 1 year-old culms. Conversely, the green moisture content was higher for the 1 year-old culms. Results allow to help future decisions for a better agroforestry and industrial valorization.

Keywords: Bamboo, physical properties, age, position, Madagascar.

POTENTIAL OF MITIGATING GREEN HOUSE GAS EMISSIONS THROUGH BAMBOO BASED AGROFORESTRY IN INDIAN SUB-CONTINENT

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Human activities are affecting the environment on a global scale. Many activities such as burning fossil fuels, transformation of forests into agricultural land, creation of artificial wetlands are releasing large amounts of so-called greenhouse gases into the atmosphere. These gases trap the heat that is radiated from the Earth's surface, thus leading to a warming of the atmosphere which is known as "Green House Effect". The Greenhouse Effect is a natural phenomenon and keeps the earth's surface some 20°C warmer than it would be if the atmosphere contained only oxygen and nitrogen. However, ever since the Industrial revolution began about 150 years ago, man-made activities have added significant quantities of GHGs to the atmosphere. Emissions of carbon dioxide, methane, nitrous oxide, ozone and long-lived industrial gases such as CFCs, HFCs, and PFCs are causing "enhanced greenhouse effect". Carbon dioxide is currently responsible for over 60% of the "enhanced" greenhouse effect; while methane, nitrous oxide, ozone and a number of industrial gases contribute the remaining 40% of the enhanced greenhouse effect.. It is ironical that the contribution of developing nations to GHG emissions is less than half of that emitted by the developed countries.

These changes in atmospheric composition are altering temperatures, precipitation patterns, sea level, extreme events and other aspects of climate on which the natural environment and human systems depend. This phenomenon is known as global climatic change. Scientists have observed that over the 20th century, the mean global surface temperature increased by 0.6 °C and snow cover has declined by some 10% since the late 1960s in the mid and high latitudes of the Northern Hemisphere. The IPCC in its report has projected that the globally averaged temperature of the air above earth surface would rise by 1.4-5.8^o C over next 100 years with an associated rise in sea level of 0.9-0.86m (IPCC, 2001).

Bamboo being one of the fast growing species, may contribute immensely towards the C-sequestration. The value of bamboo in sequestering carbon and reducing carbon dioxide emission to the atmosphere is being recognized increasingly the world over. Bamboo plantations under agroforestry systems are thus recognized to have the potential to regain some of the carbon lost to the atmosphere from different land use activities. The most important role that bamboo based agroforestry plantations may play is to offset destruction of primary forest by providing the necessary wood products from land that has already been cleared. If this can be done in a manner that provides competitive biomass accumulation rates to that of natural re-growth and is sustainable in terms of soil fertility, then plantations and bamboo based agroforestry systems could play a substantial role in CO₂ mitigation in Indian sub-continent.

The effect of irrigation on the phenology of ornamental bamboo *Phyllostachys iridescens* in Hungary.

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Phyllostachys taxa are planted as ornamentals under the continental warm temperate climate of Hungary. A two year experiment was carried out on sandy soil with a single clone of *Phyllostachys iridescens*, planted in 2009 at a spacing of 4 x 4 meters. Half of the plot was provided with drip irrigation and irrigated 3 times a week during the vegetation period from April until the end of October in 2010 and 2011. A 2:1:1 N:P:K plus microelements inorganic slow release fertilizer was spread in the whole plot in spring of both years. Sample plants were randomly chosen from the irrigated and non irrigated areas and dates of shooting, shoot mortality and culm vertical growth completion were recorded. The total precipitation and number of days with rain in the vegetation period was 703 mm and 73 days in 2010 and 227 mm and 49 days in 2011. The number of days with maximum temperatures above +30° C was 25 in 2010 and 31 in 2011. The first shoots appeared on April 24 in 2010 and April 22 in 2011 and by May 14 in 2010 and May 23 in 2011 100% of irrigated plants, while only 50 and 30% of unirrigated plants produced shoots. By June 7 in 2010 and June 13 in 2011 all unirrigated plants produced shoots, meaning irrigation hastened shooting time by 21-24 days. 100% of irrigated first shoots finished their longitudinal growth phase by June 23 in 2010 and June 20 in 2011, while it was delayed in the unirrigated plants by 57 days until August 15 in 2011. The length of drought stress in the unirrigated plants, reflected by the complete folding of leaves lasted 17 days from July 8-24 in 2010 and 67 days between July 7-19 and August 25 to October 20 in 2011.

Influence of Gregarious Bamboo Flowering on Shifting Cultivation in Northern Laos

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Bamboo species often flower gregariously, which can have a significant impact on the vegetation of an area. Although, in the tropics, the scale of gregarious flowering is smaller and its cycle is shorter than that in the temperate zone, few details are known about the effects of bamboo flowering on surrounding crops or vegetation. In northern Laos, local livelihoods depend on products from shifting cultivation fields and fallow forests, and bamboo species are dominant in such areas. To examine the influence of bamboo flowering on the local vegetation and livelihoods in this area, I visited 50 villages and conducted interviews to determine the bamboo flowering history of the past 30 years. The interviews indicated that more than 30 species of bamboo have been observed in northern Laos. Although the flowering dynamics vary among species, 36 villages experienced gregarious flowering, and the bamboos of 20 of these villages were attacked by rodents. Thus, bamboo flowering and associated rodent attack is not uncommon in northern Laos. To examine the influence of these phenomena in greater detail, an intensive study was conducted in KK village in Luang Phabang Province. *Dendrocalamus* sp. flowered gregariously in 2010 and 2011 in the village. Flowering *Dendrocalamus* sp. culms accounted for 37.3% of all trees and culms of all species in the village. The proportion of flowering clumps to the total number of clumps was 61.2%, and flowering culms accounted for 96.3% of the total number of culms in a flowering clump. In 2010 and 2011, upland rice, Job's tears, and maize grown in the village were attacked by rodents and production declined by 60–70%. The damage was distributed mainly in the southeastern parts of the fields. The rodents attacked twice in the cultivation period and each attack period lasted about 1 week.

