Theme: 1.2-Commercial Plantation and Management

In vitro Propagation of an Edible Bamboo *Dendrocalamus latiflorus* Munro Using Nodal Explants

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Abstracts

An efficient and reproducible protocol for mass propagation of an important edible bamboo species *Dendrocalamus latiflorus* Munro has been developed through *in vitro* culture. Nodal segments containing single axillary bud were used as explants for shoot multiplication. Administration for 10 minutes of 0.1% mercuric chloride to explants collected in February-March facilitated optimum culture establishment and bud break. The nodal explants were inoculated on Murashige and Skoog (MS) medium supplemented with different concentrations of 6- benzylaminopurine (BAP). 100% axillary bud break was obtained in MS medium supplemented with 5.0mg/l BAP within 10-14 days. Highest shoot multiplication rate (10.67 folds) with average shoot number (32 shoots per culture) was obtained when *in vitro* raised shoots were cultured on MS medium supplemented with 5.0mg/l BAP. For *in vitro* root induction, cluster of 3-4 proliferated shoots were transferred to MS medium containing different concentrations of auxins such as α- naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA). Maximum rooting (80%) with highest average root number (7.20 roots per shoot) was achieved when 5.0 mg/l IBA was added in MS medium. Rooted plantlets were successfully hardened and acclimatized by transplanting first in plastic cups containing a mixture of sand, soil and peat moss in a ratio of 1:1:1 in lab condition for 4 weeks and then in pots containing garden soil kept in glasshouse for 1 month resulting 90% survival rate. This protocol will be helpful for large scale production of this edible bamboo completing its high demands for use in different purposes.

Keywords: *Dendrocalamus latiflorus*, *In vitro*, Nodal explants, Mass propagation.

Introduction

Dendrocalamus latiflorus Munro, known as Taiwan giant bamboo, is an evergreen bamboo species native to southern China, Taiwan and Myanmar which is also cultivated in India, Thailand, Japan, the Philippines and Indonesia. It is a dense clumping bamboo occuring naturally in subtropical humid

areas at slightly higher altitudes up to 1000m. It can also be grown successfully in lowland to moderate elevations in the tropics (Hsu et al. 2000). In Manipur, it grows luxuriantly in hills and plain areas on alluvial and sandy soils. The culms are erect with a pendulous tip, 14-25m tall and 8-20cm in diameter at base, pale green and covered with white waxy scurf when young. Mature culms are used as water pipes, made into small rafts, baskets, for paper pulp, house construction, etc. (Naithani et al. 2010). Succulent young shoots are edible (Lin et al. 2007) and sweet in taste, thus it is also called as Sweet Dendrocalamus. In Manipur, bamboo shoots are highly used vegetable items and the shoots of *Dendrocalamus latiflorus* are mostly preferred because of its delicious taste and less irritant sensation in the mouth. The young shoots are rich in proteins, carbohydrates, vitamins, fibres and bioactive compounds such as phenols and phytosterols which have many nutraceutical properties (Thounaojam et al. 2017) and demand for this bamboo is on the rise due to its multifarious uses. Indiscriminate exploitation and lack of cultivation have resulted in severe depletion of wild stock, resulting in critically low population levels of this bamboo species. Hence, it is very much necessary to conserve this natural resource for sustainable development.

Bamboo has monocarpic habit i.e., flowers only once during its lifetime, which occurs at the end of its first fruiting season (Nadgauda et al. 1990). Due to its long, irregular flowering habit, low seed viability and poor seed set, improvement of this species is not possible through the conventional breeding methods. Similarly, bulkiness and limited availability of propagules, difficulties in transportation, low survival rate and limited rooting of the propagules are the major constraints in bamboo propagation through vegetative methods (Arya and Sharma 1998; Godbole et al. 2002). In such condition, *in vitro* methods remained an alternative solution for mass propagation. Plant regeneration of *Dendrocalamus latiflorus* was done through callus induction using young inflorescences (Lin et al. 2007), anther (Qiao et al. 2013) and young shoots (Ye et al. 2017). So far there has been no report on micropropagation of *Dendrocalamus latiflorus* using nodal explants. Therefore, the present study was undertaken to develop a method on *in vitro* propagation of an important edible bamboo, *Dendrocalamus latiflorus* Munro using nodal explants of adult grown clump.

