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Somatic Embryogenesis From Mature *Bambusa ventricosa*

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Introduction

Bamboo provides economic benefits for about 2.5 billion people around the world (INBAR 1999). It continues to play a key role in small landowner income creation and in assisting marginalized groups by enabling them to participate in high value-added processing activities. As worldwide demand for forest resources grows, bamboo has emerged as a leading non-wood forest product. Bamboo plantations provide environmental and economic benefits through conservation and enhanced processing and production opportunities (Lobovikov et al. 2007).

Figure 1. *Bambusa ventricosa* mature plant

Traditional propagation techniques, such as rhizome division, are inefficient for large-scale production. Micropropagation can improve plantation economic viability by increasing propagation productivity and homogeneity, and by providing consistent and dependable plant quality (Gielis et al. 2002).

Bamboo plants grown from tissue culture have shown superiority in rate of culm growth and development over conventional culm cuttings (Rao et al. 1990; Sood 2002). However, reliable, efficient, economical protocols that are suitable for commercially propagating mature, elite plants are scarce (e.g., Gillis et al. 2007, Jimenez et al. 2006, Lin et al. 2004, Sood et al. 2002, Godbole 2002, and Saxena & Bhojwani 1993). Gillis et al. (2007) reported a 98% success rate for embryogenic callus induction, 90% germination rate for somatic embryos, 46% maturation rate of embryos into plantlets, 100% acclimatization of plantlets into soil, and an overall success of 40%. Their process reduced costs up to 57% compared to conventional micropropagation using axillary branching. However, the Gillis et al. protocol is limited to the use of pseudo-spikelets, dormant buds in the inflorescence, as starting materials. Such materials are not readily available. Alternative tissue culture protocols for bamboo are needed to allow the use of more commonly available tissue, such as vegetative buds, as explants. In addition, browning and necrosis of cultures and poor rooting are major problems in bamboo micropropagation (Huang et al. 2002; Ramanayake 2006).

This paper describes a bamboo tissue culture protocol based on explants from mature plants that minimizes the browning and rooting problems identified as bottlenecks in previous methods. The positive results of this initial research can be further optimized for commercial application.

Materials and methods

The plant source material was a mature bamboo, about 6 ft. in height, acquired on the island of O'ahu (Figure 1). Three months prior to aseptic culture, the mother plant

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Figure 2. Severe pruning to revitalize plant

Figure 3. Source of explant materials

Figure 4. Newly sprouted axillary shoots

was divided into five 3-gal. pots to revitalize the plant's health. All shoots were severely pruned to 1 ft. above ground to induce sprouting of new axillary branches (Figure 2).

Plants were fertilized with controlled-release fertilizer Nutricote® (13-13-13) and watered twice daily by drip irrigation. In a greenhouse environment, insects and other potential plant pathogens were monitored; as a precaution TetraSan®/Akari spray mix was applied to treat and control mites, and Pyronyl™ Crop Spray was applied for aphids. The procedure then followed for micropropagation can be divided into 6 stages.

Stage 1. Initiation of aseptic cultures

As a first step, newly sprouted axillary shoots of 4–6 in. with distinct internodes and buds were excised from the mother plant (Figure 3).

Sterilization of plant material was conducted as follows. Plant material was soaked in 70% alcohol for 5 min., followed by 10% (v/v) Clorox® bleach for 40 min., followed by three rinses with sterile deionized water. Stems were cut into single-node segments of 1 in. and cultured in Murashige and Skoog (MS) basal medium (Murashige & Skoog 1962) with vitamins (*Phyto*tech Labs® product M519), combined with 3% sucrose, 3 mg/l BAP, and 3 g/l Gelrite®. Explants were cultured under 16/8-h light/dark photoperiod (cool white 40W fluorescent lamp) with an average temperature of 25°C.

Stage 2. Axillary bud induction and multiplication

New axillary buds sprouted from nodal segments as early as 3 weeks after culture initiation and continued to sprout over a period of 3 months. About 80% of the stem segments produced axillary buds (Figure 4).

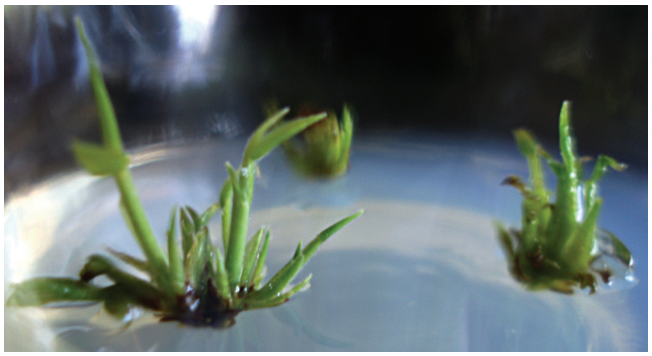

Figure 5. Multiplying cultures

The axillary bud was separated from the mother tissue and transferred to fresh medium every four weeks. Culture mass increased at an approximate rate of 250% per month on this medium (Figure 5).

