In vitro Propagation of the Giant Bamboo *Dendrocalamus giganteus* Munro

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Abstract

Dendrocalamusgiganteus belongs to the family Poaceae, is one of the largest bamboo species in Bangladesh with a diversified economic value. Conventional propagation methods of bamboo are generally inefficient due to their low multiplication rate, time consuming, laborintensive and costly. The study was therefore designed with the objective to develop a comprehensive protocol for the micropropagation of *D. giganteus*. Using seeds as explants, shoot initiation, shoot multiplication, rooting and acclimatization were carried out. Surface sterilized *D. giganteus* seeds were germinated in semi-solid MS medium for culture initiation. MS medium augmented with 6-Benzylamino-purine (BAP) at different concentrations (4, 7, 13 and 18 µM) were used separately for shoot initiation and multiplication. Half strength MS media supplemented with various concentrations (2, 5, 10 and 20 µM) of indole-3-butyric acid (IBA) was used separately for rooting. Plant growth regulator (PGR)-free MS and half strength MS medium was used as control treatment for shoot initiation, shoot multiplication or rooting experiment respectively. All viable seeds were germinated in-vitrowithin 4-10 days of placing them in germination media. After transferring the germinated shoots, MS media supplemented with 18µM BAP was found bestfor shoot generationand multiplication while PGR-free MS medium showed longest mean shoot length and took maximum number of days for shoot generationwith poor multiplication. The highest rooting percentagewas achieved fromthe half strength liquid MS media supplemented either with 10µM or 20µM IBA solution. Maximum number of root per clump was found with 20µM IBA and fastest rooting with 10µM IBA. Finally, the survival percentage of plantlets in greenhouse condition was found to be 92.5% after 30 days of acclimatization. The study enables to develop an effective micro-propagation protocol for the species.

Keywords:Acclimatization, BAP,*Dendrocalamusgiganteus*, IBA,micropropagation, sterilization

Introduction

Bamboo is the strongest and fastest-growing perennial grass species (BPG2012) and is unique with complex branching patterns; woody culms and gregarious, monocarpic flowering plant (Singh et al. 2013) belongs to the subfamily Bambusoideae under the family Poaceae. More than 1,439 species of bamboo categorized under 115 genera are found in the world (BPG 2012). In Bangladesh there are 9 genera and 33 species (Banik 1998) of bamboo growing throughout the country. Most of these species belong to the genera *Bambusa*and *Dendrocalamus*. Seven species of bamboo grow naturally in the forests of Bangladesh (Banik 1998). Rests are cultivated in the villagesthroughout the country. *Dendrocalamusgiganteus*, known as Giant Bamboo or Dragon Bamboo and locally called as BhudumBanshis one of important the village bamboos cultivated in the South-eastern part of thecountry. It is a giant tropical and subtropical clumping species native to Myanmar (Burma), Bhutan, China and Thailand and introduced in Bangladesh, Indonesia, Laos, Vietnam, Australia, New Zealand (Schröder 2010), Madagascar (Contu 2013) and in Sri Lanka (Schröder 2010) in 1856 is considered as the tallest bamboo in the world(Ramanayake and Yakandawala 1997).Schröder (2010) reported that, *D. giganteus* is one of the twelve high yielding bamboos worth raising as a large scale bamboo plantation, as it is very good for construction, paper production and young shoots are good for

vegetable products. Culms are also used for scaffolding, boat masts, rural housing, water pipes, vases, buckets, water pitchers, matting, boards and parquet, furniture, water pots. Culm sheaths are used to make hats. The stems are especially suitable for the production of bamboo boards, which is an ideal material for room decoration and other interior applications such as walls, ceilings, floors, doors, shelves, etc. *Dendrocalamusgiganteus* can be planted to protect soil against erosion. As one of the largest bamboo species, it has a high ornamental value. This bamboo species also produces large amount of biomass. It can give an annual yield of 20 to 30 t/ha, which is 2.7 times higher than that of *D.latiflorus*(Schröder 2010).

Conventionally, bamboos are propagated through seeds, clump division, rhizome, offset and culm cuttings (Banik 1994; 1995).However, gregarious flowering at long intervals followed by the death of clumps (Austin and Marchesini 2012), short viability of seeds (Bereket 2008), presence of diseases and some pests (Singh et al. 2013) are limiting factors to use seeds as valuable source of propagation. Vegetative propagation methods have limitation for mass propagation *i.e.* propagules are difficult to extract, bulky to transport and planting materials are insufficient in number for large-scale plantation (Kassahun 2003; Mudoiet al. 2013). Seasonal dependence, low survival rate and limited rooting of the propagules are other limitations (Singh et al. 2013).