Material and Methods

Single nodal segments (3-4cm in length) with unsprouted buds of *Dendrocalamus latiflorus* were collected from lateral branches of bamboo plants growing in natural places of Thoubal District, Manipur. The lateral branches for experiments were collected in different seasons. After removing the leaf sheaths, nodes containing lateral spines were removed by scraping carefully with scalpel blades. The nodal explants were then swabbed with 70% alcohol and washed with fresh soapy water of surgical hand wash (Chlorhexidine gluconate solution) for 10 minutes followed by 3-5 times washing

with distilled water. Explants were treated with solution containing bavistin (0.5% w/v), streptocyclin (0.1% w/v) and rifampicin (0.1% w/v) for 10 minutes, followed by immersion in 0.1% mercuric chloride for 10 minutes. Explants were then rinsed 5 times with autoclaved double-distilled water. Both ends of the sterilized nodal segments were trimmed to remove any traces of sterilant and inoculated vertically in culture tubes containing 20ml of Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with various concentrations of 6-benzylaminopurine (BAP), sucrose (3% w/v) and agar (0.8% w/v) for bud sprouting. pH of the medium was adjusted to 5.8 with 1N NaOH and 1N HCl before autoclaving at 121ºC for 20 minutes. Cultures were incubated in culture room at 25±2ºC temperature and 16 hours illumination with a photon flux density of 2500 lux from white fluorescent tubes. Experiments were repeated for three times and for each experiment a minimum of 20 replicates were taken. Observations were recorded after two weeks of inoculation.

The proliferated shoots were excised from the mother explant and transferred to culture jars containing 50ml of MS medium supplemented with different concentrations of BAP (1-7mg/l) for further *in vitro* shoot proliferation. When sufficient shoots were obtained, the proliferating shoots were cut into a cluster of 3 shoots and subcultured on same multiplication medium. The number of propagules cultured and propagules derived at the end of subculture is regarded as the rate of multiplication. Data were recorded after four weeks of subculture. The multiple shoot cultures were maintained by repeated subculture on to the fresh medium for every four weeks.

For studies on *in vitro* rooting, a cluster of 3-4 shoots were separated and transferred on MS medium supplemented with different concentrations of α- naphthalene acetic acid (NAA) and Indole-3-butyric acid (IBA). After 4 weeks of culture on this medium, rooting percent and number of roots per propagule were recorded.

For acclimatization, *in vitro* raised plantlets were removed from the rooting medium and washed thoroughly under running tap water to remove all traces of adhering medium. The plantlets were transferred to plastic cups filled with a mixture of sterilized sand, soil and peat moss in the ratio of 1:1:1. They were covered with transparent plastic cups to prevent transpiration and to maintain relative humidity at 80% for a few days and kept in the culture room, irrigated with half strength MS solution (without organics). After 4 weeks, the plantlets were transferred to pots containing garden soil and acclimatized for 1 month in glasshouse before subsequent transplantation to the field.

Results and Discussion

Culture Initiation

The sterilization procedure followed in this study yielded 79% aseptic cultures. Based on the frequency of bud-break and rate of contamination, 0.1% mercuric chloride for 10 minutes was

regarded as the best treatment to initiate aseptic cultures of nodal segments from mature plants. The explants collected during February-March showed highest rate of bud break (75%) with less frequency of contamination. Rainy season had almost an equal frequency of bud break but the rate of contamination was very high. Least contamination was recorded during winter season but the percentage of bud break was very low. Seasonal effects on *in vitro* bud break was earlier reported by Ramanayake and Yakandawala (1997) in *Dendrocalamus giganteus*, Das and Pal (2005) in *Bambusa balcooa*, Devi and Sharma (2009) in *Arundinaria callosa*, Banerjee et al. (2011) in *Dendrocalamus asper* and Anand and Brar (2013) in *Bambusa bambos*. Size of explants also influenced the frequency of bud break. Small sized explants did not respond well in cultures and large sized explants produced higher phenolic exudates. It was noted that medium sized explants (25mm) gave better response compared to small and large sized explants.

Axillary bud break was achieved within 10-14 days in all the aseptic cultures on MS medium supplemented with 1-7mg/l BAP (Figure 1a). On basal medium, the frequency of bud-break was 50% (Table 1). Incorporation of BAP into the medium improved the percentage of bud break and shoot proliferation (Table 1). 100% bud break response was obtained in MS medium supplemented with 5.0mg/l BAP. In all treatments, 1-4 shoots proliferated from the axillary bud (Figure 1b). The use of BAP for nodal bud break was more efficient than other cytokinins as also reported by Arya et al. (2001); Devi and Sharma (2009); Banerjee et al. (2011); Waikhom and Louis (2014.)

Table 1. Effect of BAP concentrations on bud break frequency and shoot formation from nodal explants of *Dendrocalamus latiflorus*. Data recorded after 2 weeks of inoculation.

