RESEARCH ARTICLE

A Rapid *in vitro* Micro Propagation of *Bambusa Vulgaris* Using Inter- Node Explant

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ABSTRACT- *B. vulgaris (Bambusa vulgaris Schrad. ex Wendl)* has been promoted in order to solve the deforested environments and economic problems. The present experimentation was conducted at a rapid *in vitro* propagation of *Bambusa vulgaris*, commonly called as Buddha bamboo, with internode as explant. The growth had a significant effect on the development of the plants with three cytokinins tested (IAA, NAA, and 2,4 D) along with 0.3 mg/l BAP was found to be most effective in inducing bud break and multiple shoot formation. The growth hormones NAA, IAA, 2,4-D, and BAP shown effective on root and shoot formation.

Key-words- BAP, *B. vulgaris*, IAA, Internode, NAA, Plant growth hormones, 2,4-D

INTRODUCTION

Plant micropropagation is one of the most promising methods in plant biotechnology for the development of large-scale production of crops, such as bamboo species [1,2]. Bamboo is one of the fastest growing renewable resources in the world with great scope in reforestation [3]. Liquid media in the micropropagation processes was considered as an ideal solution for reducing cost of plantlet production.

Bambusa vulgaris var. wamin, commonly called as Buddha bamboo, is a native of China [4]. The plant is 4-8 m tall, ornamental bamboo with no reports on flowering [5]. Culms are usually dark green in color, have short with much swollen (pitcher shaped) internodes. Some internodes of bamboos remain in vegetative state for indefinite periods. The rate of over exploitation of various economic trees like bamboo, in the world is yielding to a bleak future of various tree plants of significant importance [6].

Production by ex-situ conservation is not yet a viable option and conservation of bamboo diversity depends upon the protection of natural habits [6,7]. Propagation through macro proliferation technique is a major breakthrough, but is again the limitation of requirement of seeds. Hence the modern method of conservation like Micropropagation provides an alternative for regeneration of new plants rapidly in plants like bamboo [8,9].

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Bambusa vulgaris var. *Striata* (Yellow bamboo) is a moderate sized bamboo with culms reaching a height of 8–20 m and a diameter of 5–10 cm. Branching is usually from mid-culm to top; nodes prominent, internodes up to 45 cm long. It is easy to propagate by culm and branch cuttings [10,11]. Cuttings taken from 1–2-year-old culms, planted in summer months may give maximum rooting. Multiple shoot production has also been reported from mature shoots in MS medium supplemented with coconut milk, kinetin and BAP. Pre-rooted rhizome and culm cuttings can also be used. Ground layering and air layering are also found successful. *Bambusa vulgaris* is used for paper-making, scaffolding, poles, fencing, curios, handicraft, edible shoots, medicine, etc [12]. Rings prepared from the split culms are put into ear perforations by the Naga tribes of Manipur. Pulp made from this species is used for mixing with hardwood pulps.

Fig. 1: *Bambusa vulgaris var striata* (Yellow variety)

MATERIALS AND METHODS

Collection of Plant Material

Healthy plant yellow bamboo spp. *(Bambusa vulgaris)* is collected from the Raja nursery Jarhabhata chowk, Bilaspur (CG), India at the green to brownish stage and the experiment was done in the Department of Microbiology and Bioinformatics, Bilaspur University, Bilaspur (CG).

Preparation of Explant

Inter-nodal region of stem were cut upto 3 inches (*Bambusa vulgaris Schrad. ex Wendl*) with sterilized blade. The upper layers of explant were scrubbed off to remove the dust and wax. The internode explant was then washed in running tap water for 10 minutes. The explant was washed with distilled water containing 1% of detergent (Tween 20) for 5 min and rinsed 2–3 times with sterile distilled water and then soaked in fungicide (Bavistin 1%) for 10 min followed by rinsing with sterile distilled water. Thereafter, the explants were surface disinfected with 70% ethanol for 1 min and rinsed 2–3 times with sterile distilled water, treated with 0.1% aqueous mercuric chloride ($HgCl_2$) for 5 min and thoroughly washed 4–5 times with sterile distilled water under aseptic condition.