Considering problems encountered both in sexual and asexual conventional propagation of the *D. giganteus* and growing interest of the country on the economic and ecological benefit of bamboo, a method that brings about rapid large scale production of bamboo is highly desirable. In this regard different scholars recommended micropropagation as an excellent means to achieve this aim. The benefitsofmicropropagation of plants with tissue culture technique is that the plants can be disease free, multiplying large number of plants in a small space by starting from a few explants, reduced water and nutrient needs for micropropagation of plants, rapid multiplication of tissues that can in turn be used to yield more tissue culture material, ease to transport and delivery of plantlets (Burr et al. 2013). Large number of studies have been undertaken on micropropagation of bamboos using seeds, somatic embryogenesis, nodal culture, and in vitro flowering. Different researchers have been publishing scientific articles on successful micropropagation protocol through seed culture in different bamboo species. For instance, Arya et al. (1999) on *D. asper*, Bag et al. (2000) on *T. spathiflorus*, Arya et al. (2012) on *D. hamiltonii*, Devi et al. (2012) on *D. giganteus*. Most of these studies are for the experimental purposes and lack of crucial parts, establishment in the field. They were neither very efficient nor applicable for other bamboo species or both (Mudoi et al. 2013). More over these techniques of bamboo propagation haven't been tried or standardized in Bangladesh. Therefore, the current protocols described haven't been applied for the large scale plantation programs of the bamboo for commercial/industrial purposes which indicates the requirement of research project for micropropagation via tissue culture methods would be able to solve most or at least many of these problems in propagation of bamboo. The present research project has therefore been designed to develop an efficient and comprehensive regeneration methods for the commercially important bamboo species *Dendrocalamusgiganteus* through tissue culture methods from seed originated shoot explants.

Materials and Methods

Study Areaand Sources of Experimental Materials

The study was conducted at the Plant Tissue Culture Laboratory under Silviculture Genetics Division of Bangladesh Forest Research Institute (BFRI), Chittagong, Bangladesh from July 2016 to June 2017. The study area is located at the intersection of 22°22′0″N Latitude and 91°48′0″E longitudein South-eastern Bangladesh. The area is characterized by hot humid summer and dry cool winter. The mean maximum and minimum temperature of the area is 30.2°C and 12.6°C respectively with a relative humidity 79% and annual rainfall of 2919.1 mm. *Dendrocalamusgiganteus* seeds were obtained from the Bangladesh Forest Research Institute (BFRI) which were collected from Mehergona Range under Cox's Bazar North Forest Division on 13 March, 2016. Healthy seeds were selected carefully and used as explants for initiating experiments. To maintain the viability,seeds were stored in plastic bag in refrigerator at +4°C temperature until use.

Experimental Procedures

Media Preparation

MS media was prepared by mixing 4g/l MS powder, 4%of sucrose and plant growth regulatorsin double distilled water (DDW). The media was adjusted to pH 5.8 (Arya et al. 2012) using 0.1N KaOH or 0.1N HCl before adding 2.8 g/l gelrite. About 30ml of the media was dispensed in each culture vessel (150 ml), about 10ml in test tubes for experiment initiation and 50ml of the mediawas dispensed in each culture jar (250 ml) for multiplication and rooting experiments. The culture vessels containing the medium were plugged tightly with autoclavable lids prior to autoclaving at 121°C with 0.15KPa pressure for 20 minute. Then it was allowed to cool at room temperature and stored in culture room until used.

Explants Surface Sterilization

Before seeds are inoculated on a medium, they were surface sterilized to get rid of all microorganisms. Seeds were washed with tap water to remove debris and dehusked. The dehusked seeds were rinsed in distilled water and soaked for 2 hour. Then the imbibed seeds were washed by DDW with liquid soap with 2-3 drops of Tween-20 for 25 minutes with agitation to physically remove most microorganisms and debris (if any). The seeds were then treated with 70% ethanol for 30 seconds under laminar air flow cabinet. After pretreatment with ethanol, the explants were rinsed with autoclaved distilled water three times, to lower the toxic effect of ethanol followed by treatment with 0.1% Mercuric Chloride for 20 minute with gentle shaking. Finally the seeds were washed with DDW thrice to remove the traces of Mercuric Chloride.

Proliferation of Culture Shoots

Sterilized seeds were cultured with 10 ml MS medium in test tubes. Ten days after placing the seeds on the germination media (when germinated seedlings were around 2.0 cm in length) (Figure 1)shoots were excised from the seeds and transferredin tothe vessels containing 30 ml MS medium fortified with 4% sucrose, 2.8 g/l gelritewith various concentrations of BAP (0, 4, 9 and 13 µM) for shoot generations. MS medium without PGRs was used as control. All vessels were placed on shelf ofthe growth room with a photoperiod of 16h/8h light/dark cycle using cool -white fluorescent lamps (photon flux density, 40µmol m^{-2} s^{-1} irradiance) at 25 ± 2°C and RH of 70- 80%. Number of days for shoot generation, shoot generation rate and percentagewere recorded 6 weeks after setting them in the shoot generation media.

Shoot Multiplication in Solid MS Media

To avoid the carry over effect of shoot generation media during shoot multiplication, initiated shoots were excised from the clump and sub-cultured on MS media supplemented with or without PGRs for two weeks. Each propagule was placed vertically into 50 ml MS medium in 200ml vessels supplemented with 4% sucrose (Arya et al. 2012; Tuan et al. 2012), 2.8 g/l gelrite and six concentrations of BAP [M1 (0 µM), M2 (4 µM), M3 (7 µM), M4 (9 µM), M5 (13 µM) and M6 (17 µM)] considered as six treatments. MS medium without PGRs was used as control. The adopted experimental design was completely randomized with three replicates and 15 explants per treatment (n= 45). All vessels were placed on the shelf of growth room with a standard photoperiod, temperature and humidity. Number of shoot per explant, number of leaves per shoot, and shoots length (cm) were recorded 6 weeks after setting the experiment.