BAP (mg/l)	Bud break (%)	Average no. of shoots/explants
0.0	50	0.83±0.98
1.0	66	1.00±0.89
2.0	66	1.30±0.82
3.0	83	1.50±1.22
4.0	83	1.50±1.05
5.0	100	1.83±1.47
6.0	83	1.17±0.75
7.0	66	1.00±0.00

Mean of six replicates ± SD

Shoot Multiplication

Multiplication of proliferated shoots was obtained after 3-4 weeks when cluster of 3 shoots were transferred to MS medium supplemented with 1-7mg/l BAP (Figure 1c). On BAP-free MS medium

the subcultured shoots did not multiply. Of the various treatments, 5.0mg/l BAP gave the best multiplication rate: 10.67 folds with an average shoot number of 32.00 (Table 2). Increased concentrations of BAP (6.0mg/l and 7.0mg/l) showed steady decline in shoot multiplication rate as well as in shoot number. Earlier reports on *in vitro* multiplication of bamboos, where BAP had been used extensively for shoot multiplication (Ramanayake et al. 2006; Yasodha et al. 2008; Ramanayake et al. 2008; Arya et al. 2001; Mudoi and Borthakur 2009; Sharma and Sarma 2013), support the present results for shoot multiplication of *Dendrocalamus latiflorus.* The *in vitro* shoots were maintained by regular subculturing in freshly prepared MS medium supplemented with 5.0mg/l BAP after every 4 weeks. Delaying of sub-culturing period resulted in gradual browning of the shoots. Hence, sub-culturing period was recorded as one the most crucial factor for obtaining desired level of regeneration of shoots. It was also observed that the propagule size of 3-4 shoots had higher multiplication potential than the propagules with less or more than 3-4 shoots.

Table 2. Effect of BAP concentrations in MS Medium on shoot multiplication of *Dendrocalamus latiflorus*.

BAP (mg/l)	No. of shoots	Multiplication rate
Control	0.00±0.00	0.00±0.00
1.0	9.80±9.42	3.27±3.14
2.0	18.80±13.07	6.27±4.36
3.0	25.40±7.30	8.47±2.43
4.0	25.60±7.80	8.53±2.60
5.0	32.00±15.15	10.67±5.05
6.0	22.00±7.21	7.33±2.40
7.0	18.00±6.00	6.00±2.00

Mean of five replicates ± SD

Table 3. Effect of different treatments on rooting of *Dendrocalamus latiflorus* on MS Medium.

Treatments (mg/l)	Rooting (%)	No. of roots
Control	0	0.00±0.00
1.0 NAA	20	1.60±3.58
2.0 NAA	40	2.60±3.71
3.0 NAA	60	3.80±3.56
3.0 IBA	60	4.40±4.10
5.0 IBA	80	7.20±4.32
10.0 IBA	80	5.80±3.77

