Review

Micropropagation of important bamboos: A review

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Bamboos are versatile, arborescent, perennial and non-wood forest trees with tremendous eco-sociological and commercial importance. Different propagation techniques are available for bamboo, such as seed propagation, clump division, rhizome and culm cuttings, but these classical techniques suffer from serious drawbacks for large or mass scale propagation. For mass scale propagation, these are largely insufficient and inefficient and micropropagation is the only viable method. Indeed, the order of magnitude of the demand for bamboo planting material indicates that micropropagation will inevitably be necessary for mass scale propagation. The potential of micropropagation for mass scale propagation of bamboo has raised high hopes and a lot of research has been focused on the development of protocols for large and rapid scale propagation. These encompass optimization and establishment of *in vitro* culture techniques including micropropagation, somatic embryogenesis, *in vitro* flowering, macro proliferation, field performance and clonal fidelity. This review briefly provides the state-of-the-art information on tissue culture mediated biotechnological interventions made in bamboo for large scale micropropagation, that being the need of the hour.

Key words: Bamboo, micropropagation, somatic embryogenesis, *in vitro* flowering, macroproliferation, field performance, clonal fidelity.

INTRODUCTION

Bamboo an important non-wood forestry products is one of the most important agricultural plants worldwide (Liese, 1987). It is a fast growing world's greatest natural and renewable resources gaining approximately 75 to 400 mm per day, whose rate of biomass generation is unsurpassed in the plant kingdom. In total, about 18 million ha of bamboo are distributed in world ecosystems in Asia, Africa and America. Bamboo is a vernacular term for the members of subfamily *Bambusoideae* of the family *Poaceae,* the grasses. It is an important forest tree with multifarious use in daily life, apart from having largest use in the paper and pulp industry (Varmah and Pant, 1981). One of the main problems with bamboo is that it has been regarded as a resource, which is simply those to take, as has been for thousands of years by people in

rural economies. However, in industrial economies, such practice leads to considerable over exploitation and rapid depletion of bamboo resources. Estimates regarding future use of bamboo also indicate that there will be a huge shortage of bamboo planting material in long terms (Subramanlam, 1994; Nadgauda et al., 1997). To cope with this forecasted shortage, micropropagation via tissue culture thus attracted lot of attention. It was believed that this method could solve most or at least many problems in propagation of bamboo.

Bamboos are distributed all over the world with 75 genera and 1250 species, but majority occur in the tropics although, they are found naturally in all subtropical and temperate zones except in Europe. Research on tissue culture of bamboo was fairly recent. Extensive research on micropropagation of bamboo species had carried out using juvenile (zygotic embryo, seed or seedling) and mature clump derived (nodal buds) tissues with more than 40 species of bamboo. A large number of papers on *in vitro* studies of bamboo have been published

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but all lacked crucial parts. They were either not very efficient, or not applicable on other bamboos, or both. It is the author's opinion that there are now sound reasons to anticipate that micropropagation via tissue culture methods could solve most or at least many problems in propagation of bamboo. We intend to explain the reasons for this renewed optimism against the background of knowledge accumulated that will be relevant to any ultimate success in exploiting these new approaches.

Problems associated during *in vitro* culture of bamboo

Browning is a major problem that was associated during *in vitro* culture of bamboo due to phenolic exudation. As a result, the multiple shoots were going to be blackish brown and ultimately dried up. This problem was solved by the addition of some additives along with plant growth regulators (Ganesan and Jayabalan, 2005). Browning of excised plant tissues as well as nutrient media occurs frequently and remains a major basis for recalcitrance *in vitro*. The severity of browning has varied according to species, tissue or organ, and nutrient medium and other tissue variables (Huang et al., 2002). Browning problem is encountered either during culture initiation of bamboo or in sub-culturing stage. To overcome the harmful effect of browning, different types and concentration of antioxidants are incorporated into the media or soaking the explants in liquid solution of those mentioned (Zamora et al., 1988; Mehta et al., 1982; Saxena, 1990; Saxena and Dhawan, 1999). It was also noticed that though incorporation of antioxidants may reduce the browning percentage, frequent transfer/subculture to the fresh medium is the most effective than the above all treatments.

MICROPROPAGATION

Conventional methods of propagation of bamboo are based on seeds and vegetative methods. However, availability of seed is limited to certain specific period. Many bamboo plants develop flower and seed only two to three times in a century and viability period of the seed is very short. Hence, the propagation is done vegetatively by using different parts of the bamboo like off-set and rhizome planting, branch and culm cuttings, marcotting and layering but, vegetative propagation through cuttings, offsets and rhizomes are bulky and not available in sufficient number, expensive and also cumbersome to handle and liable to desiccation. Moreover, the shortage of supply has been compounded by absence of inadequate replantation. In this case, Plant tissue culture offers special advantages as it ensures a continuous supply of planting material in a short span of time and helps in multiplication, as well as, conserving wild germ-

plasm.

Research on tissue culture of bamboo was fairly recent. Micro propagation via tissue culture attracted a lot of attention since it was believed that this method could solve most or at least many problems in propagation of bamboo. Extensive research on micropropagation of bamboo species has been carried out using juvenile (Zygotic embryo, seed or seedling) and mature clump derived (nodal buds) tissues with more than 40 species of bamboo. A large number of papers on micropropagation of bamboo have been published as original papers or reviews including more general aspects. Apart from micropropagation, these publications had focused on somatic embryogenesis, genetic improvement and *in vitro* flowering. Micropropagation through juvenile explants (Table 1a) and mature clump derived tissues (Table 1b) could help the regeneration of large number of plants in a relatively short time.

The first report on successful tissue culture of bamboo was by Alexander and Rao (1968) who described embryo culture of *Dendrocalamus strictus.*

Micropropagation from juvenile explants

Two major advantages of using seedlings are that it is a new generation as well as easier technique for *in vitro* multiplication, but its disadvantages are, insufficient knowledge of genetic background, restricted availability of seeds and loss of germination capacity etc.

In 1968, Alexander and Rao reported the aseptic germination of bamboo seeds (*D. strictus*) heralding the start of tissue culture of bamboo in White (1963) major and minor elements. Since then, White's basal medium was used by Nadguada et al. (1990) in *Bambusa arundinacea* and *D. strictus*; Mascarenhas et al. (1988) in *D. strictus*; Ravikumar et al. (1998) in *D. strictus* for zygotic embryo germination. Nadgir et al. (1984) reported plantlet production from seedling shoot explants of *D. strictus*. Murashige and Skoog (1962) (MS) basal medium was routinely used by Joshi and Nadguada (1997) in *B. arundinacea*; Yasodha et al. (1997) in *Banksia nutans* and *Dendrocalamus membranaceus*; Saxena (1990) in *Bambusa tulda* for the above purpose. Moreover, Joshi and Nadguada (1997) used half strength of MS basal medium for embryo germination of *B. arundinacea*.