Continuous Shoot Multiplication in Liquid Media

The shoot multiplication was also found to be slow in the solid media and the newly generated shoots were too small to root in the rooting media. Therefore, after multiplication of sufficient shoots (Figure 2a),in the solid media, shoot bunch were transferred in to liquid MS medium (having 4% sucrose) supplemented with three different concentrations of BAP [CS1 (0 µM), CS2 (4 µM) and CS3 (9 µM)] were used for continuous shoot multiplication. For the purpose, shoot clumps (Figure 2b) were excised in to small bunches (having three shoots in each bunch) (Figure 2c) and were placed in vessels

with 15 ml growth media (Figure 2d). The vessels were well wrapped with parafilm and placed in Laboratory Shaker Incubator with 55 strokes per minute (Ramanayake and Yakandawala 1997), with similar photoperiod and light intensity. Cultures were replaced to fresh medium when they were brown in colour. During subculture, the brown parts (if any) of the plantlet were removed carefully. The number of proliferated shoots and the longest shoot lengths were recorded.

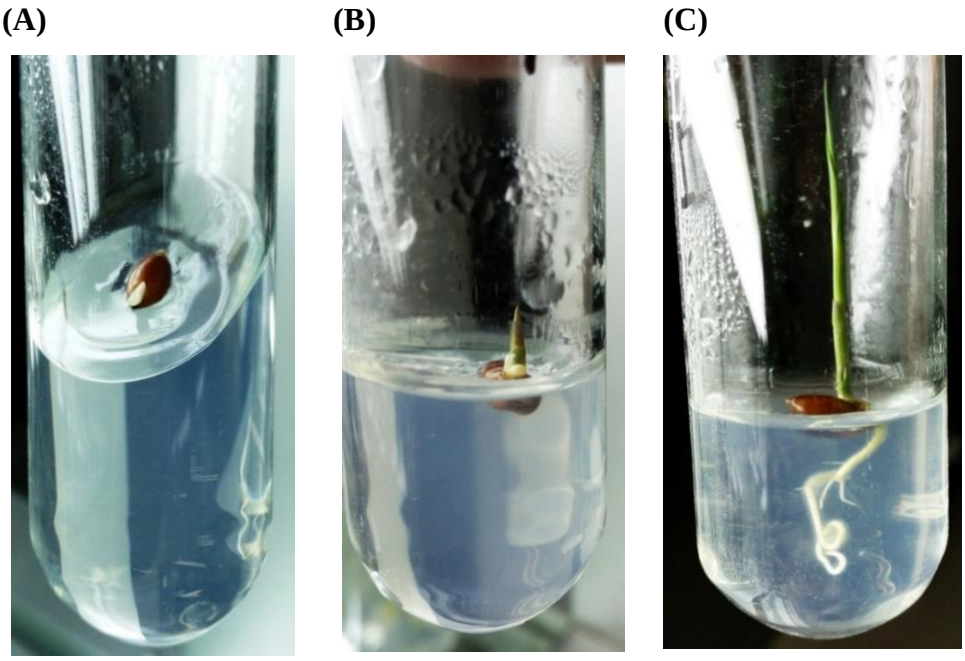


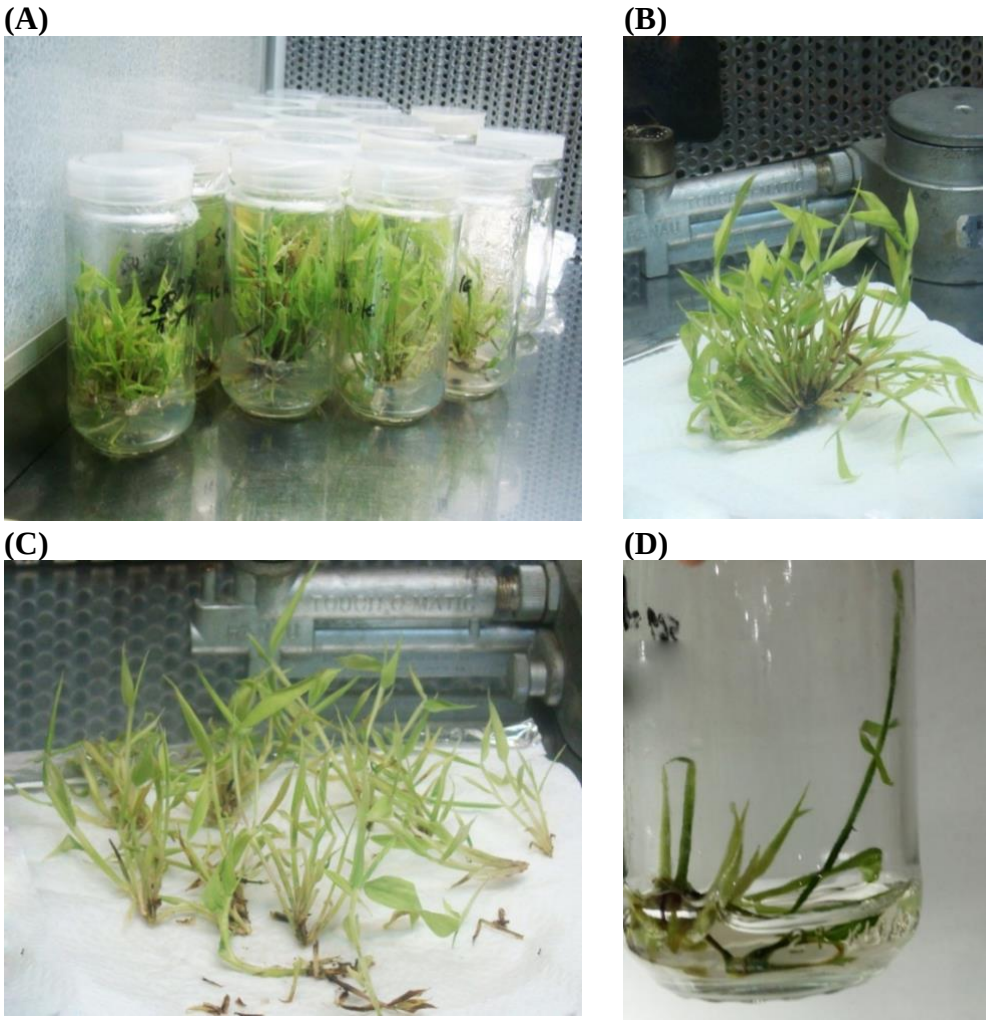
Figure 1 Various stages of seed germination and shoot development. Four days (a), seven days (b) and ten days (c) after placing the seeds on the germination media.

