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A Rapid *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 have 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. Sub-culturing at lower concentration of Kn (0.25 µg/l, 0.50 µg/l 1.0 µg/l and 1.50 µg/l respectively) fortified medium caused stagnant and unhealthy of the shoots, leading to ultimate death of the cultures. Among the three cytokinins tested, BAP was selected as the most suitable hormone to induce shoot multiplication. 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 highest and maximum shoot generation. Shoot regeneration was also achieved in the MS medium containing Kn (1.0, 1.5 and 2.0 µg/l). Highest multiplication is observed in the concentration of Kn (2.0 µg/l). The shoot multiplication rate is moderate with shoot length of 2.00±0.5 cm. Multiplication potentiality was observed in the cluster having more than 2–3 shoots. Best period for recycling of multiplying shoots is 2–3 weeks in old culture. Delaying of sub-culturing period resulted in gradual browning of the shoots. The sub-culturing period was recorded as the most crucial factor for obtaining optimal and desired level of regeneration of shoots. The well grown prop gules in the present experimentation were shown successful regeneration. Hence, the experiment concluded with best growth pattern observed in media containing auxins like BAP and cytokinins like Kinetin.

Keywords: *Bambusa balcooa. Roxb, BAP, kinetin, in vitro propagation*

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INTRODUCTION

Bamboo grows naturally in several types of forest lands and is also cultivating in many areas of India [1, 2]. 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 implements [3–6] The main bamboo species used for papermaking in India is *Dendrocalamus strictus* [7, 8]. The leaves of bamboo have good quality of forage and were also used in preparation of toys in traditional days. In different parts of North-east India, the young shoots of *D. strictus* also used for eating purpose because of its high nutritive values and the medicinal benefits that help to get rid of certain diseases due to its antioxidant capacity [9]. An *in vitro* micro propagation includes the rapid vegetative multiplication of valuable plant material for agriculture and forestry [10].

Since from last few decades, many researchers and companies around the world have performed research to develop efficient micro propagation technology for tropical and temperate bamboos [11]. As bamboo is a prime renewable resource using for biomass production and solving issue of global climatic variations, a high end research has been focused in the development of standard protocol and obtaining healthy plantlets [12, 13].

Bambusa balcooa Roxb. (*Poaceae*: *Bambusoideae*) is a subcontinent multipurpose native Indian bamboo species that reaches a

height of 12-23 m, diameter of around 18–25 cm, and grows to 600 m altitude [14]. 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* [15].

MATERIALS AND METHODS

Collection of Plant Material

Healthy plant spp. (*Bambusa balcooa*.) was collected from the forest nursery, near Sakri Bypass Road, Bilaspur at the green stage.

Preparation of explants

Shoot tip inter nodal region of 2–3 cm^2 (*Bambusa balcooa*) were cut with 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 min. The explants were washed with distilled water containing 1–2 drops of detergent (Twine 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 1min. The presence of these microbes usually resulting increased culture mortality. Explants were subjected to repeated washing in distilled water. After that, the explants were treated with an antibiotic Streptomycin sulphate of 0.1% concentration which acts as antibacterial agent and antifungal agent like fluconazole of 0.1% concentration for 15 minutes. Rinse 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 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. 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.

Preparation on Murashige and Skoog (MS) Media with Different Concentration of Growth Regulators

MS + 3% Sucrose + 6 BAP – 0 to 2 µg/l + IAA – 0.1%
MS + 3% Sucrose + 6 BAP – 0.5 to 2 µg/l + Kn – 0.1%
MS + 3% Sucrose + 3µg/l BAP +3 µg/l IAA
MS + 3% Sucrose + 3µg/l BAP +2 µg/l IAA

Establishment of Inter Node

Surface sterilized immature and semi-hard wood shoot tip were 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 16 hours photo period 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.

RESULTS AND DISCUSSION

In the present work, various results were obtained from *in vitro* propagation using explants were presented in Tables 1–3.

