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Research Article

Micropropagation of bamboo (*Bambusa vulgaris*) through nodal segment

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ABSTRACT : Worldwide interest in bamboo as a source of biomass in sustainable agriculture and agro forestry system has increased rapidly in recent years. An efficient and reproducible procedure for the large scale propagation of *Bambusa vulgaris* is described. Nodal segments from field grown culms were used as explants to develop a method of *in vitro* regeneration in *Bambusa vulgaris*. Optimum bud spread development was after 25 – 28 days cultivation of Murashige and Skoog medium supplemented with 2.5 mg/ l of BAP (Benzyl Amino Purine) and 2.5 mg/l kinetin. The MS medium supplemented with 7.5 mg /l IBA (Indole Butyric Acid) was most suitable for rooting of shoots. The *in vitro* regenerated plantlets, after hardening and acclimatization, showed 80% survival when transferred to the field.

KEY WORDS : Bamboo, Micropropagation, Tissue culture

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INTRODUCTION

Bamboo (*Bambusa vulgaris*) is a rhizomatous plant. The genus of bamboo such as *Arundinaria, oxytenanthera, Oreobambos* and Bamboos are present through out the continent under rainfall ranging from 700 to 1500 mm (Sene, 1998) mass utilization of bamboo resources for paper and pulp industries, inadequate management practice, interference from biotic factors such as grazing and browsing, forest fires and over exploitation by the rural and tribal communities for domestic and commercial use are some of the major factors contributing to the scarcity of bamboos in India (Shirin and Rana, 2007).

The production of seeds is irregular as flowering occurs on culms of 100- year-old plants. Like other bamboo species it is also known to be monocarpic, *i.e.* flowering once before culm death. Regeneration of *Bambusa vulgaris* by seeds is impossible due to lack of flowering. Vegetative propagation is commonly practiced in bamboo cultivation but the plants developed will all be as old as their stock and will tend to flower and die simultaneously as the actual age is the same in every part of the bamboo. Propagation through tissue culture seems to be most effective. Micropropagation of bamboos has allowed to develop a new type of ornamental bamboos that can be year round with a high quality and distributed more widely than classically propagated ornamental bamboos.

Micropropagation using tissue culture techniques offers substantial advantage over other methods. The plant material can be multiplied rapidly on a large scale using explants from physiologically young, field tested elite plants. Explants for bamboo species could be taken from Juvenile materials as zygotic material (or) it could be from adult (or) mature

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material as node, leaf, sheath (Gielies, 1995). *In vitro* propagation of *Dendocalamus hametonii* using nodal explants from elite seedlings of proven field performance was investigated by Tsay *et al.* (1990). Prutpongse and Gavinlertvatana (1992) successfully micro propagated *in vitro* 54 bamboo species including *Dendocalamus giganteus* was achieved from node segments cultured on MS medium containing 5.0 mg/l BA and 0.16 mg/l NAA. Ndiaye *et al.* (2006). Who developed procedures for the regeneration of *Bambusa vulgaris* using nodal segments on modified Murashige and Skoog (MMS) medium supplemented with 2 mg/l of BAP. A synergistic effect of the two cytokinin was observed and the best interaction giving the highest rate of shoot multiplication was obtained for a combination of 5µM BA and 15µM kinetin using nodal segments of *Bambusa glaucescens* (Shirin and Rana, 2007). Recently, Diab and Mohamed (2008) also reported the highest shoot multiplication was achieved on six week on MS medium with 5.0 mg/l BA an 0.2 mg/l NAA.

In the present study an attempt has been made to develop an efficient *in vitro* micropropagation method from excised zygotic embryos as well as from nodal explants taken from 1year old plant of *Bambusa vulgaris*.

EXPERIMENTAL METHODS

Seed germination:

Seeds were carefully dehusked and wetted with soft detergent solution for 10 minutes and then washed with tap water. Thereafter, the seeds were rinsed in distilled water and imbibed for hours. Finally they were surface disinfested with mercuric chloride 0.1% for 7 minutes, rinsed three times with sterilized distilled water and cultures in Petriplates containing 30 ml of plant growth regulator free Murashige and Skoog basal medium supplemented with sucrose 30 g, myo - inositol 100 mg/l, thiamine Hcl 0.1 mg/l and nicotinic acid 0.5 mg/l. the pH was adjusted to 5.7 to 5.8 with Agar Agar and autoclaved at 121^0C for 20 minutes. The Petridish containing ten seeds per dish were incubated at 25 ± 2^0C in the dark (Niladri Bag *et al*., 2000).

Multiple shoot formation from excised embryos:

After 8 days of incubation in the dark, Petridishes containing germinated seeds were placed under white fluorescent light for 4 days and the emerging shoots were allowed to grow to a height of 5-6 mm. At this stage the germinating embryos were carefully excised under aseptic conditions and further cultured in 250 ml conical flack containing 60 ml of MS medium supplemented with 2% sucrose with benzyl amino purine and kinetin in various combinations. After 8 weeks of culture the number of explants responding to various treatment, rate of shoot multiplication and the length of the shoot were recorded.

Bud sprouting from nodal segments:

Single node segment (1.5 – 2.5 mm in diameter, 20 -25 mm long) taken from a one year old seedling were used for this experiment. Nodal segments with healthy axillary buds, with sufficient internodal portion on either side, were cultured on ½ strength MS medium after removal of the leaf sheath and surface disinfestation with 0.1% mercuric chloride. After one week, infection free explants were transferred to the multiplication medium containing BAP and IBA and incubated for 12-15 days. The sprouted buds were then excised from nodal explants and cultured on the same medium for multiplication.