Root Induction

The in vitro regenerated shoots (three shoots in a bunch), were excised from the multiple shoot clumps and transferred on conditioning media (media left for 2 weeks after preparation) for rooting experiment. Rooting response of shoots was examined on half strength (2g/l) MS medium supplemented with 2% sucrose, 2.8 g/l gelrite, and different concentrations of IBA [R1 (0 µM), R2 (2 µM), R3 (5 µM), R4 (10 µM) and R5 (20 µM)]. For each treatment ten vessels, three bunches in each vessel were used for root induction. All shoots were incubated on rooting medium for 4 weeks at 25±2^{o}C with standard photoperiod and light. Number of bunch rooted, number of roots per bunch, and average root length (cm) were recorded 28 days after setting them in rooting media.

Hardening of Rooted Plantlets and Acclimatization

For hardening, the in vitro rooted plantlets were first washed with distilled water to remove adhered medium (that harbors microbial growth) to prevent contamination. The washed plantlets were then planted into plastic pots filled with mixture of autoclaved soil with coconut coir (soil: coir at 1:1 ratio by volume) and transferred in to greenhouse at temperature 24±2^{o}C with relative humidity 60-70%. The transplanted plantlets in greenhouse were covered with transparent polyethylene sheets to reduce evapotranspiration for five days and occasionally sprayed with water. For the next five days the plantlets were acclimatized in greenhouse condition by removing the polyethylene sheet and watered once a day. Subsequently the plants that appeared strong and healthy were transferred to a warmer (30±2^{o}C) greenhouse room and were allowed to grow there for fifteen days with watering at every alternative days. Percentage of plantlets successfully acclimatized was recorded 30 and 60 days after transplanting. Finally, the plants were transferred to bigger polybags and placed in open sun for further growth. All data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS and significant differences among the mean values were compared using Tukey's test at alpha level of 0.05.



Figue 2 Multiple shoots in vessels (a), shoot clump (b), separation of shoot clumps in small bunch of three shoots (c) and shoot bunch in liquid media for continuous shoot multiplication or for root induction (d).

Results and Discussion

In vitroSeedGermination and Shoot Generation

All viable seeds were germinated in the sterile MS media within 4-10 days of culturing. Ten days after placing the seeds on the germination media (when germinated seedlings were around 2.0 cm in length) (Figure 1), shoots were excised from the seeds and transferred in to the vessels containing 30 ml MS media supplemented with four concentrations (0, 4, 9 and 13 µM) of BAP for shoot generations. BAP concentration significantlyaffectedthe shoot proliferation (p<0.001), *i.e.*,days requiredfor proliferation, number of shoot and shoot length, (Table 1). The maximum shoot generation percentage was recorded from 13µM BAP supplemented MS medium followed by 9 µM BAP and the lowest shoot proliferation was observed in the PGRs free MS media (Table 1). Of the various concentrations of BAP tried, highest number of shoot was obtained with 13µM BAP where 5.60±1.00 shoots/explant were developed in six weeks of culturing; followed by 3.73±1.00 shoots/explant with 9µM BAP concentration (Table 1). The result was supported by Arya et al. (1999; 2012) who mentioned that the shoot initiation percentage was greatly influenced by the type and concentrations of BAP in *D. asper* and *D. hamiltonii* respectively.

Table 1 Effect of BAP fortified Basal media* on *D. giganteus* shoot proliferation from seeds

Treatment Code	BAP (µM)	Shoot Initiation %	Number of Shoots	Length of shoots initiated	Days till initiation
I1	0	$33.3^{a} \pm 5.7$	$1.0^{a} \pm 0.0$	$6.9^{d} \pm 0.3$	$7.6^{bcd} \pm 1.7$
I2	4	$50.0^{ab} \pm 10.0$	$1.9^{a} \pm 1.0$	$5.3^{c} \pm 0.4$	$6.6^{abc} \pm 1.1$
I3	9	$66.6^{bc} \pm 5.7$	$3.7^{b} \pm 1.0$	$4.5^{b} \pm 0.2$	$5.2^{ab} \pm 1.3$
I4	13	$83.3^{c} \pm 11.5$	$5.6^{c} \pm 1.0$	$3.2^{a} \pm 0.2$	$4.6^{a} \pm 0.9$

Note: Different letters within the same column indicates significantly different means using Tukey's test at alpha level of 0.05; ± = Standard Error of Mean.