Cytokinins, especially 6-benzylaminopurine (BAP) was effective for inducing shoot proliferation in several bamboos viz. *B. arundinacea, Bambusa vulgaris* and *D. strictus* (Nadgir et al., 1984); in *B. nutans and D. membranaceus* (Yasodha et al., 1997); in *B. tulda* (Saxena, 1990); in *D. asper* (Arya et al., 1999); in *Dendrocalamus brandisii* (Vongvijitra, 1988); in *B. arundinacea* and *D. strictus* (Nadguada et al., 1990); in *Dendrocalamus hamiltonii* (Chambers et al., 1991); in *D. strictus* (Maity and Ghosh, 1997); in *D. strictus*

Table 1a. Micropropagation of bamboo from zygotic embryo explants.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
Bambusa sp.	Zygotic embryo	White major and minor elements	Nil	Shoot formation	Alexander and Rao (1968)
Bambusa arundinacea	Zygotic embryo	Nitsch and Nitsch (1967) medium	# # 2,4-D (30.0 μM) # # # BAP (5.0 μM) + 2,4-D (30.0 μM) + PVP (250 mg l^{-1})	Somatic embryogenesis	Mehta et al. (1982)
B. arundinacea	Zygotic embryo	MS	** BAP (1.0-10.0 mg l^{-1}) + IBA	Plantlet formation	Nadgir et al. (1984)
B. arundinacea	Zygotic embryo	White MS	* Basal + 2% sucrose + + Basal *** BAP (0.5 PPM) + CW 5% + IBA (0.5 PPM)	*In vitro* flowering and seed set	Nadgauda et al. (1990)
B. arundinacea	Zygotic embryo	MS	* ½ MS basal • BAP (2.22 μM) • BAP (2.22 μM) + 2ip (7.21 μM)	*In vitro* flowering	Joshi and Nadgauda (1997)
B. arundinacea	Zygotic embryo	MS	• BAP (2.22 μM) + CW 5%	*In vitro* flowering	Nadgauda et al. (1997)
B. nutans	Zygotic embryo	MS	* Basal ••• Basal *** BAP (0.5 mg l^{-1}) + IBA (0.5 mg l^{-1}) - ½ MS	Mass propagation	Yasodha et al. (1997)
B. tulda	Zygotic embryo	MS	* Basal ** BAP (3×10^{-6}M) or (6×10^{-6}M) *** BAP (8×10^{-6}M) + Kn (4×10^{-6}M) + IAA (1×10^{-5}M) + Coumarin (6.8×10^{-5}M)	*In vitro* propagation	Saxena (1990)
B. vulgaris	Zygotic embryo	MS	** BAP (1.0-10.0 mg l^{-1}) + IBA	Plantlet formation	Nadgir et al. (1984)
B. vulgaris	Zygotic embryo	MS	# 2,4-D (3.0 mg l^{-1}) + Kn (0.25 mg l^{-1}) # # ½ MS + Kn (0.5mg l^{-1}) + Ads (10.0 mg l^{-1}) + 2,4-D (2.0 mg l^{-1}) # # # ½ MS + Kn (0.5 mg l^{-1}) + Ads (10.0 mg l^{-1}) + 2,4-D (2.0 mg l^{-1}) *** Ads (0.5 mg l^{-1}) + IBA (0.25 mg l^{-1}) + GA$_3$ (0.5 mg l^{-1}) • Ads (0.5 mg l^{-1}) + IBA (0.25 mg l^{-1}) + GA$_3$ (0.5 mg l^{-1})	Somatic embryogenesis and *in vitro* flowering	Rout and Das (1994)
Dendrocalamus asper	Zygotic embryo	MS	• BAP (1.0-10.0 mg l^{-1}) *** BAP (3.0 mg l^{-1}) + IBA(10.0 mg l^{-1}); NAA (3.0 mg l^{-1})	Micropropagation	Arya et al. (1999)

Table 1a. Contd.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
D. brandisii	Zygotic embryo	MS	* BAP (6 × 10^{5}M) *** BAP (0.5-2 × 10^{5}M) + NAA (1×10^{5}M)	Micropropagation	Vongvijitra (1988)
D. brandisii	Zygotic embryo	White MS	* Basal + + Basal *** BAP (0.5 PPM) + CW 5% + IBA (0.5 PPM)	*In vitro* flowering and seed set	Nadgauda et al. (1990)
D. giganteus	Zygotic embryo	MS	# 2,4-D (3.0 mg l^{-1}) + Kn (0.25 mg l^{-1}) # # ½ MS +Kn (0.5gm l^{-1}) + Ads (10.0 mg l^{-1}) + 2,4-D (2.0 mg l^{-1}) # # # ½ MS + Kn (0.5 mg l^{-1}) + Ads (10.0 mg l^{-1}) + 2,4-D (2.0 mg l^{-1}) *** Ads (0.5 mg l^{-1}) + IBA (0.25 mg l^{-1}) + GA$_3$ (0.5 mg l^{-1}) • Ads (0.5 mg l^{-1}) + IBA (0.25 mg l^{-1}) + GA$_3$ (0.5 mg l^{-1})	Somatic embryogenesis and *in vitro* flowering	Rout and Das (1994)
D. hamiltonii	Zygotic embryo	MS	*** BAP (4.4 µM) • BAP (4.4 µM); BAP (22.2 µM)	Micropropagation and *in vitro* flowering	Chambers et al. (1991)
D. hamiltonii	Zygotic embryo	MS	# 2,4-D (1.0-3.0 mg l^{-1}) *** BAP (2.0 mg l^{-1}) + Kn (1.0 mg l^{-1}) + NAA (1.0 mg l^{-1}) + IBA (5.0 mg l^{-1})	Somatic embryogenesis and organogenesis	Zhang et al. (2010)
D. membranaceus	Zygotic embryo	MS	* Basal *** BAP (0.5 mg l^{-1}) + IBA (0.5 mg l^{-1}) - ½ MS	Mass propagation	Yasodha et al. (1997)
D. strictus	Zygotic embryo	MS	** BAP (1.0-10.0 mg l^{-1}) + IBA	Plantlet formation	Nadgir et al. (1984)
D. strictus	Zygotic embryo	White MS	* Basal + IAA, NAA, IPA (0.05-5.0 PPM)	Micropropagation	Mascarenhas et al. (1988)
D. strictus	Zygotic embryo	MS	# 2,4-D (3.0 mg l^{-1}) + Kn (0.25 mg l^{-1}) # # ½ MS + Kn (0.5 mg l^{-1}) + Ads (10.0 mg l^{-1}) + 2,4-D (2.0 mg l^{-1}) # # # ½ MS + Kn (0.5 mg l^{-1}) + Ads (10.0 mg l^{-1}) + 2,4-D (2.0 mg l^{-1}) *** Ads (0.5 mg l^{-1}) +IBA (0.25 mg l^{-1}) + GA$_3$(0.5 mg l^{-1}) • Ads (0.5 mg l^{-1}) + IBA (0.25 mg l^{-1}) + GA$_3$ (0.5 mg l^{-1})	Somatic embryogenesis and *in vitro* flowering	Rout and Das (1994)
D. strictus	Zygotic embryo	MS	* GA$_3$ (1.0 mg l^{-1}) *** BAP (2.0 mg l^{-1})	Plant regeneration	Maity and Ghosh (1997)
D. strictus	Zygotic embryo	MS and White	** BAP and Kn (0.5 mg l^{-1} each) + CW (200 mg l^{-1}) *** BAP and Kn (0.5 mg l^{-1} each) * Basal medium • IBA (0.25 mg l^{-1})	Plant regeneration	Ravikumar et al. (1998)