Mean of five replicates ± SD

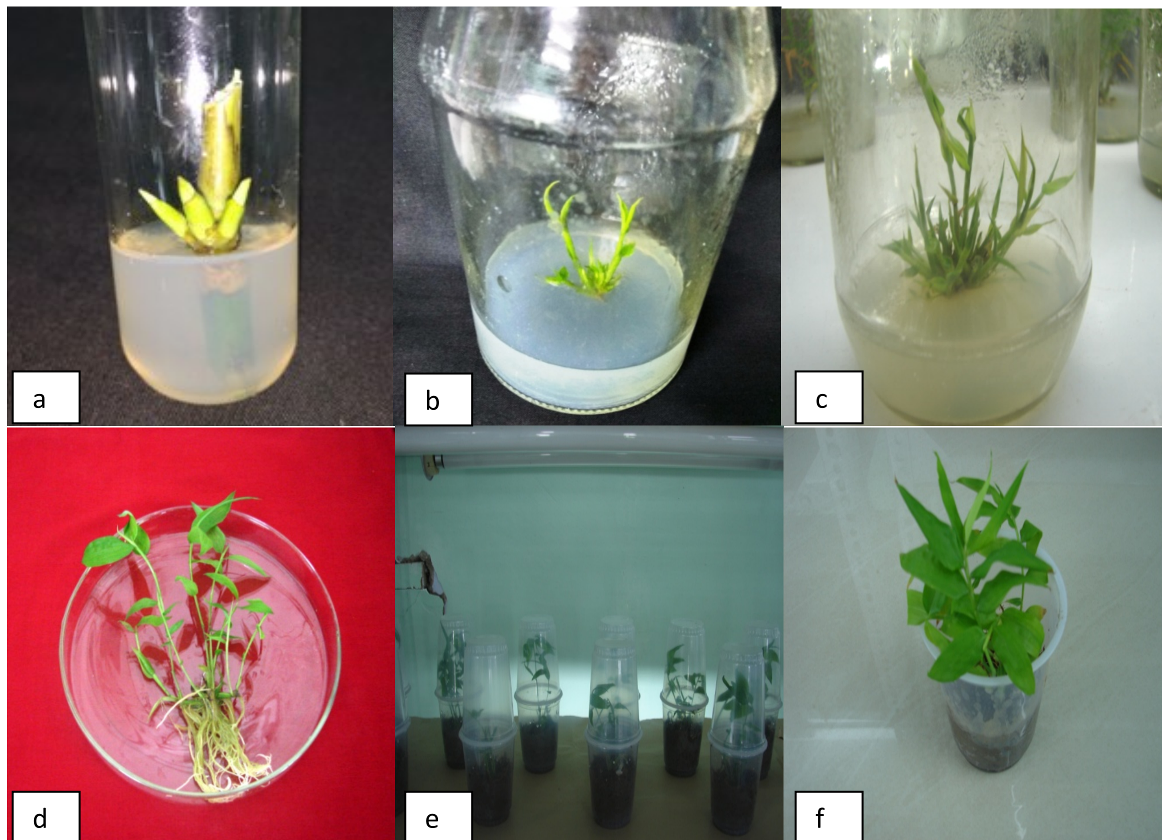

Figure 1. Micropropagation of *Dendrocalamus latiflorus* (a) axillary bud break, (b) shoot proliferation in MS medium (c) multiple shoot formation in MS + 5.0 mg/l BAP (d) root formation after transfer to MS + 5.0 mg/l and 10mg/l IBA (e) hardening of *in vitro* raised plantlets (f) an acclimatized plant.

Root induction

In vitro rooting was attempted by culturing the *in vitro* multiplied shoots (cluster of 3-4 shoots) on MS medium supplemented with different concentrations of auxins such as NAA (1-3mg/l) and IBA (3-10mg/l). It was observed that rooting was more effectively induced when clusters of shoots were used rather than individual shoot. A significant rooting upto 80% was obtained within 4 weeks when 5.0mg/l and 10.0mg/l IBA was added in MS medium (Figure 1d). 5.0mg/l IBA produced highest average root numbers (7.20 roots per shoots) with 80% rooting efficiency (Table 3). Root induction was also noticed in medium supplemented with NAA but rooting percentage was low as compared to IBA. These results are in line with earlier reports on several bamboo species such as *Bambusa vulgaris* (Rout and Das 1997), *B. oldhamii* (Lin et al. 2005), *Dendrocalamus asper* (Arya et al. 2001), *Dendrocalamus hamiltonii* (Arya et al. 2012) and *Bambusa bambos* (Anand and Brar 2013).

Hardening and Acclimatization

Two-step hardening procedure following gradual exposure of *in vitro* raised rooted plantlets in (i) controlled conditions of culture room for 4 weeks and (ii) semi controlled conditions of glasshouse for 4 weeks produced 90% healthy acclimatized plantlets (Figure 1e-1f). Coverage of transparent polythene (Thomas and Ravindra 1997) with regular irrigation ensured high humidity and increase in temperature which evidently encouraged the rapid acclimatization process. The use of farm yard manure for acclimatization also played a significant part in retaining the moisture (Gantait et al. 2010).

Conclusion

The present study provides an efficient and rapid method for *in vitro* propagation of an edible bamboo, *Dendrocalamus latiflorus* Munro from the nodal segments of the field grown mature culm, with high multiplication efficiency, proper rooting and easy establishment in the field condition. *In vitro* bud-break and shoot multiplication were enhanced by supplementation of BAP. MS medium with 5.0mg/l BAP gave 100% axillary bud break and highest multiplication rate (10.67 folds). Maximum rooting (80%) with highest average root number (7.20 roots per shoot) was achieved when 5.0mg/l IBA was added in MS medium. Hence, the combination of 5.0 mg/l BAP and 5.0 mg/l IBA for shooting and rooting is the best for micropropagation of *Dendrocalamus latiflorus*. The plant survival rate was 90% when acclimatization was carried out by transplanting first in plastic cups containing a mixture of sand, soil and peat moss in a ratio of 1:1:1 in lab condition for 4 weeks and then in pots containing garden soil kept in glasshouse for 1 month. This protocol can be adopted commercially for mass propagation of this edible bamboo species which will contribute to meeting its growing demand for true-to-type, disease-free, high-quality plant material and edible shoots.

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