The longest (7.50 cm) and shortest (2.70 cm) shoots were recorded from PGR-free and 13µM BAP fortified MS medium respectively (Table 1). In BAP fortified media mean shoot length was negatively correlated with level of cytokinin concentrations. While increasing BAP concentration from 4µM to 13µM in the MS medium, shoot length was decreased from an average of 6.6 cm to 4.6 cm (Table 1). This result might be due to explants cultured on those medium produced less number of shoots (1-2 shoots), thus there was less nutritional competition in the control and toxic effects of ethylene at higher cytokinin concentration (Lorteau 2001). The result was consistent with the report of Arya et al. (1999) on decreasing of shoot length developed from the explants as cytokinin concentration was increased.

Shoot Multiplication in Solid Media

The various concentration of BAP tested significantlyaffected ($p< 0.001$) the number of shoot and shoot lengthof *D. giganteus* (Table 2). Cytokininsare known to promote the function of other growth regulators like 2iP and zeatin (Gaspar et al. 1996). In our study, application of exogenous BAP resulted increased multiplication rate and higher number of shoot over PGR-free MS medium (Table 2). Among the various concentration of BAP investigated, MS media supplemented with 18µM BAP showed highest multiplication,17.1±4.7 number of shoot per explantfollowed by 13µM and 9µM BAP (Table2 and Figure 3). The effect of BAP in inducing multiple shoots has already been reported in bamboo species like *Arundinariacallosa* (Devi and Sharma 2009), *D. hamiltonii* (Sood et al. 2002) and *B. oldhamii*(Thiruvengadam et al. 2011). The superiority of BAP in shoot induction may due to its ability to induce natural hormones such as zeatin within the tissues than other synthetic cytokinins (Zaerr and Mapes 1982). Accordingly, the ability of plant tissues to metabolize the natural hormones is faster than artificial growth regulators.

Table 2 Effect of BAP supplemented basal media on *D. giganteus* shoot multiplication

Treatment Code	BAP (µM)	Number of Shoots	Length of Shoots (cm)
M1	0	$1.00^{a} \pm 0.00$	$7.86^{a} \pm 1.23$
M2	4	$6.20^{abc} \pm 1.30$	$5.57^{b} \pm 1.27$
M3	7	$8.40^{bd} \pm 1.82$	$5.22^{b} \pm 1.06$
M4	9	$9.80^{cde} \pm 1.92$	$4.67^{b} \pm 1.39$
M5	13	$15.02^{ef} \pm 4.36$	$3.93^{b} \pm 0.71$
M6	18	$17.06^{f} \pm 4.69$	$3.81^{b} \pm 1.22$

Note: Different letters within the same column indicates significantly different means using Tukey's test at alpha level of 0.05; ± = Standard Error of Mean.

