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In vitro propagation of *Bambusa balcooa* by plant tissue culture technique

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Abstract

Bambusa balcooa is a common plant grown in Bihar, Orissa, Jharkhand and Uttarakhand states of India having good cultural importance. The present experimentation on nodal explants of *B. balcooa* on MS media supplement under particular culture conditions has shown shunted growth in 30 days. Shoot tip explants of *B. balcooa* seedlings produced multiple shoots on MS medium supplemented with different plant growth regulators (PGRs) individually and in combination. Shoot tip explants of *B. balcooa* requires 30 days to initiate shoots. Among the three cytokinins tested, BAP was selected as the most suitable hormone to induce shoot multiplication. The highest shoot multiplication is found by incorporation with BAP (1.5 µg/L and 2 µg/L). The shoot multiplication rates are good with shoot length 2.5±0.3 cm and 3.9±0.4 cm with the highest and maximum shoot generation. The shoot multiplication rate is moderate with a shoot length of 2.00±0.5 cm. Multiplication potentiality was observed in the cluster having more than 2–3 shoots. The best period for recycling multiplying shoots is 2–3 weeks in the old culture. Delaying of the sub-culturing period resulted in gradual browning of the shoots. The sub-culturing period was recorded as the most crucial factor for obtaining the optimal and desired level of regeneration of shoots. The well-grown propagules in the present experimentation were shown successful regeneration. Hence, the experiment concluded with the best growth pattern observed in media containing auxins like BAP and cytokinins like kinetin.

1. Introduction

Bamboo grows naturally in several types of forest lands and is also cultivating in many areas of India (Zhou *et al*., 2005; Kaladhar *et al*., 2017). About 50% of the total annual production of bamboo in our country is used by several industries like paper, pulp, mat boards, rayon, house construction, making baskets, bridges, coffins, beds, toys and weapons and agricultural implement (Soderstrom and Calderon 1976; Singh, 2008; Reddy *et al*., 2008; De Flander and Rovers, 2009). The main bamboo species used for papermaking in India is *Dendrocalamus strictus* (Scurlock *et al*., 2000; Pandey and Singh, 2012). The leaves of bamboo have a good quality of forage and were also used in the preparation of toys in traditional days. In different parts of North-east India, the young shoots of *D. strictus* are also used for eating purposes because of their high nutritive values and the medicinal benefits that help to get rid of certain diseases due to their antioxidant capacity (Chongtham *et al*., 2011). An *in vitro* micropropagation includes the rapid vegetative multiplication of valuable plant material for agriculture and forestry (Bennett *et al*., 2014). Since from last few decades, many researchers and companies around the world have performed research to develop efficient micropropagation technology for tropical and temperate bamboos (Lim *et al*., 2012). As bamboo is a prime renewable resource used for biomass production and solving the issue of global climatic variations, high-end research has been focused on the development of the standard protocol and obtaining healthy plantlets (Guta, 2012). *Bambusa balcooa Roxb*. (Poaceae: Bambusoideae) is a subcontinent multipurpose native Indian bamboo species that reaches a height of 12-23 m, the diameter of around 18–25 cm, and grows to 600 metres altitude (Patel *et al*., 2015) The flowering cycle of *B. balcooa* is about 60 years, and the plant usually dies after flowering without having seeds setting. Hence, asexual propagation is the only way for its propagation of *B. balcooa* (Banik, 1985). In our research, the propagation of *B. balcooa* in MS medium with plant

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growth regulators (PGRs) at different concentrations are studied at particular culture conditions.

2. Materials and methods

A collection of healthy plant material (*Bambusa balcooa*) was taken from the forest nursery, near Karakambadi Road, Biotechnology research centre.

2.1 Preparation of explant

Shoot tip internodal region of 2–3 cm^2 (*Bambusa balcooa*) was cut with a sterilized blade. The upper layers of explants were scrubbed off to remove the dust and wax. The explants were then washed in running tap water for 10 mins. The explants were washed with distilled water containing 1–2 drops of detergent (Tween 20) for 5 mins 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. The presence of these microbes usually results in increased culture mortality. Explants were subjected to repeated washing in distilled water. Then explants were treated with 0.1% aqueous mercuric chloride ($HgCl_2$) for 5 mins and thoroughly washed 4–5 times with sterile distilled water under aseptic conditions.