Preparation of MS Media

Culture medium and growth conditions MS (Murashige and Skoog 1962) medium with 2% (w/v) sucrose was used for the present study. The medium was further amended with BAP (0.3mg/L) in combination with 3 mg/l of IAA, NAA and 2,4-D respectively. The pH of the medium was adjusted to 5.6 before gelling with 1% agar. The chemicals used in this study are prepared media (Hi-media, Qualigens and SD fine chemicals, India). Murashige and Skoog (50 ml) each was dispensed into 150 ml sterilized conical flask (Borosil) and plugged with non-absorbent cotton plug.

Storage of Prepared Media

After preparation the media were autoclaved and the left for a while to reach an ambient temperature and stored in the refrigerator at 6°C.

Volume of Culture Media used in Culture Jar

For normal propagation plantlet regeneration experiment, 20 ml of semi-solid culture media were dispensed in each conical flask.

Establishment of Shoot

Surface sterilized immature and semi-hard wood shoots were cultured on MS media with and without 0.1 % activated charcoal and the survived explants were transferred to regeneration media. Percentages of browning and survivals as well as the number of shoot buds initiated**,** the new leaves formed and callus formation was recorded over a period of 4 weeks. Then, the cultured explants were maintained inside the plant tissue culture room at 25 ± 26^0C, and 16 h photoperiod were provided by cool white fluorescent tubes. The relative humidity was 50–55%.

RESULTS AND DISCUSSION

The present experimentation on a rapid *in vitro* propagation of *Bambusa vulgaris*, commonly called as Buddha bamboo, with internode as explant was conducted in lab conditions. Table 1 represents various culture conditions, taken for *in vitro* cultivation of *Bambusa vulgaris* Schrad. ex Wendl by plant tissue culture. Inter node explants of *Bambusa vulgaris* internode survived on MS medium supplemented with IAA NAA and 2,4-D and shoot initiated at 3 weeks.

Table 1: Culture condition required for *in vitro* cultivation of *Bambusa vulgaris* Schrad. ex Wendl

Ex-plant	Temp.	Moisture	Light period	Time of regenretion
Inter node	25±2	50–55	16 hours	3 weeks

Table 2 represents the survival shoot initiation and the regeneration of explant "inter node" in MS media. In the present experimentation, *B. vulgaris* internode produced multiple shoots on MS medium supplemented with different plant growth regulators in combination. Internode explants took 25 days to initiate shoots. The type and concentration of cytokinin influenced the average number of inter node produced per explant as well as mean length of the shoots. The growth had a significant effect on the development of the plants with three cytokinins tested combined with 0.3 mg/l BAP. The reports were found to be most effective in inducing bud break and multiple shoot formation from the explants by producing maximum of (2 cm) shoot lets/explant as an average.

Table 2: Culture of explant (Internode region) on MS media in BAP (0.3mg/L) in combination with 3 mg/l of IAA (R1), NAA (R2) and 2,4-D (R3) respectively

Explant in MS Agar Media	Percentage (%) of Explant survival			Average No. of shoot initiation		
	R1	R2	R3	R1	R2	R3
Inter Node	66%	66%	66%	2/3	2/3	2/3

Note: IAA (R1), NAA (R2) and 2,4-D (R3)

Fig. 2: Shoot initiation and regeneration of internode of bamboo

Table 3: Effect of plant growth regulators on multiple shoot induction

S. No.	Plant growth regulator (mg/l)	Shoot	Root
1	NAA (3) + BAP (0.3)	++	+
2	IAA (3)+ BAP (0.3)	+ +	++
3	2,4-D (3) + BAP (0.3)	+ +	_ _

Table 3 shows the growth hormones NAA, IAA, 2,4-D 3mg/l concentration, respectively with BAP 0.3 mg/l and its effect on shoot. All the plant growth regulators showed good results in shoot regeneration. Root regeneration was found better in IAA combined with BAP. During the acclimatization phase, the *in vitro* plants showed 75% survival.

CONCLUSIONS

The present report has shown positive effect of growth in the *B. vulgaris* var. *Striata* (Yellow bamboo) by *in vitro* propagation.

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