Table 1: *Culture Condition Required for in vitro Cultivation of Bambusa balcooa.*

Ex-plant	Temperature	Moisture	Light period	Time of regeneration
Leaf disc	25±2	50-55	16 hours	2 weeks
Inter node	25± 2	50-55	16 hours	2 weeks

Table 1 represents various culture conditions taken for *in vitro* cultivation of *(Bambusa balcooa) by* plant tissue culture. Inter node explants of *B. balcooa* survived on MS medium supplemented with IAA and IBA for shoot initiation in 3 weeks. The leaf disk explants of *B. balcooa* given response at cultural conditions. The explants on MS supplement with particular culture condition *B. balcooa* from nodal explants shunted 30 days. Shoot tip explants of *B. balcooa* seedlings produced multiple shoots on MS medium supplemented with different PGRs individually and in combination. Shoot tip explants required 30 days to initiate shoots (Figure 1).

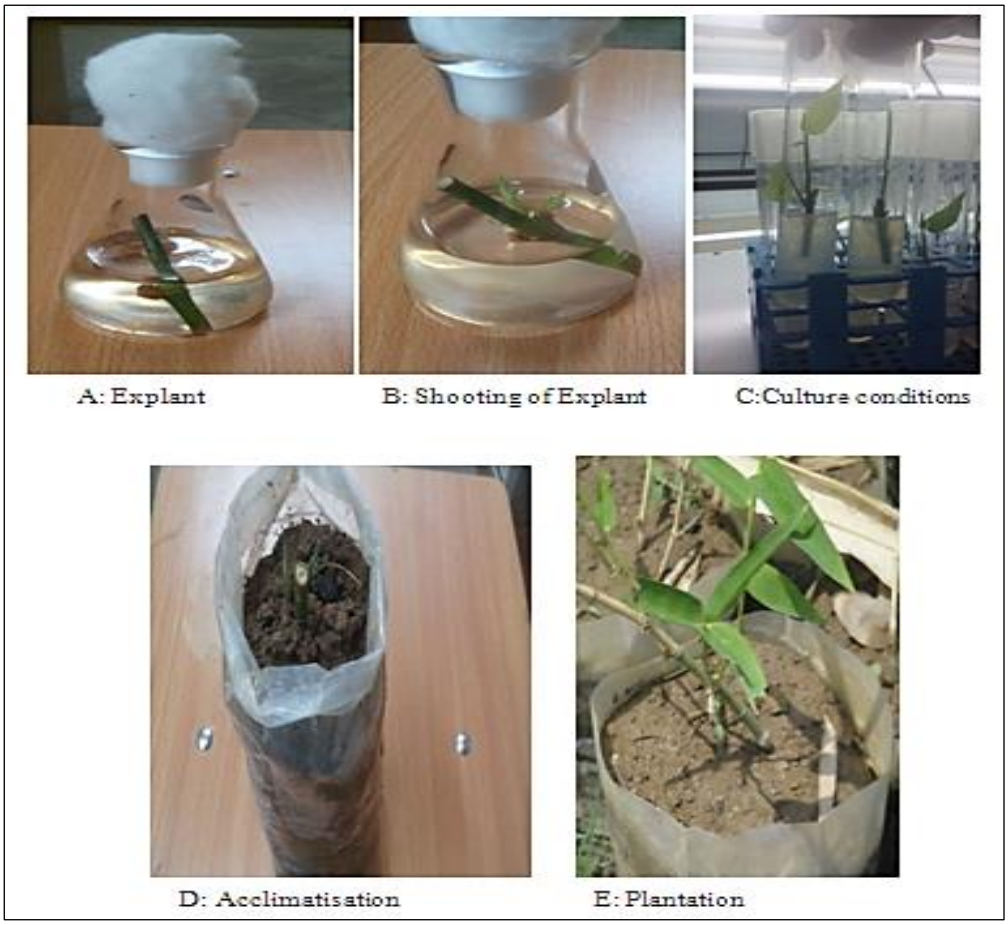

Fig. 1: *Internodes Regeneration of Bambusa balcooa.*

Table 2: *Culture of Explants Internodes Region on MS Media.*

Explants in MS (Murashige and Skoog) Agar Media	Percentage (%) of Explants Survival			Average Number of Shoots initiation			Number of Regenerated Leaf		
Leaf Disc	R1	R2	R3	R1	R2	R3	R1	R2	R3
	50% (4 Shoots)	25% (2 Shoots)	Nil	50%	25%	Nil	100% (4 Nodes)	100% (4 Nodes)	Nil
Inter Node	100%	50%	Nil	100%	50%	Nil	Nil	Nil	Nil

Note: *R1- 3µg/l BAP +3 µg /l IAA R2-3µg/l BAP +2 µg/ l IAA, R3- Control*

Table 2 represents survival shoot initiation and regeneration of explants "inter node" in MS media. 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 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 maximum of 2 cm shoot lets/explants as an average.

In vitro bud breaking of two bamboo species (*Dendrocalamus giganteum and Bambusa vulgarism*) were extensively studied by Ramanayake and other researchers from April, 1994–1995 and found seasonal effect on bud breaking [16, 17]. After the bud break, the elongated shoots were separated from nodes by sharp scalpel and transferred in the same fresh medium [18]. Initially the sprouted nodal buds produced thick shoots with clusters of shoots are of varied number. The excised shoots (either single or two- three together) clusters, established from nodal buds of parent bamboo were used as explants bamboo were used as explants [19].