Table 1a. Contd.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
D. strictus	Zygotic embryo	MS	# 2,4-D (3×10^{-5}M) ## 2,4-D(1×10^{-5} M)+ Kn (5×10^{-5} M)+ soluble Polyvinylpyrrolidone PVP (250 mg l^{-1}) ### NAA (5×10^{-6} M) + Kn (5×10^{6} M) + soluble PVP (250 mg l^{-1})	Large-scale propagation	Saxena and Dhawan (1999)
D. strictus	Zygotic embryo	MS	* ½ MS + TDZ (0-1.0 mg l^{-1}) *** TDZ (0.01 mg l^{-1}) • TDZ (0.5 mg l^{-1}; 1.0 mgl^{-1})	*In vitro* flowering	Singh et al. (2000)
D. strictus	Zygotic embryo	MS	* 2,4-D (2.0 mg l^{-1}) *** ½ MS + BAP (2.0 mg l^{-1}) + ½ MS+IBA(2.0 mg l^{-1})	Clonal propagation	Reddy (2006)
D. strictus	Zygotic embryo	MS	* 2,4-D	Plantlet formation	Zamora et al. (1988)
Dinochloa sp.	Zygotic embryo	MS	* 2,4-D	Plantlet formation	Zamora et al. (1988)
Gigantochloa levis	Zygotic embryo	MS	* 2,4-D	Plantlet formation	Zamora et al. (1988)
Otatea acuminate aztecorum	Zygotic embryo	MS Gamborg et al.1968 (B$_5$)	# 2,4-D (0.3 mg l^{-1}) + BAP (0.5 mg l^{-1}) ## 2,4-D (3.0 mg l^{-1}) + BAP (0.5 mg l^{-1}) ### 2,4-D (3.0 mg l^{-1}) ### 2,4-D (3.0 mg l^{-1})	Somatic embryogenesis and plantlet regeneration	Woods et al. (1992)
Schizostachyum lima	Zygotic embryo	MS	* 2,4-D	Plantlet formation	Zamora et al. (1988)
S. lumampao	Zygotic embryo	MS	* 2,4-D	Plantlet formation	Zamora et al. (1988)
Sinocalamus latiflora	Zygotic embryo	MS	## 2,4-D (6.0 mg l^{-1}) + Kn (3.0 mg l^{-1}) + PVP (250 mg l^{-1}) ### 2,4-D (3.0 mg l^{-1}) + Kn (2.0 mg l^{-1})	Somatic embryogenesis and plant regeneration	Yeh and Chang (1987)

*, Embryo germination; **, shoot bud initiation; ***, shoot multiplication; #, callus formation; # #, somatic embryogenesis; # # #, embryoids formation; •, *in vitro* flowering; ••, rhizome formationl; •••, organogenesis; +, rooting; ++, hardening; + + +, protoplast isolation and culture; x, inflorescence proliferation; xx, cell suspension culture.

(Ravikumar et al., 1998) either singly or in combination with auxin and complex additives. Moreover, half strength of MS with BAP was responsible for the same in *D. strictus* (Reddy, 2006). In certain cases, Adenine sulphate (Ads) in combination with indole-3-butyric acid (IBA) and gibberellic acid (GA$_3$) was responsible for shoot proliferation in *B. vulgaris, Dendrocalamus giganteus* and *D. strictus* (Rout and Das, 1994). Moreover, thidiazuron (TDZ) was suitable for the same in *D. strictus* (Singh et al., 2000).

Seedling explant was also used for multiple shoot induction in *B. tulda* (Saxena, 1990); *D. brandisii* (Vongvijitra, 1988); *D. strictus* (Shirgurkar et al., 1996); *B. nutans and D. membranaceus* (Yasodha et al., 1997); *Thamnocalamus spathiflorus* (Zamora, 1994) and *D.*

hamiltonii (Sood et al., 2002).

Micropropagation from mature tissue explants

In order to supplement conventional methods of propagation, an efficient *in vitro* propagation method by using explants from selected mature plants would offer a desirable alternative for large-scale propagation of bamboo. Micropropagation through axillary bud proliferation by nodal explants resulted true to type plant population without intervening the callus phase and thus maintain the clonal fidelity. Being a clonal method, it highly reduces or eliminates the variation inherent in seed-raised population but, endogenous contamination, hyper-hydricity and instability of multiplication rates affect the flow

Table 1b. Micropropagation of bamboo from mature clump derived tissues.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
Bambusa arundinacea	Nodal explants	MS	** BAP (5.0 mg l⁻¹) *** BAP (5.0 mg l⁻¹) + NAA (3.0 mg l⁻¹)	Mass multiplication	Arya et al. (2002b)
B. atra	Axillary shoots from adult field culms	MS	+ Basal	Root induction	Ramanayake et al. (2008)
B. balcooa	Nodal segments	MS	*** BAP (11.25 µM) + Kin (4.5 µM) + ½ MS + IBA (1.0µM)	*In vitro* regeneration	Das and Pal (2005)
B. balcooa	Nodal buds	MS	** BAP (1.0 mg l⁻¹) *** BAP (1.0-5.0 mg l⁻¹) + ½ MS + NAA (1.0-3.0 mg l⁻¹) + IBA (1.0-5.0 mg l⁻¹)	Mass propagation	Islam and Rahman (2005)
B. bambos	Nodal segments from nursery raised 3 yr old plant	MS Lloyd and Mc Crown (1980) medium (WPM)	*** BAP (5.0 mg l⁻¹) + NAA (3.0 mg l⁻¹) • NAA (3.0 mg l⁻¹)	Micropropagation	Arya and Sharma (1998)
B. bambos var. *gigantea*	Caryopsis	MS	*** BAP (5.0µM) •• BAP (2.0-5.0µM + GA₃ (0.1µM) + NAA (50.0 µM)	*In vitro* rhizome induction and plantlet formation	Kapoor and Rao (2006)
B. edulis	Segments of *in vitro* spikelets	MS	• TDZ (0.5 µM) + NAA (53.8 µM) + 2,4-D (4.48 µM)	*In vitro* flowering and plantlet survivability	Lin et al. (2003a)
B. edulis	Single nodal segments excised from proliferated branches of 10-yr old field grown culms	MS	**BAP (1.0 mg l⁻¹) ** BAP (1.0 mg l⁻¹) + NAA (1.0 mg l⁻¹) *** TDZ (0.01 mg l⁻¹) + TDZ (0.01 mg l⁻¹) + 2,4-D (0.5 mg l⁻¹) + TDZ (0.01mg l⁻¹) • TDZ (0.01 mg l⁻¹)	Micropropagation and *in vitro* flowering	Lin et al. (2004)
B. edulis	Multiple shoots	MS	*** TDZ (0.1mg l⁻¹)	*In vitro* flowering	Lin et al. (2007)
B. glaucescens	Nodal segments	MS	*** BAP (5.0 µM) + Kin (15 µM) + IBA (1.0 µM)	*In vitro* regeneration	Shirin and Rana (2007)
B. nana	Small branch cuttings	MS	*** BAP (3×10⁻⁵M)	*In vitro* culture	Vongvijitra (1988)
B. nutans	Nodal buds	MS	** BAP (1.0 mg l⁻¹) *** BAP (1.0-5.0 mg l⁻¹) + ½ MS + NAA (1.0- 3.0 mg l⁻¹) + IBA (1.0-5.0 mg l⁻¹)	Mass propagation	Islam and Rahman (2005)
B. nutans	Nodal segments	MS	** BAP (2.22 µM) + IBA (49.0 µM) + Glucose	*In vitro* rooting	Yasodha et al. (2008)