Rooting:

Clumps of three to four microshoots (3.5 – 4.0 cm) in height were separated from the mother clump and placed on ½ strength MS medium supplemented with 0-300 µM IBA. These shoot clamps were cultured for 14 days on medium supplemented with IBA before transfer to PGR free ½ strength MS medium. After 8 weeks of culture on this medium per cent of rooting, number of roots per clump and the length of the longest root were recorded.

Hardening and acclimatization:

The rooted shoots were taken out from the culture flasks and washed thoroughly with running tap water to remove all traces of medium attached to the roots. These plants were transplanted in plastic pots containing a mixture of soil and sand.

EXPERIMENTAL RESULTS AND ANALYSIS

The two media tested, showed significant difference in the number of shoot newly formed. Among the two medium MS medium showed highest rate of regeneration followed by MMS. Ndiaye *et al.* (2006) reported that optimal shoot growth was obtained on Modified Murashige and Skoog (MMS) medium supplemented with 2 mg/l of BAP.

Surface sterilization of nodal segments with different concentration of $HgCl_2$ and duration to standardize the duration of sterilization and concentration of $HgCl_2$. Surface sterilization of nodal segments with 0.15% $HgCl_2$ for 15 min yielded 64% aseptic cultures Increase in concentration of $HgCl_2$ (0.20%) and increase in the duration of sterilization (15 -20 min) resulted in a high frequency of aseptic cultures, but a affected adversely the percentage of bud break.

Seed germination:

With in 12days of inoculation 50% of seeds germinated on PGR - free MS medium. The excised germinating embryos

did not survive for more than 3-4 weeks on PGR free medium. The presence of PGRs (BAP and Kn) in the medium improved the survival as well as shoot multiplication. Addition of BAP (2.5 – 10 mg/l) and Kn (2.5 – 5 mg/l) to the medium, multiple shoot formation was induced from excised embryos. Among various combinations best results were recorded on medium containing 2.5 mg/l BAP and 2.5 mg/l Kn noticed 85% of explants proliferated with 12 shoots per explant in 8-10 weeks. But increased the concentrations of BAP and kinetin adversely affected the shoot multiplication rate.

Bud sprouting from nodal segment :

Nodal segment without a leap sheath, sprouted within 4-5 days of culture on ½ strength MS medium, generally two to three buds were present on a nodal explant and almost all the buds sprouted, but occasionally one (or) two remained suppressed.

After 3- 4 weeks, sprouted buds of 25 – 30 mm length were excised and cultured on medium supplemented with BAP and Kn. Highest shoot multiplication was obtained on MS medium supplemented with 2.5 mg/l BAP and 2.5 mg/l Kn (Table 1). No multiplication was observed during the first 25 – 30 days incubation of these excised buds on the multiplication medium. However, following subculture, shoot multiplication started and gradually increased with time.

Traditionally bamboos are propagated through seeds, off sets and calm cuttings. Propagation by seeds is unreliable due to the long and unpredictable flowering habit and also undesirable on account of a large variation found in the seedling population. There fore, in order to supplement the conventional methods, an efficient *in vitro* propagation method using explants taken from established and selected mature plants would offer a desirable alternative for large scale multiplication of lite bamboo (Sood *et al*., 1992; Chaturvedi *et al.*,1993; Arya and Arya, 1996; Sharma and Arya, 1998).

Rooting:

The *in vitro* multiplied shoots failed the root on a harm one free basal medium. Roots were induced in the presence of auxin (NAA (or) IBA). Among various concentrations of IBA tested, rooting (65-85 %) was observed at concentrations ranging from (5.0 – 10.0 mg/l). Maximum rooting (87%) was obtained at 7.5 mg/l IBA (Table 2). IBA has been used for rooting of other bamboo species like *Dendrocalamus strictus* (Nadgir *et al*., 1984), *Bambusa arundinacea* (Nadguada *et al*., 1990), *Dendrocalamus giganteus* and *Dendrocalamus strictus* (Das and Rout, 1991).

Thus, IBA proved to be essential for providing initial stimulus for *in vitro* rooting, while advanced root and shot growth was noticed only on transfer to auxin free MS medium. Similar results have been reported by Nadgir *et al*. (1984) in *Dendrocalamus strictus* and by Saxena and Bhojwani (1991) in *Bambusa vulgaris*. In this study, it was observed that

Table 1 : Effect of BAP and kinetin and their interaction on auxillary bud proliferation of nodal explants

BAP	Kinetin	% response of germinating embryo	No of shoots / embryo	Average length
0.0	0.0	0.0	0.0	0.0
2.5	2.5	85	12	30
5.0	2.5	70	10	32
7.5	2.5	50	8.2	26.2
10.0	2.5	50	9.0	24.5
2.5	5.0	32	3.0	20.2
5.0	5.0	45	4.8	21.6
7.5	5.0	30	3.2	22.5
10.0	5.0	52	7.5	23.4

Table 2 : Effect of IBA supplemented half strength MS medium on in vitro root induction

IBA (mg/l)	% of root induction	Number of induced per propagule	Length of root (cm)
1.0	38.15	1.90	0.76
2.5	45.82	2.12	0.92
5.0	68.65	4.26	1.60
7.5	87.12	5.16	1.54
10.0	66.50	5.15	1.14
S.E.±	6.7	0.4	0.1
C.D.	19.0	1.2	0.3
CV (%)	132.6	107.8	54.3

continuous culture on a medium with higher doses of IBA for a relatively longer period, adversely affected growth and subsequent survival of shoots. *In vitro* rooted plantlets were hardened and acclimatized prior to transplantation in the field.

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