In vitro propagation of Bambusa balcooa by plant tissue culture technique

1,⁎Anbuselvi, S., 1Priyanka, P.S., 1Monitha, B. and 2Saroja Preethy, R.

1Department of Agricultural Biotechnology, Bharath Institute of Higher Education and Research, Chennai-73, Tamilnadu, India
2Research scholar, Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Chennai-73, Tamilnadu, India

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 Caldeon 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
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 cm² (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 (HgCl₂) 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

| Treatment | % of respond shoots | Contamination % | Shoots Avg. Data |
|-----------|---------------------|-----------------|------------------|
|           | I Week | II Week | III Week | I Week | II Week | III Week | No. | Length (Cm) | No. | Length (Cm) | No. | Length (Cm) |
| Control - Running water + Extrim - 2 g + Bavistin - 2 g + 0.1% in HgCl₂ | 93% | 88% | 86% | Nil | 13% | 13% | 1.3 | 1.2 | 2.2 | 2.8 | 3.1 | 3 |
| 1 BAP 1 mg/L - Running water + Extrim - 2 g + Bavistin - 2 g + 0.1% in HgCl₂ | 100% | 100% | 90% | Nil | 10% | 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).

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.

References
Banik, R.L. (1985). Techniques of bamboo propagation with special reference to prerooted and pre-rhizomed branch cuttings and tissue culture, presented at the International Bamboo Workshop, 6-14 October, p. 160-169. Hangzhou, People’s Republic of China: IDRC.

Bennett, I.J., McComb, J.A. and Tonkin, C.M. (1994). Alternating cytokinins in multiplication media stimulates in vitro shoot growth and rooting of *Eucalyptus globulus* Labill. *Annals of Botany*, 74(1), 53–58. https://doi.org/10.1006/anbo.1994.1093

Chongtham, N., Bisht, M.S. and Haorongbam, S. (2011). Nutritional properties of bamboo shoots: potential and prospects for utilization as a health food. *Comprehensive Reviews in Food Science and Food Safety*, 10(3),153–168. https://doi.org/10.1111/j.1541-4337.2011.00147.x

De Flander, K. and Rovers, R. (2009). One laminated bamboo-frame house per hectare per year. *Construction and Building Materials*, 23(1), 210–218. https://doi.org/10.1016/j.conbuildmat.2008.01.004

Guta, D.D. (2012). Assessment of biomass fuel resource potential and utilization in Ethiopia: sourcing strategies for renewable energies. *International Journal of Renewable Energy Research*, 2(1), 131–139.

Islam, S.A.M.N. and Rahman, M.M. (2005). Microcloning in commercially important six bamboo species for mass propagation and at a large scale cultivation. *Plant Tissue Culture Biotechnology*, 15(2), 103–111.

Kaladhar, D.S.V.G.K., Tiwari, P. and Duppala, S.K. (2017). A Rapid in vitro Micro Propagation of *Bambusa vulgaris* Using Inter-Node Explant. *International Journal Life Sciences and Scientific Research*, 3(3), 1052–1054. https://doi.org/10.21276/ijlssr.2017.3.3.14

Lim, J.S., Manan, Z.A. and Alwi, S.R.W. (2012) A review on utilisation of biomass from rice industry as a source of renewable energy. *Renewable and Sustainable Energy Reviews*, 16(5), 3084–3094. https://doi.org/10.1016/j.rser.2012.02.051

Pandey, B.N. and Singh, N.B. (2012). Micropropagation of *Dendrocalamus strictus* Nees from mature nodal explants. *Journal of Applied Natural Sciences*, 4(1), 5–9. https://doi.org/10.31018/jans.v4i1.213

Patel, B., Gami, B. and Patel, N. (2015). One step pre-hardening micropropagation of *Bambusa balcooa* Roxb. *Journal of Phytology*, 7, 1–9. https://doi.org/10.5455/jp.2015-06-02

Pratibha, S. and Sarma, K.P. (2014). *In vitro* propagation of *Bambusa nutans* in commercial scale in Assam, India. *Journal of Environmental Research and Development*, 9(2), 348–355.

Reddy, K.N., Pattanaik, C. and Reddy, C.S. (2008). Plants used in traditional handicrafts in north eastern Andhra Pradesh. *Indian Journal of Traditional Knowledge*, 7(1), 162–165

Scurlock, J.M.O., Dayton, D.C. and Hames, B. (2000). Bamboo: an overlooked biomass resource? *Biomass and Bioenergy*, 19(4), 229–244. https://doi.org/10.1016/S0961-9534(00)00038-6

Sharma, P. and Sarma, K.P. (2011). *In vitro* propagation of *Bambusa balcooa* for a better environment, presented at the International Conference on Advances in Biotechnology and Pharmaceutical Sciences, December 2011, p. 248-252. Bangkok,
Thailand.

Singh, O. (2008). Bamboo for sustainable livelihood in India. *Indian Forester*, 134(9), 1193-1198.

Soderstrom, T.R. and Calderon, C.E. (1979). A commentary on the bamboos (Poaceae: Bambusoideae). *Biotropica*, 11(3), 161–172. https://doi.org/10.2307/2388036

Trees, F. (2013). Tropical Agricultural Science. *Pertanika Journal of Tropical Agricultural Sciences*, 36(1), 1–26.

Zhou, B.Z., Fu, M.Y., Xie, J.Z., Yang, X.S. and Li, Z.C. (2005). Ecological functions of bamboo forest: research and application. *Journal of Forestry Research*, 16(2), 143–147. https://doi.org/10.1007/BF02857909