Table 1b. Contd.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
B. oldhamii	Bamboo mosaic virus (BaMV)-free meristems	MS	*** TDZ (0.45 µM) + NAA (10.74 -26.85 µM)	Multiple shoot proliferation	Lin et al. (2007)
B. salarkhanii	Nodal buds	MS	** BAP (1.0 mg l^{-1}) *** BAP (1.0-5.0 mg l^{-1}) + ½ MS + NAA (1.0-3.0 mg l^{-1}) + IBA (1.0-5.0 mg l^{-1})	Mass propagation	Islam and Rahman (2005)
B. tulda	Nodal explant	MS	*** BAP (12.0 µM) + IAA (0.1 µM) + Glutamine (100 µM) + Coumarin (40.0 µM)	Micropropagation	Mishra et al. (2008)
B. ventricosa	Shoot tips	MS	** BAP (4.44 µM) *** BAP (4.44 µM) + NAA (5.4 µM) + BAP (0.44 µM)	*In vitro* propagation	Huang and Huang (1995)
B. vulgaris	Nodal explants	MS	** BAP (5.0 mg l^{-1}) *** BAP (5.0 mg l^{-1}) + NAA (4.0 mg l^{-1})	Mass multiplication	Arya et al. (2002b)
B. vulgaris	Nodal buds	MS	** BAP (1.0 mg l^{-1}) *** BAP (1.0-5.0 mg l^{-1}) + ½ MS + NAA (1.0-3.0 mg l^{-1}) + IBA (1.0-5.0 mg l^{-1})	Mass propagation	Islam and Rahman (2005)
B. vulgaris var *striata*	Nodal buds	MS	** BAP (1.0 mg l^{-1}) *** BAP (1.0-5.0 mg l^{-1}) + ½ MS + NAA (1.0-3.0 mg l^{-1}) + IBA (1.0-5.0 mg l^{-1})	Mass propagation	Islam and Rahman (2005)
B. vulgaris	Nodal explants	MMS	* BAP (2.0 mg l^{-1}) *** BAP (2.0 mg l^{-1}) + IBA (20.0 mg l^{-1})	Clonal multiplication	Ndiaye et al. (2006)
B. vulgaris 'striata'	Axillary buds (single node stem segments) from field grown culms plant	MS	** BAP (2.0 mg l^{-1}) *** BAP (4.0 mg l^{-1}) + IBA (3.0 mg l^{-1}) + IBA (3.0 mg l^{-1}) + TDZ (0.5 mg l^{-1})	*In vitro* shoot proliferation and rooting	Ramanayake et al. (2006)
Dendrocalamus asper	Stem cuttings	MS	** BAP (0-2.0 mg l^{-1}) + CW (0-20.0 mg l^{-1}) *** BAP (5.0 mg l^{-1}) + NAA (0.5 mg l^{-1})	*In vitro* culture	Suwannamek (1992)
D. asper	Small branch cuttings	MS	*** BAP (3×10^{-5}M)	*In vitro* culture	Vongvijitra (1988)
D. asper	Nodal segments	MS	** BAP (0.1-15.0 mg l^{-1}) * BAP (1.0-10.0 mg l^{-1}) *** BAP (3.0 mg l^{-1}) • NAA (3.0 mg l^{-1}) • IBA (10.0 mg l^{-1})	Large scale plantlets production	Arya et al. (2002 a and b)

Table 1b. Contd.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
D. asper	Explants from immature and mature inflorescence	MS	*** BAP (3.0 mg l⁻¹) ** BAP (1.0-4.0 mg l⁻¹) + NAA (2.0 mg l⁻¹) + IBA (10.0 mg l⁻¹)	Shoot multiplication	Arya et al. (2008)
D. giganteus	Nodal explants	MS	** BAP (2.0-5.0 mg l⁻¹) *** Kin (10.0μM) + BAP (10.0μM)	Mass multiplication	Arya et al. (2002b)
D. giganteus	Nodal segments collected from young shoot of mature culm	MS	** BAP (30.0μM) *** BAP (20.0μM) + IBA (25.0μM) + BAP (0.05μM)	Rapid multiplication	Arya et al. (2006)
D. giganteus	Explants derived from a 70-yr- old adult field clump	MS	••• 2,4-D (33.9 μM) + NAA (16.1 μM) xx 2,4-D (33.9 μM) + NAA (40.3 μM)	Organogenesis	Ramanayake and Wanniarachchi (2003)
D. giganteus	Single node segments of secondary branches were collected from a 5-yr- old field grown clump	MS	** BAP (2.0 mg l⁻¹) + Kn (0.1 mg l⁻¹) + Benlate (1.0 gm l⁻¹) *** BAP (6.0 mg l⁻¹) + Kn (0.1 mg l⁻¹) + CW 8.0% + IBA (3.0 mg l⁻¹) + Major salts half + IBA (3.0 mg l⁻¹) + Coumarin (10.0 mg l⁻¹)	Micropropagation	Ramanayake and Yakandawala (1997)
D. giganteus	Axillary shoots from adult field culms	MS	+ IBA + TDZ + Coumarin	Root induction	Ramanayake et al. (2008)
D. hamiltonii	Epicotyl tissue	MS	*** BAP (4.4 μM) • BAP (4.4 μM); BAP (22.2 μM)	Micropropagation and *in vitro* flowering	Chambers et al. (1991)
D. hamiltonii	Sprouting buds of nodal explants	MS	# BAP (1.0 mg l⁻¹) + 2,4-D (1.0 mg l⁻¹) # # ½ MS+BAP(1.0mg l⁻¹) # # # BAP (1.0 mg l⁻¹) + 2,4-D (1.0 mg l⁻¹) + Basal + 8% sucrose	Somatic embryogenesis and plantlet formation	Godbole et al. (2002)
D. hamiltonii	Seedlings	jnjnjnjnM½ MS	** ½ MS + BAP (0.5-5.0 mg l⁻¹) *** ½ MS (2.5 mg l⁻¹) * BAP and 2,4-D (1.0 mg l⁻¹ each) # # # BAP and 2,4-D (1.0 mg l⁻¹ each)	Large scale *in vitro* propagation	Sood et al. (2002)
D. hamiltonii	Nodal explants	MS	** BAP (2.0-12.0 μM), NAA (10.0 μM) *** BAP (8.0 μM) + NAA 1.0 μM) • IBA (100 μM)	*In vitro* regeneration	Agnihotri and Nandi (2009)