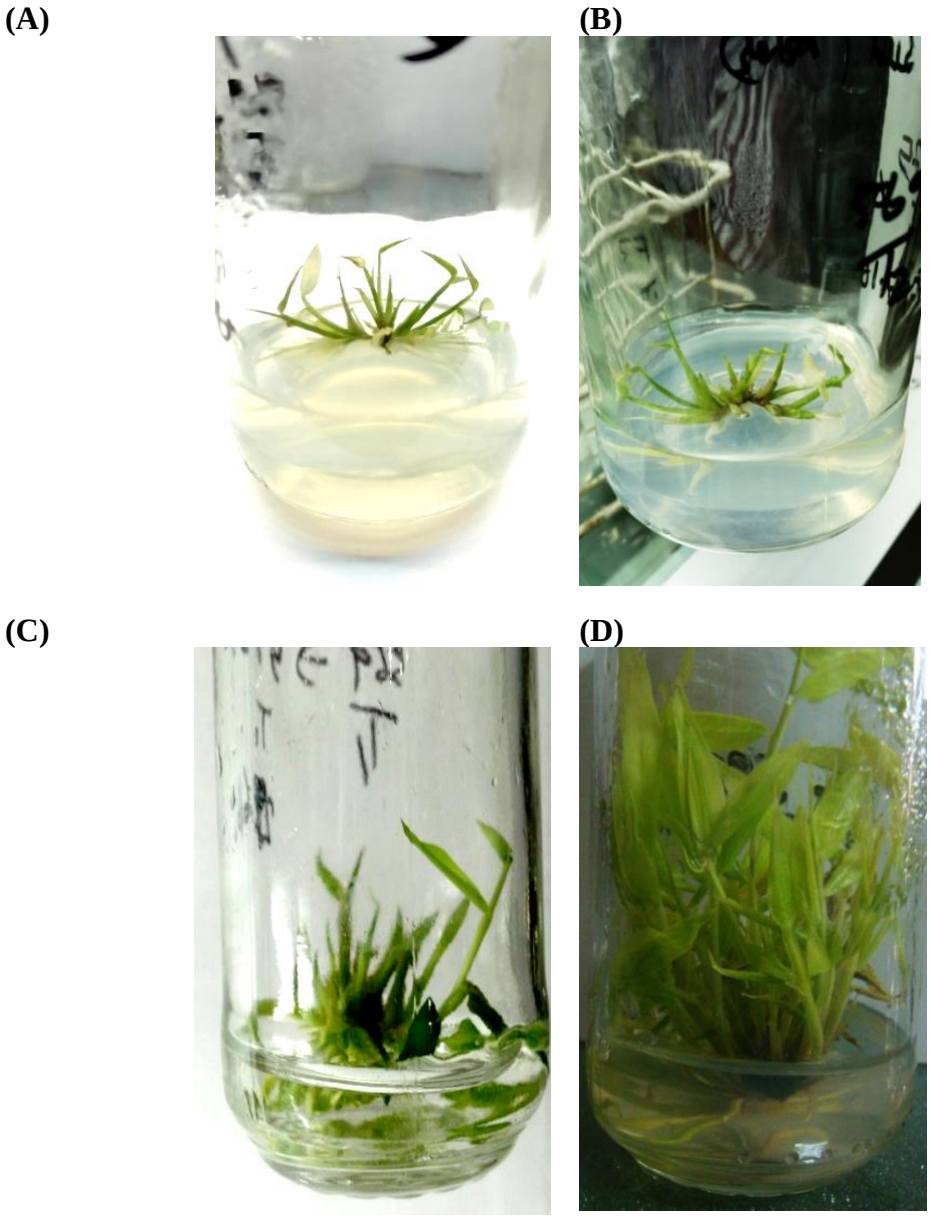


Figure 3 Stages of shoot multiplication in MS media supplemented with 18 µM of BAP. Shoots developed after one (a), two (b), four (c) or six (d) weeks of placing the shoot bunch in the multiplication media.

Continuous Shoot Multiplication

Shoot multiplication and growth was examined in liquid media supplemented 9µM or 13µM BAP. Shoot number was recorded for 4 consecutive cycles consisting of 30 days each. In the first cycle the number of shoot was low which corresponded to axillary shoot induction and the growth of enough axillary shoots to proliferate. This lag stage went up to Cycle 2 where mean shoot number was 5.83 and 6.63 for CS1 and CS2 respectively (Table 3). This was followed by a rapid growth phase when the shoot number increased (Figure 4). Subsequently a period of constant growth was achieved with periodic fluctuations. The mean shoot number was found higher in BAP 13µM than BAP 9µM at every cycle. It was noticed that in both the liquid media shoot number was low in the lag phase which was supported by Ramanayake and Yakandawala (1997). After the lag phase there is a subsequent growth at a high rate. Moreover, the shootsin liquid media were growing faster in height and diameter compared to shoot developed in the solid media

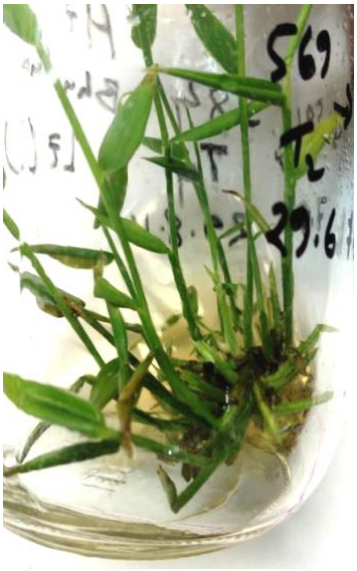


Figure 4 Continuous shoot multiplication and elongation in liquid medium supplemented with 13µM BAP

Table 3 Mean number of shoot for four subculture cycles in two media

Treatment Cycle	Mean Shoot Number	
	CS1	CS2
Cycle 1	3.3	4.5
Cycle 2	5.8	6.6
Cycle 3	10.7	12.0
Cycle 4	15.0	16.1

Note: Each cycle consists of 30 days; Treatment codes CS1 and CS2 indicate the MS media supplemented with 9µM and 13µM BAP respectively.

Effect of IBA on Root Induction

The development of healthy roots is required for the successful establishment of in vitro regenerated shoots to adapt the external environments. Therefore, in the present study,micropropagated shoots of*D. giganteus*were investigated for their rooting ability. Bunches of shoots(three shoots each) were planted in half strength MS media supplemented with five concentrations of IBA (0, 2, 5, 10, 20 µM) for rooting trials. Rooting percentage and number of roots in *D. giganteus* shoots were greatly enhanced with the application of exogenous rooting hormone IBA but the number of days required for root induction were indifferent among the treatments (Table 4). In most of the treatments, root induction was started 7 days after planting them in rooting media. However, rooting in the media with higher IBA concentration (20µM) delayed up to 14 days.

Among the various concentrations of IBA investigated, MS media supplemented with 10µM and 20µM of IBA showed highest percentage of root induction (66.7%), followed by 5µM IBA (60.0 %) (Table 4) and the lowest was in the control, PGR-free treatment (
Table , R1). With regard to average number of roots developed per bunch in half strength MS media with 20µM IBA proved its superiority over the other treatments by producing 3.0±1.6 root per bunch (Figure 5c) followed by 2.0±0.7 (Figure 5b) and (1.6±0.6) with 10µM IBA (Figure 5a) and 5µM IBA, respectively (Table 4). Parthiban et al. (2013) reported that the highest root number for *B. Balcooa* was obtained from higher IBA concentration supplemented MS medium. In the present study, the

treatment R5 (MS medium supplemented with 20µM IBA) was found superior both in rooting percent and number of roots produced, which was in agreement with Parthiban et al. (2013) and Diab and Mohamed (2008). According to the preliminary observation (data not included) from this study, further increase in IBA concentration (40µM) causes poor root induction and condensed root length. Differential effectiveness among auxins might be attributed to the concentration of free auxin that reached the target competent cells, and the metabolic stability of the auxins. Caboche et al. (1987) observed an inhibitory and toxic effect of NAA at above the optimum concentration. IBA, because of its longer side chain (Nordström et al. 1991; Strader and Bartel 2011), is more stable than IAA which rapidly oxidizes and metabolize in plant tissues. IBA is not only more stable than IAA but also convertible to IAA (Strader and Bartel 2011). Therefore, its stability on one hand and convertibility to IAA on the other hand enable IBA to sustain auxin longer and enhances rooting in the culture media than NAA.