Multiplying shoots were transferred to fresh medium every two weeks. Persistent browning occurred throughout the culture period. At each subculture, necrotic mother tissue was carefully excised to reduce further browning (Figure 6).

Stage 3. Induction of embryogenic callus

Elongated shoots of 1 to 2 inches were cut transversally into ⅛- to ¼-inch segments (Figure 7). These segments were cultured in basal medium (*Phyto*tech Labs® M519), supplemented with 3 mg/l 2,4-D, 2 mg/l kinetin, 3% sucrose, 3 g/l Gelrite®, and pH 5.6.

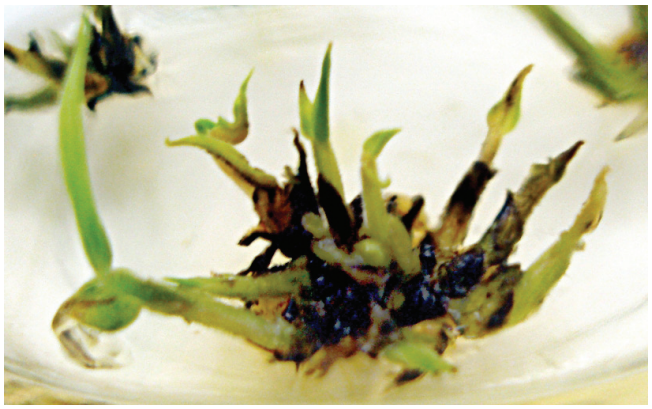

Figure 6. Persistent browning in multiplying cultures

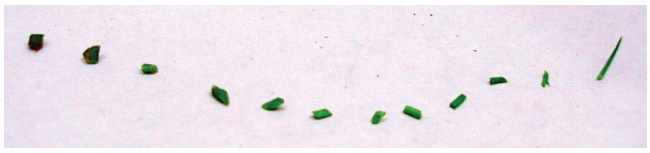

Figure 7. Shoot segments ready for culture

Six weeks after culture, white, opaque, and translucent embryogenic calli were produced from the stem segments (Figure 8).

The embryogenic calli were separated from the stem segments and transferred to medium supplemented with 1 mg/l 2,4-D, and 1 mg/l kinetin. Medium was refreshed every week until rapidly proliferating embryogenic calli were produced (Figure 9).

Stage 4. Somatic embryo induction

Somatic embryos were induced from embryogenic calli after transfer to medium supplemented with 1.5 mg/l BAP and 0.2 mg/l IBA (Figure 10).

Stage 5. Plantlet formation

Somatic embryos were transferred to Lloyd and McCown woody plant basal salt medium (Lloyd & McCown 1980) supplemented with MS vitamins and 0.5 mg/l BAP. Somatic embryos developed into a bipolar structure that germinated into complete plantlets with shoot and root systems (Figure 11, 12).

Stage 6. Established plants

Bamboo plantlets were acclimatized in greenhouse conditions on a Pro-Mix/black cinder 1:1 potting mix under a misting system (Figure 13).

In Brief

This tissue culture system follows a simple 6-step regeneration system that requires about 50 weeks from induction of calli to complete plantlets. This is only a foundation protocol, using Buddha Belly bamboo. The bamboo family is very diverse, and the protocol will need to be redesigned for each particular variety, by increasing or decreasing each of the hormone combinations at every step.

The results of this research also highlight three important aspects. First, the pretreatment of mother plants by severe pruning enables some degree of rejuvenation of plant materials before culture initiation. This technique for rejuvenation has been commonly used in micropropagation of woody plants such as pine, fir, maples, and fruit trees (e.g., Sánchez-Olate et al. 2004). Second, subculturing for three months prior to embryo induction seems to increase the ability of the cells to induce embryogenic calli. Finally, weekly subculturing results in rapidly proliferating embryogenic calli.