Table 1b. Continued.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Result	Reference
D. hookeri	Axillary shoots from field culms	MS	+ IBA + TDZ + Coumarin	Root induction	Ramanayake et al. (2008)
D. latiflorus	Albino mutant inflorescence	MS	x TDZ (0.45 μM) + NAA (26.82 μM) ** 2,4-D (4.52 μM) + Picloram (4.14 μM)	Albino inflorescence proliferation	Lin et al. (2006)
D. longispathus	Single nodal segments and thin sections of internodes	MS B5	** BAP (12.0 μM) + Kn (3.0 μM) *** BAP (15.0 μM) + IBA (1.0 μM) + CW 10% + IAA (1.0 μM) + IBA (1.0 μM) + Coumarin (68.0 μM) # # 2,4-D (0.1-10.0 mg l-l) + 2,4,5-T (0.1-10.0 mg l-l)	Somatic embryo and Multiplication	Saxena and Bhojwani (1993)
D. membranaceus	Nodal explants	MS	** BAP (1.0-5.0 mg l-l) + NAA (0.5 mg l-l) *** BAP (1.0-5.0 mg l-l) + NAA (0.5 mg l-l) + NAA (3.0 mg l-l) + IBA (10.0 mg l-l)	Mass multiplication	Arya et al. (2002b)
D. strictus	Stem segments	MMS	** IAA (0.5 mg l-1) + Ads (15.0 mg l-l) • IBAandNAA(1.0 mg l-l each) + 2,4-D (0.5 mg l-l) + Phloroglucinol (1.0 mg l-l)	Shoot multiplication	Chaturvedi et al. (1993)
D. strictus	In vitro grown seedlings	MS and White	* ½ MS + BAP (0 -2.0mg l-l) * White basal *** ½ MS + BAP (0.5mgl-l) + ½ MS	In vitro propagation and rhizome formation	Shirgurkar et al. (1996)
D. strictus	Nodal segments	MS and White	** BAP and Kn (0.5 mg l-l each) + CW (200 mg l-l) *** BAP and Kn (0.5 mg l-l each) * Basal medium • IBA (0.25 mg l-l)	Plant regeneration	Ravikumar et al. (1998)
D. strictus	Nodal explants	MS	** BAP (2.0-5.0 mg l-l) *** BAP (2.0-5.0 mg l-l)	Mass multiplication	Arya et al. (2002b)
Dendrocalamus sp. (6 Nos)	Single nodal segments and thin sections of internodes	MS B5	** BAP (12.0 μM) + Kn (3.0 μM) *** BAP (15.0 μM) + IBA (1.0 μM) + CW 10% + IAA (1.0 μM) + IBA (1.0 μM) + Coumarin (68.0 μM) # # 2,4-D (0.1-10.0 mg l-l) +2,4,5-T(0.1-10.0mg l-l)	Somatic embryo and Multiplication	Saxena and Bhojwani (1993)
Guadua angustifolia	Nodal explants	MS	** BAP (3.0 mg l-l) *** BAP (3.0mg l-l) + BAP (3.0 mg l-l)	In vitro propagation	Jiménez et al. (2006)
P. meyeri	In vitro node culture	Modified MS (MMS)	* ½ MMS + Plant Preservative Mixture 0.1% + ½ MMS	Clonal propagation	Ogita et al. (2008)
Thyrsostachys oliveri	Small branch cuttings	MS	*** BAP (3×10-5M)	In vitro culture	Vongvijitra (1988)

Table 1b. Continued.

Species	Explant source	Basal medium used	Growth regulators (as indicated)	Results	References
T. oliveri	Nodal buds	MS	** BAP (1.0 mg l^{-l}) *** BAP (1.0-5.0 mg l^{-l}) + ½ MS + NAA (1.0-3.0 mg l^{-l}) + IBA (1.0-5.0 mg l^{-l})	Mass propagation	Islam and Rahman (2005)

*, Embryo germination; **, shoot bud initiation; ***, shoot multiplication; #, callus formation; # #, somatic embryogenesis; # # #, embryoids formation; •, *in vitro* flowering; ••, rhizome formationl; •••, organogenesis; +, rooting; ++, hardening; + + +, protoplast isolation and culture; x, inflorescence proliferation; xx, cell suspension culture.

of *in vitro* mass propagation. However, tissue culture protocols from mature tissues of bamboo were very limited to only few species.

Multiple shoots were produced from mature tissue explants (more than 20 years) of *B. arundinacea* (Arya et al., 2002b); *Bambusa balcooa* (Das and Pal, 2005; Dutta Mudoi and Borthakur, 2009); *D. giganteus* (Arya et al., 2006); *D. strictus* and *B. vulgaris* (Nadgir et al., 1984) and *D. hamiltonii* (Godbole et al., 2002) in BAP supplemented MS medium. 15 to 20 years old tissues of *D. hamiltonii* (Agnihotri and Nandi, 2009) resulted shoot multiplication on combination of BAP and α-naphthalene acetic acid (NAA) fortified MS medium, whereas, *B. vulgaris* (Ndiaye et al., 2006) on only BAP enriched medium. From 8 to 10 years old mature tissues, *in vitro* shoot regeneration of *D. strictus* (Chaturvedi et al., 1993) was observed on modified MS of indole-3-acetic acid (IAA) and Ads supplemented medium. In *D. strictus* (Ravikumar et al., 1998) combination of BAP and Kinetin that is, 6-furfurylaminopurine (Kn) was responsible; but in *B. nutans* (Yasodha et al., 2008) only BAP was responsible for shoot multiplication. Moreover *Boletus edulis* (Lin et al., 2004) showed shoot multiplication either on BAP, NAA or TDZ and 2-4-dichlorophenoxyacetic acid (2, 4-D) supplemented MS medium.

Nodal explants (1 to 5 years) of *B. balcooa, B. nutans, Bambusa salarkhanii, B. vulgaris, B. vulgaris* var *striata, Thyrsostachys oliveri* (Islam and Rahman, 2005); *Bambusa bambos* (Arya and Sharma, 1998); *D. hamiltonii* (Chambers et al.1991; Sood et al. 2002); *Guadua angustifolia* (Jiménez et al., 2006) was cultured on BAP fortified MS medium for shoot proliferation, but, *B. vulgaris* (Ramanayake et al., 2006) exhibited shoot multiplication on BAP and IBA, and *D. giganteus* (Ramanayake and Yakandawala, 1997) on BAP, Kn, Benlate and coconut water (CW) supplemented MS medium. Thereafter, Ogita et al. (2008) reported multiple shoot formation of *Phyllostachys meyeri* on Plant Preservative Mixture (PPM) enriched half strength of modified MS medium. Mehta et al. (2010) established multiple shoots from nodal segments of *B. nutans* on BAP supplemented MS medium. Single node segments from 5-year old *B. nutans* were cultured in BAP, Kn and IBA fortified MS medium (Negi and Saxena, 2011).

Induction of roots

Roots were induced on shoots within 20 to 45 days of explanting period either on auxin or on a combination of both auxin and cytokinin enriched medium. For successful root induction, apart from the optimum concentration of growth regulators, selection of appropriate size of shoot propagule was also more important factor. A propagule of two to three shoots should be selected from profusely growing healthy multiple shoots having1.0 to 2.0 cm in length. Longer shoots (> 2.0 cm) with folded leaf lamina showed a lower rooting percentage. Placing of single shoot in rooting media, failed to induce root formation. Similar to our case, Arya et al. (2002a) reported that a propagule of three shoots (1 to 2 cm long) was the best for root induction of *D. asper*.