CLONING AND CHARACTERISTIC ANALYSIS OF TRANSPOSABLE ELEMENTS IN THE BAMBUISOIDEAE SUBFAMILY**

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Transposable elements (TEs) are the sequences of DNA that can move around to different positions within the genome of a single cell. We comprehensively and systematically investigated the distributions, diversities and evolution of Ty1-*copia* retro-elements, *PIF/Pong*-like elements and *mariner*-like elements in 69 representative bamboo species from 38 genera within six subtribes mainly found in China. 1) Seventy-two Ty1-*copia* retro-elements (GU350877-GU350949), 139 *PIF*-like elements (DQ861453-DQ861591), 82 *Pong*-like elements (GU350795-GU350876) and 82 *mariner*-like elements (DQ528658-DQ528739) were characterized in 44 representative bamboo species. Phylogenetic analysis showed they were widespread, diverse, abundant and polyphyletic in origin in Bambusoideae subfamily (Zhou et al. 2010a, b, c; Zhong et al. 2010). 2) Seventy-nine full-length *mariner*-like transposases (HM101484-HM101562) were cloned from 44 representative bamboo species, 78 of which were uniform in length and highly homologous and contained intact DNA-binding motifs and DD39D catalytic domains with few obvious mutations revealing that they might be derived from a same ancestor and young in horizontal transfer. Yeast transposition array showed *Agmar1* was indeed an active TE which could provide a foundation for the future development of a new genetic tool available for the plant gene tagging (Zhou et al. 2011). 3) Transposon display revealed high-level insertional polymorphism for copies of *mariner*-like elements with different methylation patterns in *Ph. pubescens* and eight intra-species cultivars. The study has argued that TEs and atypical cytosine methylation patterns might have played a role in the mutations of *Ph. pubescens* and the differentiation of *Ph. pubescens* into distinct cultivar groups. All the results provided the deeper understanding for impact of TEs on bamboo genomes during bamboo evolution.

Keywords : Transposable elements, Bambusoideae, distribution, diversity, transposition

Reference

- [1] Zhong, H., Zhou, M. B., Xu, C. M., Tang, D. Q. 2010. Diversity and evolution of Pong-like elements in Bambusoideae subfamily. *Biochem Syst Ecol*, 38: 750–758
- [2] Zhou, M. B., Lu, J. J., Zhong, H., Liu, X. M., Tang, D. Q., 2010a. Distribution and diversity of PIF-like transposable elements in the Bambusoideae subfamily. *Plant Sci*, 179, 257–266
- [3] Zhou, M. B., Lu, J. J., Zhong, H., Tang, K. X., Tang, D. Q. 2010b. Distribution and polymorphism of mariner-like elements in the Bambusoideae subfamily. *Plant Syst Evol*, 289, 1–11
- [4] Zhou, M. B., Zhong, H., Tang, D. Q. 2011. Isolation and characterization of seventy-nine full-length mariner-like transposases in the Bambusoideae subfamily. *J Plant Res*, 124, 607–617
- [5] Zhou, M. B., Zhong, H., Zhang, Q. H., Tang, K. X., Tang, D. Q. 2010c. Diversity and evolution of Ty1-*copia* retroelements in representative tribes of Bambusoideae subfamily. *Genetica*, 138, 861–868

Towards Innovative Processing and Sustainable Utilization of Bamboo Resources in Ghana in a Climatic Threatened Environment: The Technical Issues.

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Abstract

The sustainable use of bamboo resources is an important part of forest management because bamboo is believed to be an essential tool to balance technological advancement with environmental sustainability in most tropical countries like Ghana. In this present study, the technical issues involved in the enhanced processing and the development of a viable bamboo based industry in Ghana were addressed. The technological properties of native bamboo species in southern Ghana- thermogravimetric, phytochemical and selected physical and mechanical properties of *Bambusa vulgaris* from different sites in Ghana- were examined. Preliminary phytochemical screening revealed the absence of alkaloids (an important decay resistance indicator) and the presence of anthraquinone glycosides.

The results of the thermogravimetric analysis indicate a rapid weight loss between temperature of 200 °C and 400 °C in all samples. There was no marked trend in the shrinkage for outer diameter and culm wall thickness of bamboo samples and were not statistically significant at 5%. The information on the technical properties of bamboo in Ghana will be relevant for innovative processing and utilization of our bamboo resources. The study concluded with recommendations for capital investments supported by research to establish some bamboo- based industries in southern Ghana where over 200,000 hectares of bamboo covers exist. This step is relevant towards developing location-specific adaptation tools to the impact of climate change as the forest cover in Ghana dwindles at alarming rate.

Key words: bamboo, technical properties, processing, innovation



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