Table 4 Rooting ability of *D. giganteus* shoots in half strength MS media supplemented with various concentrations of IBA six weeks after planting them in the rooting media.

Treatment Code	IBA (µM)	Rooting %	Root Number	Days till Root Induction
R1	0	$33.3^{a} \pm 11.6$	$0.8^{a} \pm 0.5$	$12.8^{a} \pm 2.2$
R2	2	$46.7^{a} \pm 6.6$	$1.4^{ab} \pm 0.6$	$9.5^{ab} \pm 4.4$
R3	5	$60.0^{b} \pm 0.0$	$1.6^{ab} \pm 0.6$	$9.3^{ab} \pm 5.0$
R4	10	$66.7^{b} \pm 9.6$	$2.0^{b} \pm 0.7$	$7.3^{ab} \pm 2.1$
R5	20	$66.7^{b} \pm 10.6$	$3.0^{b} \pm 1.6$	$7.8^{b} \pm 3.1$

Note: Different letters within the same column indicates significantly different means using Tukey's test at alpha level of 0.05; ± = Standard Error of Mean.

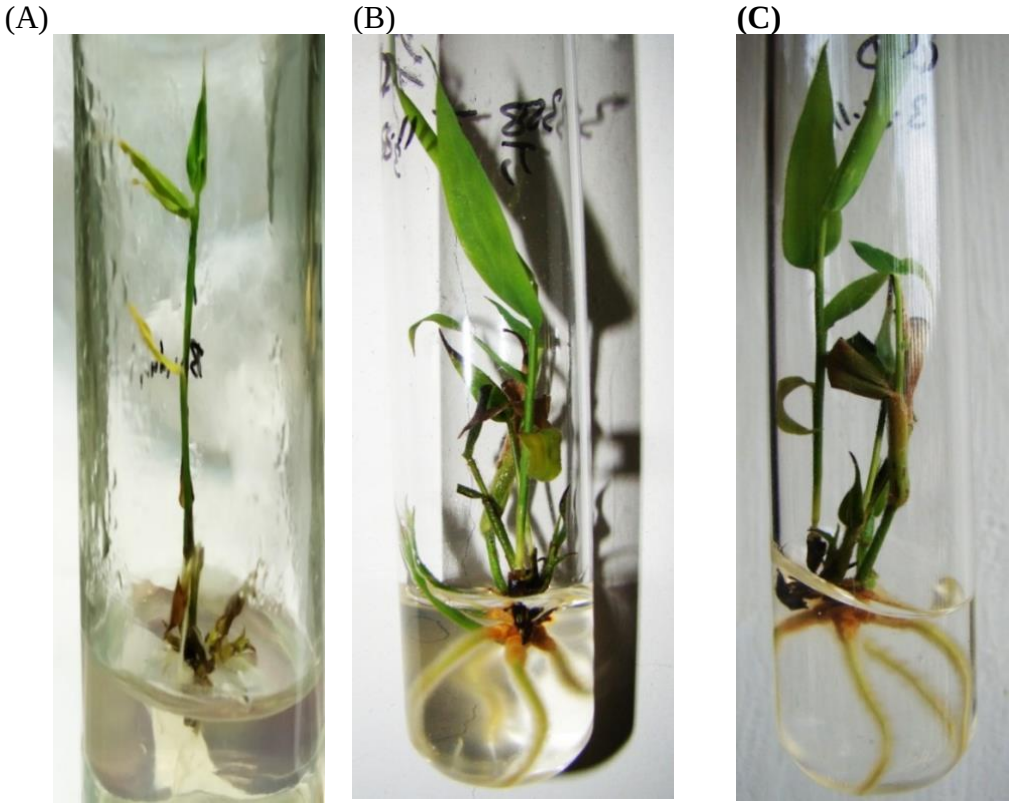


Figure 5 Root induction in the micropropagated shoots in rooting media supplemented with 0 µM (a) 10 µM (b) or 20 µM (c) IBA.

Hardening and Acclimatization

In vitro developed plantlets have morphological and physiological abnormalities due to the in vitro culture conditions (Pospišilova et al. 1999). Direct transfer of in vitro plantlets to ex vitro environment may result in rapid wilt and death (Lesar et al. 2012). Therefore plants grown in vitro were gradually acclimated to the external environmental condition in the greenhouse.For this study 40 in vitro rooted *D. giganteus* plantlets were successfully acclimatized in the mixture of autoclaved forest soil, and coconut coir (1:1). The survival rate was 92.5% and 87.5% after 30 and 60 days of hardening, respectively (Table 5). In the present study, during hardening stage some plantlets were found wilting in the first three days of transferring and some leaves were dried up and subsequently detached from the shoots. This may be due to unrestricted loss of water from their leaves or low hydraulic conductivity of roots and root-stem connections (Kumar and Rao 2012). Therefore, just after transferring the in vitro rooted shoots in to the small pots were covered with clear polyethylene with 2-3 small holes for first five days. After ten day of hardening two-three new leaves were developed from each shoots. Gradually the plantlets started growing and the leaf number increased as the plant height increased. Progressively, as the acclimatization process continue, color of the leaves turned to deep green and size of the leave increased with the size of the plant. Furthermore, proliferations of new tillers were observed after 30 days of hardening (Figure 6).