Induction of roots was observed on zygotic embryo derived shoots of IBA supplemented medium in *B. arundinacea* and *D. brandisii* (Nadguada et al., 1990); *B. nutans* (Yasodha et al., 1997); *D. asper* (Arya et al., 1999) and *D. membranaceus* (Yasodha et al., 1997). IAA, NAA and indole-3-propionic acid (IPA) enriched medium was also responsible for root induction in *B. tulda* (Saxena, 1990) and *D. strictus* (Mascarenhas et al., 1988). Gibberellic acid (GA$_3$) was also suitable for induction of rooting in *B. vulgaris, D. giganteus* and *D. strictus* (Rout and Das, 1994).

Moreover, NAA treatments were favourable for promoting rooting on shoots of mature tissue derived bamboos viz. *B. arundinacea* (Arya et al., 2002b); *B. balcooa* (Islam and Rahman, 2005); *B. bambos* (Arya and Sharma, 1998); *B. edulis* (Lin et al., 2003a, 2005); *Bambusa oldhamii* (Lin et al., 2007); *Bambusa ventricosa* (Huang and Huang, 1995); *B. vulgaris* (Arya et al., 2002b); *D. asper* (Suwannamek, 1992; Arya et al., 2008); *Dendrocalamus latiflorus* (Lin et al., 2006); *D. membranaceus* (Arya et al., 2002b) and in *T. oliveri* (Islam and Rahman, 2005).

Similarly, IBA treatments were used for promoting rooting on shoots of mature tissue derived bamboos viz. *B. balcooa* (Das and Pal, 2005; Islam and Rahman, 2005); *Bambusa glaucescens* (Shirin and Rana, 2007); *B. nutans, B. salarkhanii, B. vulgaris* var *striata* (Islam and

Rahman, 2005); *B. vulgaris* (Islam and Rahman, 2005; Ndiaye et al., 2006); *D. asper* (Arya et al., 2008); *D. brandisii* (Mukunthakumar et al., 1999); *D. giganteus* (Ramanayake and Yakandawala, 1997); *D. hamiltonii* (Agnihotri et al., 2009); *D. latiflorus* (Lin et al., 2006); *D. membranaceus* (Arya et al., 2002b) and *T. oliveri* (Islam and Rahman, 2005).

A combination of TDZ and 2,4-D in *B. edulis* (Lin et al., 2004); NAA and 2,4-D in *B. edulis* (Lin et al., 2005); NAA and IBA in *B. edulis* (Lin et al., 2005); IBA and Glucose in *B. nutans* (Yasodha et al., 2008); BAP and NAA in *B. ventricosa* (Huang and Huang, 1995) and *B. balcooa* (Dutta Mudoi and Borthakur, 2009); IBA and TDZ in *B. vulgaris* 'striata' (Ramanayake et al., 2006); IBA and BAP in *D. giganteus* (Arya et al., 2006) and IBA and Coumarin in *D. giganteus* (Ramanayake and Yakandawala, 1997) promoted rooting of bamboo shoots. Use of combination of IAA, IBA and Coumarin in *D. longispathus* Kurz (Saxena and Bhojwani, 1993) enhanced rooting in this species. Moreover, more than 20 years old explants of *B. atra, D. hookeri* and *D. giganteus* (Ramanayake et al., 2008) induced rooting on MS medium while incorporated with IBA, TDZ and Coumarin. Addition of GA_3 in *B. vulgaris and D. strictus* (Rout and Das, 1994); TDZ in *B. edulis* (Lin et al., 2004); 2,4-D in *B. edulis* (Lin et al., 2003a); Coumarin in *B. tulda* (Mishra et al. 2008) and BAP in *G. angustifolia* (Jiménez et al., 2006) singularly enhanced rooting in these bamboo species.

Though majority of workers observed root induction in either singular or combined hormonal treatments, yet few workers also obtained rooting on hormone free basal medium e. g. in *B. atra* (Ramanayake et al., 2008); *D. hamiltonii* (Godbole et al., 2002); *D. strictus* (Shirgurkar et al., 1996) and in *P. meyeri* (Ogita et al., 2008).

Rooting percentage (%)

All the treatments with plant growth regulators promoted rooting; however, variations were observed among the treatments for percent rooting of microcuttings. Hundred per cent root induction was not observed in most of the bamboo species. It was 20% (Nadgir et al., 1984); 80% (Mascarenhas et al., 1988); 50% (Shirgurkar et al., 1996) and 85-90% (Ravikumar et al., 1998) in *D. strictus.* It was reported as 80 to 85% (Arya et al., 2002b); 45.85% (Ndiaye et al., 2006) and 92% (Ramanayake et al., 2006) in *B. vulgaris*. It was 27.8% in *P. meyeri* (Ogita et al., 2008); 80 to 85% in *B. arundinacea* and *D. asper* (Arya et al., 2002a, 2002b). Moreover, it was observed as 80.3% in *B. edulis* (Lin et al., 2003a) and 25 to 30% (Sood et al., 2002) rooting recorded in *D. hamiltonii.* It was 80 to 85% (Arya et al., 2002b) and 73% (Yasodha et al., 2008) in *D. membranaceus.* Rooting percentage was observed as 30% in *B. glaucescens* (Shirin and Rana, 2007); 70% in *D. longispathus* (Saxena and Bhojwani, 1993); 77.5% in *D. giganteus* (Ramanayake and Yakandawala, 1997);

68% in *B. nutans* (Yasodha et al., 2008) and 75% in *B. balcooa* (Dutta Mudoi and Borthakur, 2009).

However, 100% rooting percentage was reported in *G. angustifolia* (Jiménez et al., 2006); *B. bambos* var. *gigantea* (Kapoor and Rao, 2006) and in *B. nutans* (Negi and Saxena, 2011).

SOMATIC EMBRYOGENESIS

Somatic embryogenesis is a process whereby somatic cells differentiate into somatic embryos morphologically. Plant cells can be induced to give rise to somatic embryos. Tissues which were not committed to an embryogenic pathway can be induced to have embryonic determination by exposure to an auxin. Somatic embryogenesis can provide a convenient and dependable source for obtaining plants for bamboo. To get somatic embryonic callus, explant selection is the most crucial factor. Calli have been raised from various explants, viz. seeds, seedlings, isolated embryos, roots, shoot apices, leaf explants, internodes and anthers.

Somatic embryogenesis in bamboo was reported for the first time from the zygotic embryo by Mehta et al. (1982) in *B. arundinacea.* Later on, many workers used zygotic embryo for inducing somatic embryogenesis and plantlet regeneration in *B. vulgaris* and *D. giganteus* (Rout and Das, 1994); in *D. hamiltonii* (Zhang et al., 2010); in *D. strictus* (Zamora et al., 1988; Rout and Das 1994; Saxena and Dhawan, 1999); in *Otatea acuminate aztecorum* (Woods et al., 1992) and in *S. latiflora* (Yeh and Chang, 1987).