2.2 Preparation of MS media

Culture medium and growth conditions Murashige and Skoog (MS) medium with 3% (w/v) sucrose was used for the present study. The pH of the medium was adjusted to 5.6 before gelling with 1% agar. Murashige and Skoog (50 mL) each was dispensed into a 150 mL sterilized conical flask (Borosil) and plugged with a non-absorbent cotton plug.

Preparation on MS Media with Different Concentration of Growth Regulators

MS + 3% Sucrose + 6 BAP – 0 to 2 µg/L + NAA – 0.1%,

MS + 3% Sucrose + 6 BAP – 0.5 to2ug/L

MS + 3% Sucrose + 3µg/L BAP +3 µg/L NAA

MS + 3% Sucrose + 3µg/L BAP +2 µg/L NAA

2.3 Establishment of inter node

Surface sterilized immature and semi-hardwood shoot tip was cultured on MS media and the survived explants were transferred to regeneration media. Percentages of green and survivals as well as the number of shoot buds initiated, the new leaves formation were recorded over a period of 4 weeks. Then, the cultured explants were maintained inside the plant tissue culture room at 25±2°C, and a 16 hrs photoperiod was provided by cool white fluorescent tubes. The relative humidity was 50–55%. The growth of internodes and the acclimatization process will be conducted after shooting and rooting.

3. Results and discussion

Explants of *Bambusa balcooa* internodes produced multiple shoots on MS medium supplemented with different PGRs in combination. Internodes explants took 25 days to initiate shoots. The type and concentration of cytokinins influenced the average number of inter-node produced per explants as well as the mean length of the shoots. Of the three cytokinins tested, 2 µg/L. BAP was found to be most effective in inducing bud break and multiple shoot formation from the explants by producing a maximum of 2 cm shoot lets/explants as an average.

In vitro bud breaking of two bamboo species (*Dendrocalamus giganteus* and *Bambusa vulgaris*) were extensively studied by Ramanayake and other researchers from April 1994–1995 and found seasonal effect on bud breaking (Islam and Rahman, 2005; Trees, 2013). After the bud break, the elongated shoots were separated from nodes by a sharp scalpel and transferred in the same fresh medium (Pratibha and Sarma, 2014), initially the sprouted nodal buds produced thick shoots with clusters of shoots are of the varied number. The excised shoots (either single or two to three together) clusters, established from nodal buds of parent bamboo were used as explants (Sharma and Sharma, 2011)

In control, there is a smaller number of shoot multiplication. From Table 1, it is recommended that

Table 1. Morphological changes in growth of *B. balcooa* using *in vitro* propagation technique

PGR Combination	Treatment	% of respond shoots			Contamination %			Shoots Avg. Data					
		I Week	II Week	III Week	I Week	II Week	III Week	I Week		II Week		III Week	
								No.	Length (Cm)	No.	Length (Cm)	No.	Length (Cm)
Control	Running water + Extrin - 2 g + Bavistin - 2 g + 0.1% in $HgCl^{2-}$	93%	88%	86%	Nil	13%	13%	1.3	1.2	2.2	2.8	3.1	3
1 BAP 1 mg/L	Running water + Extrin - 2 g + Bavistin - 2 g + 0.1% in $HgCl^{2-}$	100%	100%	90%	Nil	Nil	10%	1.6	1.5	2.6	3.1	3.2	3.5

during fresh culturing, the effect of $HgCl_2$ was found to best with 0.1 $HgCl_2$ concentration for 5 mins rather than that of 4 mins. The species of *Bambusa balcooa* showed the best response in the PGR combination 1.0 BAP 1mg/ L, so the subculturing of explants was under process in the same combination of PGR (Figure 1).

A. Collection of explants B. Surface sterilization

C. Initiation D. Shoot multiplication E. Root formation

Figure 1. Regeneration of *Bambusa balcooa*

4. Conclusion

Internode explants of *B. balcooa* segments were treated with different concentrations of phytohormones for micropropagation studies performed. The nodal cutting explants showed a maximum number of shoot multiplication, and their better response was observed at different concentrations and combinations. Further bulk production of in vitro propagation of *B. balcooa* plants is needed for better soil conservation and decrease air pollution.

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