Figure 8. Embryogenic callus produced from stem segments

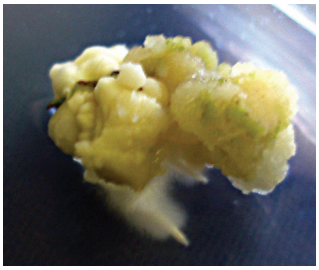

Figure 9. Rapidly proliferating embryogenic calli

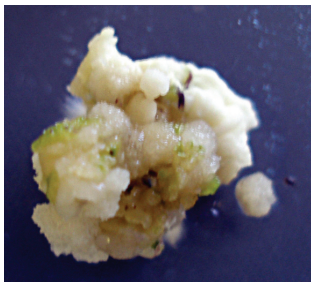

Figure 10. Somatic embryo induction

Figure 11. Germination of somatic embryos

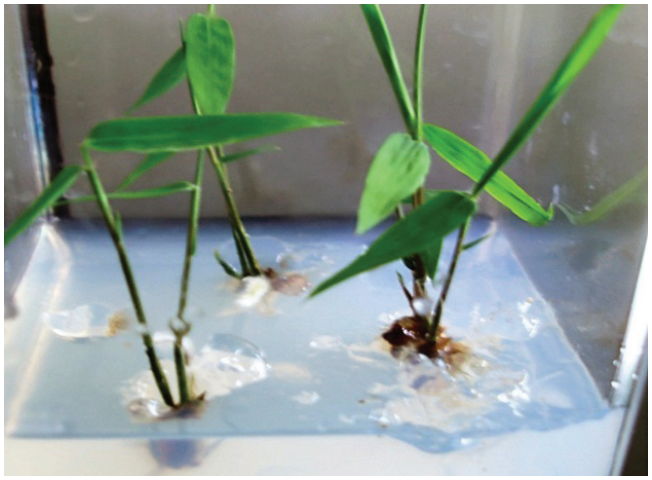

Figure 12. Elongated rooted plantlets

Figure 13. Established plants

References

Gielis, J., Peeters, H., Gillis, K.J., and Debergh, P.C. 2002. Tissue culture strategies for genetic improvement of bamboo. *Acta Hort* 552:195-203.

Gillis, K., Gielis, J., Peeters, H., Dhooghe, E., and Oprins, J. 2007. Somatic embryogenesis from mature *Bambusa balcooa* Roxburgh as basis for mass production of elite forestry bamboos. *Plant Cell Tiss Org Cult* 91:115-123.

Godbole, S., Sood, A., Thakur, R., Sharma, M., and Ahuja, P.S. 2002. Somatic embryogenesis and its conversion into plantlets in a multipurpose bamboo, *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. *Current Science* 83:885-889.

Huang, L.C., Lee, Y.L., Huang, B.L., Kuo, C.I., and Shaw, J.F. 2002. High polyphenol oxidase activity and low titratable acidity in browning bamboo tissue culture. *In Vitro Cell Dev Biol – Plant* 38:358-365.

International Network for Bamboo and Rattan (INBAR). 1999. Socio-economic issues and constraints in the bamboo and rattan sectors: INBAR's assessment. INBAR Working Paper 23. Beijing, China.

Jimenez, J.M., Castillo, J., Tavares, E., Guevara, E., and Montiel, M. 2006. In vitro propagation of the neotropical giant bamboo, *Guadua angustifolia* Kunth, through axillary shoot proliferation. *Plant Cell Tissue Org Cult* 86:389-395.

Lin, C.S., Lin, C.C., and Chang, W.C. 2004. Effect of thidiazuron on vegetative tissue-derived somatic embryogenesis and flowering of bamboo *Bambusa edulis*. *Plant Cell Tissue and Organ Culture* 76:75-82.

Lloyd, G., and McCown, B. 1980. Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip cultures. *Comb Proc Intl Soc* 30:421-427.

Lobovikov, M., Paudel, S., Piazza, M., Ren, H., and Wu, J. 2007. World bamboo resources: a thematic study prepared in the framework of the Global Forest Resources Assessment 2005. INBAR, FAO, Rome.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497.

Ramanayake, S.M.S.D. 2006. Micropropagation of tropical bamboos. In: da Silva JAT (ed.) Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues Vol 2. Global Science Books, Isleworth, UK, pp 540–550.

Rao I.U., Ramanuja Rao, I.V., Narang, V., Jerath, R.,

and Pillai, K.G. 1990. Mass propagation of bamboos from somatic embryos and their successful transfer to forest. In: Ramanuja Rao IV, Gnanaharan R, Sastry B (eds) Bamboos Current Research, Proceedings of the International Bamboo Workshop (pp 167–172). Kerala Forest Research Institute and IDRC, Canada.

Sánchez-Olate, M., Ríos, D., Rodríguez, R., Materán, M. E., and Pereira, G. 2004. Duration of the reinvigorating effect of severe pruning of mature European hazelnut plants (*Corylus avellana* L.) cv. Negretta with in vitro cultivation. Agric Téc [online] 64:338-346.

Saxena, S., and Bhojwani, S.S. 1993. In vitro clonal multiplication of four year old plants of the *D. longispathus* Kurz. *In Vitro Cell Dev Biol* 290: 135–142.

Sood, A., Ahuja, P.S., Sharma, M., Sharma, O.P., Godbole, S. 2002. In vitro protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Munro. *Plant Cell Tiss Organ Cult* 71:55–63.

Sood, A., Palni, L.M.S., Sharma, M., and Sharma, O.P. 1994. Improved methods of propagation of Maggar bamboo (*Dendrocalamus hamiltonii* Nees et. Arn. Ex Munro). In: Dwivedi BK (ed.) Biotechnology in India. Bioved Res Soc, Allahabad, pp 199–212.

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