Table 5 Survival rate of plantlets at two durations of acclimatization

Duration of acclimatization	No. of plantlets survived*	Survival rate
30 days after transferring to greenhouse	37	92.5%
60 days after transferring to greenhouse	35	87.5%

*40 plantlets were used for the acclimatization experiments

Conclusions

Tissue culture based rapid multiplication proved to be promising in different species of plants including bamboo. Therefore, optimizing efficient and reproducible in vitro multiplication protocol for *D. giganteus* would increase the social, environmental and economic benefit derived from the species. Current research work was therefore, undertaken to develop a comprehensive method for in vitro regeneration of *D. giganteus* for mass clonal propagation for industrial production. The establishment of culture, shoot raised from disinfected seeds was carried out on MS media without any growth regulators. The concentration of BAP significantly affected the duration of shoot induction, rate of shoot proliferation and the number and growth of shoots. Accordingly, MS medium supplemented with 13µM BAP showed the highest shoot proliferation percentage and maximum number of shoot (5.6) per explants in least period of time 4.6 days in shoot proliferation. Similarly, BAP 18µM produced the highest number of shoot (17.1) in multiplication. Maximum number of shoots per vessel in liquid MS medium with 13µM BAP was greater than of BAP 9µM, 16.11 and 15 respectively.The highest percent of root induction (66.7%), was recorded from half-strength MS media supplemented with 10µM or 20µM IBA. Nevertheless, the half-strength MS mediumsupplemented with 20µM IBA offered the maximum number of root. Regardless of the significance of auxin, IBA, in enhancing root induction and multiplication, the longest root (9.5 cm) was obtained from, PGR-free half-strength MS media. Compared to solid medium, the liquid medium produced more height but few number of shoots per vessel. The survival rate was as much as 92.5% after 30 days in the open greenhouse environments, and the gradual exposing to sunlight within 60 days the survival rate was 87.5% in nursery environment. It can also be recommended that in vitro rooting of giant bamboo seedlings using auxins especially IBA at 20µM was the best for root induction and development. This study can serve as baseline for germplasm conservation and further studies on this bamboo species. Further

studies on the performance of this bamboo species seeds collected from different ecotype should be used for further development of the protocol. For better rooting results, the combination effect of the studied auxin and with other hormone (auxins and/or cytokinins) should be investigated.

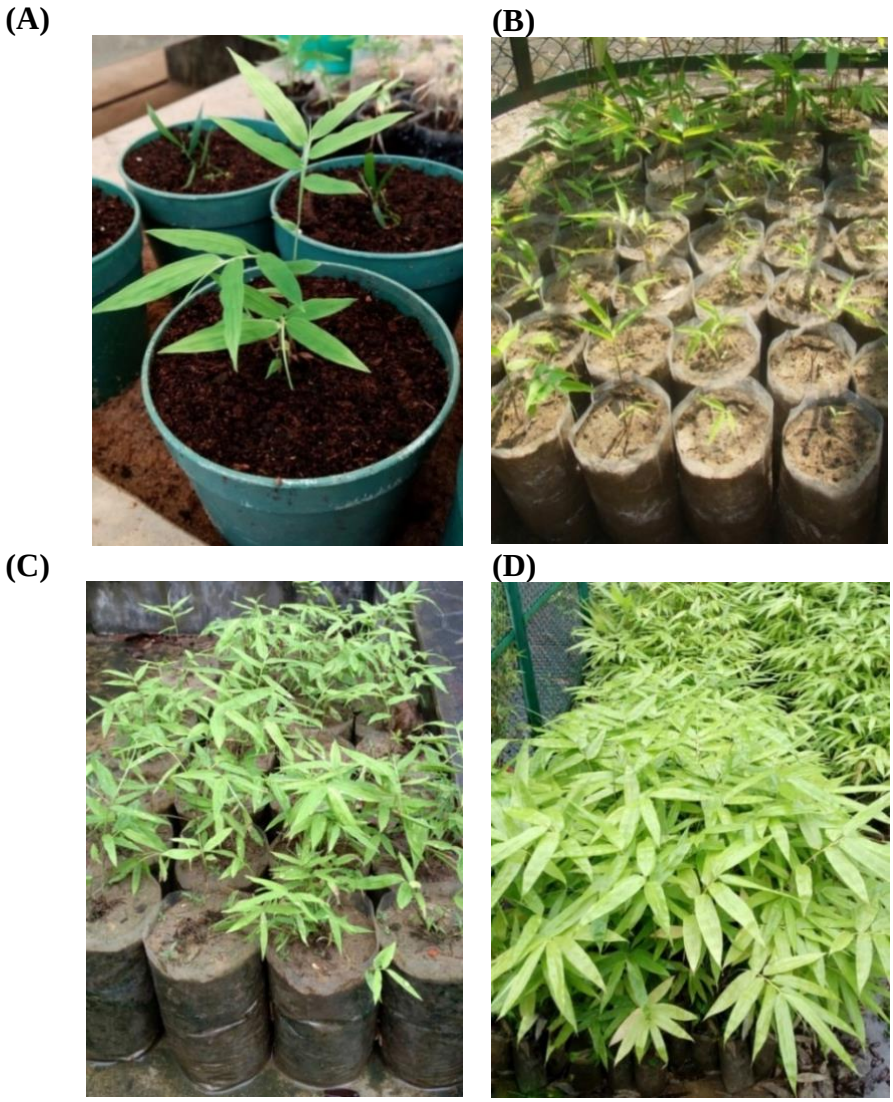


Figure 6 Various stages of hardening of plantlets; in soil:coir media (a), and in soil:compost media under shadefor 15 (b) or 30 (c) days and in open sun for 60 days of acclimation in the nursery condition (d).

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