Mature tissues from nodal and internodal region of *B. edulis* (Lin et al., 2004); *D. longispathus* (Saxena and Bhojwani, 1993) and roots of *B. beecheyana* (Chang and Lan, 1995) were responsible for somatic embryogenesis and plantlet formation. From nodal explants of *G. angustifolia* (Jiménez et al., 2006); *D. hamiltonii* (Godbole et al., 2002) and floral parts of *B. balcooa* (Gillis et al., 2007); *B. beecheyana* (Yeh and Chang, 1986b); *B. oldhamii* (Yeh and Chang, 1986a; Kanyaratt, 1991); *D. asper* (Kanyaratt, 1991); *D. latiflorus* (Kanyaratt, 1991) and *S. latiflora* (Tsay et al., 1990) somatic embryogenesis and plantlet regeneration was recorded. From young and tender shoots of *Phyllostachys nigra* (Ogita, 2005) and *P. viridis* (Hassan and Debergh, 1987) embryogenesis and plantlet development was observed. From leaf explant of *B. glaucescens* (Jullien and Van 1994); *P. viridis* (Anas et al., 1987) and seedlings of *D. hamiltonii* (Sood et al., 2002) embryogenesis and large scale plant regeneration was noticed. Embryogenesis and haploid plant regeneration was observed from anther of *S. latiflora* (Tsay et al., 1990).

Sprouted buds of *B. nutans* induced somatic embryogenesis under dark incubations with maturation and germination of well organized somatic embryos (Mehta et al., 2010). Nodal segments from secondary

branches of saplings of *Phyllostachys bambusoides* were inoculated in MS medium to assess the *in vitro* morphogenic response of leaf sheath. 4-amino-3, 5, 6-trichloropicolinic acid (Picloram) induced the callogenesis in leaf sheath of *P. bambusoides*. Although, the primary and secondary somatic embryo induction was observed from the callus, the plant development did not occur (Komatsu et al., 2011).

Organogenesis and callus culture

Organogenesis occurred in nodal explants of *B. vulgaris* and 70 years old adult field clump of *D. giganteus* (Ramanayake and Wanniarachchi, 2003). Callus culture was observed from lateral shoots of *Bambusa multiplex, B. oldhamii, Pentachaeta aurea and Sasa pygmaea* (Huang and Murashige, 1983). Moreover, callusing and rhizogenesis was induced in *B. ventricosa, Schizostachyum brachycladum* and *Thyrsostachys siamensis* (Dekkers et al., 1987) from culm internode and culm sheath base.

Cell suspension culture

A cell suspension culture system offered many advantages for examining the metabolic role of nutrients in plants of interest and for its industrial utilization. Huang et al. (1988) reported suspension cultures of *B. multiplex, B. oldhamii, P. aurea, Sasa pygmaea* and Ogita (2005) of *P. nigra*. Moreover, cell suspension culture was recorded from apical bud and nodal lateral bud explants of *B. edulis* and *oldhamii* and nodal explants of *D. giganteus* (Ramanayake and Wanniarachchi, 2003).

Artificial seed

Artificial seed production was noticed from somatic embryo of *D. strictus* (Mukunthakumar and Mathur, 1992).

IN VITRO FLOWERING

Flowering in bamboo is a botanical enigma and there is no scientific method yet developed for predicting flowering. Till now, the exact physiological mechanism or ecological factors responsible for bamboo flowering is not known precisely. Generally in most cases bamboo flowering is recorded at long infrequent intervals, which occurs two to three times in a century. There are few hypotheses propounded by experts like:

a. Parental competition,
b. Consumer satiation, this suggests that bamboo produce large quantity of seeds and storage of food reserve takes long time, and
c. Climatic periodicity, it said that flowering of bamboo is associated with climatic factors like drought.

This assumption is similar to the climatic periodicity hypothesis where bamboo flowering was mentioned to be associated with climatic factors like drought. Drought may trigger mast flowering. Since in most cases bamboo plants die after flowering, it is hypothesized that there may be genes that are involved in programmed flowering followed by cell death. Moreover, this situation may create a competition among the shoot clumps for survival, for which flowering may be formed for generation of offspring.

The first report on *in vitro* flowering of bamboo from axillary shoot cultures of *D. strictus, D. brandisii* and *B. arundinacea* (*B. bambos*), which developed viable spikelets and produced fertile seeds caused great excitement (Nadgauda et al., 1990). Since then *in vitro* flowering was observed in many varieties of bamboos, generated from seedlings viz. *D. hamiltonii* (Chambers et al., 1991); *B. arundinacea* (Ansari et al., 1996); and from shoots of somatic embryonic sources of *D. giganteus, D. strictus and B. vulgaris* (Rout and Das, 1994; Arya and Sharma, 1998). Later on, *in vitro* flowering was reported from field culm source of axillary shoot cultures of *B. bambos* (Arya and Sharma, 1998); *B. edulis* (Lin et al., 2003a, 2003b, 2004, 2007); and *B. balcooa* (Dutta Mudoi and Borthakur, 2012) etc.

Survivability in soil after hardening

One of the most important and critical steps in field transfer of micropropagated plants in their transition during hardening from *in vitro* to an *ex vitro* environment, and subsequent field performance is survivability in soil after hardening. Poor survival of a plant under *ex vitro* conditions is mainly due to poor development of cuticular waxes, non functional stomata, water loss due to poor excessive transpiration, poor root system and susceptibility to pathogens (Ziv, 1995). In course of hardening, the micropropagated plants, gradually overcome these inadequacies and adapt to *ex vitro* conditions.

After due hardening, different bamboo species were transplanted into soil and they achieved different survival rate. It was 80 to 90% (Arya et al., 2002b); 80% (Islam and Rahman, 2005) and 80 to 90% in *B. arundinacea, D. asper* and *D. membranaceus* (Arya et al., 2002a, b). Similarly, 85% survivability recorded in *G. angustifolia* (Jiménez et al., 2006); and 80 to 85% in *B. bambos* var. *gigantea* (Kapoor and Rao, 2006). Moreover, in *D. hamiltonii,* it was 80 to 85% (Sood et al., 2002); 78% (Godbole et al., 2002) and 85% (Agnihotri and Nandi, 2009). About 80% survival rates recorded in *B. nutans, B. salarkhanii, B. vulgaris* var *striata* and *T. oliveri* (Islam and

and Rahman, 2005). Accordingly, 80% (Mascarenhas et al., 1988) and 90% (Shirgurkar et al., 1996) survivability was noticed in *D. strictus*. However, 100% field survivability was recorded in *P. viridis* (Hassan and Debergh, 1987) in *B. vulgaris* (Ndiaye et al. 2006; Ramanayake et al., 2006) in *B. balcooa* (Gillis et al., 2007) in *B. edulis* (Lin et al., 2003a) and in *B. nutans* (Negi and Saxena, 2011).

MACROPROLIFERATION

Bamboo seedlings possess the capacity to proliferate. By cutting the rhizome into pieces, each with roots and shoots, each seedling can be multiplied three to seven times depending on species. Generally, this method is only suitable for species producing seeds. After successful acclimatization, macroproliferation can be very suitably adapted for well established bamboo plantlets after three to five months of transfer of the micro plants. By splitting the rooted tillers, it was possible to increase the production up to three times. This process can be continued for a number of years. By recycling of the macro-proliferation procedure, continuous plantlet production resulted. Proliferated plantlets were small in size, hence easy to handle and transport. A small initial stock could produce large numbers of plants. This is a bonus point of this experiment. Thereafter, plantlets were transferred to field condition.