Table 3: *Morphogenic Response of Nodal Explants of Bambusa balcooa at Different Concentration of Cytokinins Supplemented to MS Medium.*

Growth Regulator (µg/l)	Shoot Growth	Shoot Length (cm)	Leaves Generated
MS	-	0	-
Kn 0.25	-	**0**	-
Kn 0.50	-	1.1±0.1	-
Kn 1.00	+	1.0±0.5	+
Kn 1.50	++	1.5±0.3	++
Kn 2.00	+++	2.00±2.5	+++
BAP 0.25	-	1.0±0.5	-
BAP 0.50	+	1.5±0.4	+
BAP 1.00	++	2.5±0.40	++
BAP 1.50	+++	3.5±0.38	+++
BAP 2.00	++++	3.9±0.4	++++

NOTE: *- Nil; + Less; ++ Medium; +++ Good; ++++ Excellent*

Table 3 represent that the shoot multiplication. If once the bud break was achieved, shoot proliferation could be increased and maintained by regular sub-culturing at 4 weeks interval on MS medium with concentration of plant growth regulator. Sub-culturing at lower concentration of Kn (0.25 µg/l, 0.50 µg/l 1.0 µg/l and 1.50 µg/l) fortified medium caused stagnant and unhealthy growth of the shoots, leading to ultimate death of the cultures. Among the three cytokinins tested, BAP was selected as the most suitable hormone to induce shoot multiplication. Highest shoot multiplication is found in corporation with BAP (1.5 µg/l and 2 µg/l). The shoot multiplication rates are medium and excellent with shoot length 2.5±0.3 and 3.9±0.4 with less and maximum shoot generation. Shoot regeneration was also achieved in the MS medium containing Kn (1.0, 1.5 and 2.0 µg/l) showed shoot multiplication as low. Highest multiplication is observed in the concentration of Kn (2.0 µg/l). The shoot multiplication rate is good with shoot length 2.00±0.25 cm. Multiplication potentialities were observed in the cluster having more than 2-3 shoots. Best period for recycling of multiplying shoots is 2–3week old culture. Delaying of sub-culturing period resulted in gradual browning of the shoots. Sub-culturing period was recorded as the most

CONCLUSION

In the present study, an effective sterilization of Inter node explants of *B. balcooa* segments were shown different effect of phyto-hormones on the explants and this protocol has been established at the laboratory. Further bulk production of *in vitro* propagation of *B. balcooa* plants is needed for the better soil conservation and decrease of air pollution.

Conflict of Interest: No conflict of interest

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