Banik (1987) developed this technique for bamboo proliferation via rhizome separation. Likewise, Kumar (1994) multiplied *B. arundinacea*, *B. tulda*, *D. hamiltonii* and *D. strictus* through macroproliferation. Similarly, Singh (1995) also conducted macroproliferation of *D. hamiltonii* and this was recorded as one of the most dependable, simple and quick technique for developing large number of propagules. Dutta Mudoi and Borthakur (2009) also performed this experiment successfully in *B. balcooa* and increased the production up to three times. Recycling the macroproliferation procedure resulted in continuous plantlet production in *B. balcooa*.

FIELD PERFORMANCE OF MICROPROPAGATED BAMBOO

Field evaluation of tissue-culture raised plants and their performance (morphological and physiological evaluation) is important for long-term assessment and commercial applications. The tissue-culture raised bamboo plantlets are being assessed in terms of the height of the plant, diameter and number of culms produced. The field performance data of bamboo are being compared with those from seed-raised check plants of the same age. Bamboo culm formation was observed within one year in micropropagated plants compared with two years in seedling-derived plants. In

plantlets derived from tissue culture, the height of the culm, the number of culms per plant, the number of nodes of the main culm, and girth of the second internodes were nearly double those of seedling-derived plants (Mascarenhas et al., 1988).

Sood et al. (2002) recorded field performance of the tissue culture raised six-year-old bamboo of *D. hamiltonii*. The observations pertaining to shoot number (total culms produced), height of culms and thickness of culms at third internode from the base were made in October for six years on yearly basis. The field performance of *D. hamiltonii* was also studied by recording plant height, number of culms and leaf characteristics within one year and six months of field transfer. Plant height was found to be significantly increased with time and almost six-fold increment was observed.

CLONAL FIDELITY

Identification of somatic clones of plants derived through tissue, with respect to their trueness to their mother or between themselves can be done in various ways. Use of highly discriminatory methods for the identification and characterization of genotypes in this respect is very much essential. Identification of somatic clones derived from tissue culture raised plantlets, with respect to their trueness to their mother or between them can be done in different way. The use of highly discriminatory methods for the identification and characterization of genotypes is essential for breeding programmes. In the last ten years, several generations of molecular markers become increasingly precise, this requires a new approach every time. Several cytological and molecular markers have been used to detect the variation and/or confirm the genetic fidelity in micropropagated plants using allo-zymes, random amplified polymorphic DNA (RAPD), microsatellites or simple sequences repeats (SSR), inter simple sequences repeats (ISSR) etc. The big boom of molecular markers came with polymerase chain reaction (PCR)-technology with RAPD and related techniques (Gielis et al., 1997a). RAPD have been used in *Yushania* (Hsiao and Riesebergh, 1994) and *Phyllostachys* (Gielis et al., 1997b). The need for assessing genetic stability in bamboo tissue culture using molecular techniques had highlighted by Gielis et al. (2002).

Primers of ISSR (14 to 22 bp), longer than RAPD primers (10 bp), have higher annealing temperatures. Higher annealing temperatures mean more stringent primer annealing conditions, which lead to greater consistency; however, a low annealing temperature may increase non-specific amplification, leading to artifact bands. Since simple sequence based repeat primers target the fast evolving hypervariable sequences (Tautz, 1989), ISSR markers are considered suitable to detect variations or ascertain clonal fidelity among tissue culture produced plants.

Earlier, Das and Pal (2005) had reported about the establishment of clonal fidelity of regenerants of *B. tulda* and *B. balcooa* by random amplified polymorphic DNA (RAPD) analysis. It was also well established that as compared to RAPD, ISSR marker assay reveals larger number of polymorphic fragments per primer than RAPD because of the occurrence of abundant SSR regions. A study was conducted by Negi and Saxena (2011) to screen the tissue culture raised plantlets of *B. balcooa* for somaclonal variation by employing ISSR marker assay. This study had stated that there was no variability found among the tissue culture raised plantlets by the ISSR marker assay; therefore they said that the ISSR markers are highly efficient to ascertain the clonal fidelity of tissue culture raised progenies of *B. balcooa.* They also stated that the *in vitro* clonal propagation using preformed organs such as axillary buds circumvents de-differentiation or re-differentiation of cells or tissues, avoiding genomic aberrations and consequently maintaining the clonal fidelity of *in vitro* raised plantlets of *B. balcooa.*

ISSR had confirmed the genetic uniformity of the tissue culture raised plants up to 27 passages in *B. nutans* (Negi and Saxena, 2011). This result corroborated the observation of Mehta et al. (2010).

CONCLUSION AND FUTURE PROSPECTS

Bamboo is one of the most valuable forest plants nature has given to mankind, but to exploit its full potential, more fundamental research is needed urgently to lay the foundations for the future. It has been realized long back that *in vitro* propagation is essential to meet the ever-increasing demand of planting stock of bamboo. In this respect, basic research on bamboo tissue culture for production of quality propagules should be encouraged.

For mass propagation of bamboo, micropropagation is the only technique. According to Gielis and Oprins (2002), micropropagation is the best available technique and will become the standard for mass scale propagation of bamboo in the near future. It is now possible to micropropagate almost any selected bamboo at mass scale in a short time frame. In order to develop appropriate micropropagation systems, further research is clearly required on propagation techniques to increase the multiplication rates of bamboo. Tissue culture protocols based on juvenile tissue explants of bamboo have the advantages of having greater number of genotypes in culture, from where propagation may precede ensuring greater diversity of the species. While, mature tissue explants derived *in vitro* protocols of bamboo have the advantage of propagation of identified superior genotypes, establishment of callus cultures and obtention of plants regenerated from calli or cell suspensions via somatic embryogenesis hold a potential for the production of novel somaclonal variants of bamboo.

Though, *in vitro* flowering from numbers of bamboo species was reported, practical and commer-cially exploitable results have not been reported yet. Factors that trigger flowering in bamboo are still not clear. As it is not possible to observe the cyclic nature of flowering due to their long life spans, so it can be taken as a new area for study of the breeding of bamboo as *in vitro* flowering can open up the possibility of controlled flowering. Although, many hurdles still need to be taken before the methods really become applicable at agricul-tural scales.

Furthermore, the hardening and acclimatization are two important aspects for micro propagated bamboo species. It is observed that multiplication via tissue culture, if followed by macro proliferation, could further increase the rate of propagation of bamboo plants. Though a large numbers of reports are available regarding micro propagation of bamboo; yet a very few of them have studied up to macro proliferation and field performance study. It is necessary to define the plant quality standard to characterize the *in vitro* plant quality and to estimate the establishment of plants in an accurate and economic way. Moreover, a suitable quality control strategy is necessary to ensure the performance of the bamboo plants and the genetic fidelity of clonal planting stocks. The lack of reports on ascertaining the genetic fidelity of tissue culture raised plantlets could lead to serious con-sequences, especially in perennials like bamboo where any undesirable variant would last for several years. Several areas of work in the tissue culture of bamboo have opened up new vistas and much progress could be expected in the future.

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