Journal of Tropical Forest Science 13(2): 391–393 (2001)

CALLOGENESIS AND ORGANOGENESIS IN SANDAL (*SANTALUM ALBUM*)

S. Radhakrishnan, K. Vanangamudi* & K. T. Parthiban

Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam - 641 301, Tamil Nadu, India

The sandal tree (*Santalum album*) is valued for the sweet fragrance and medicinal properties of its heartwood. The oil from the heartwood has over 2000 years of uninterrupted perfumery trade. It is indigenous to peninsular India with an estimated natural distribution of 9040 km^2 and is confined predominantly to the two states of Karnataka and Tamil Nadu (Venkatesan *et al.* 1995). In view of the high price its wood fetches, live sandal trees in their endemic habitats are ruthlessly felled. In addition, the sandal tree suffers from a large number of diseases, of which the spike disease (caused by mycoplasma-like organisms) is the most destructive, greatly reducing the yield of heartwood (Bapat & Rao 1989). Dysgenic selection followed by pests and diseases has considerably narrowed the gene pool and leaves only an emaciated, uneconomical population comprising trees with a predominance of sapwood. The propagation of sandal trees is limited due to poor germination, which is also affected by heat, grazing, fire and adverse climatic changes (Bapat 1993). Recent techniques of biotechnology are being widely used for rapid multiplication of elite plants either through the process of direct plant regeneration from cultured explants or from callus and cell suspension. *In vitro* propagation of sandal hypocotyl segment has already been reported (Bapat & Rao 1989). The present investigation reports on the callogenesis and organogenesis in mature explants of *S. album.*

Nodal segments of 1.5 to 2.0 cm length were collected from 20-y-old identified sandal candidate plus trees growing in sandal estates in Kavalur area of Javadi hills, Tamil Nadu. A minimum of five trees were selected based on morphometric traits (height, girth at breast height, clean bole height). The explants were washed in running tap water for 30 min and then with soap solution to get rid of surface contaminants. This was followed by a sterilant treatment with 4% sodium hypochlorite for 4 min and then thorough washing with sterile distilled water for 2 to 3 min under aseptic condition. The nodal segments were dissected to 1 to 1.50 cm lengths and cultured on Murashige and Skoog (1962) medium with different concentrations and combinations of benzyl amino purine (BAP) and 2, 4-dichlorophenoxy acetic acid (2, 4-D) (Table 1). In all the media, 2% sucrose was added as carbon source. The pH of all the media was adjusted to 5.7 prior to the addition of agar (0.9%) and sterilisation at 1.05 kg cm^{-2} for 20 min. The media were dispensed into culture tubes. The cultures were exposed to a light and dark cycle of 16 and 8 h respectively. Temperature was maintained at 25 ± 2 °C and the intensity of light was approximately 1000 lux. The callus developed in the media was graded by giving 0 point for no callusing, 1 for poor, 2 for slight, 3 for moderate and 4 for profuse callusing.

Six-week-old callus cultures of yellow to light green colour were transferred to MS medium supplemented with various concentrations and combinations of BAP and indole-3-butyric acid (IBA) for multiple shoot induction. For rooting studies, the multiple shoots produced from the callus cultures were transferred to root induction medium containing no additive (control), 1, 2 and 3 mg l^{-1} IBA, and the seed extract of *Cajanus cajan* (red gram), in separate treatments.

Received December 1998

**Professor, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore - 641003, Tamil Nadu, India*

For all the experiments four replications were used. The data obtained from this study were subjected to Duncan's multiple range test (DMRT) after Gomez and Gomez (1984).

Callus initiation was the highest (27.8%) in the treatment MS + BAP 4.0 mg l^{-1} with a relative growth rate of 3.0 (Table 1). The next best treatment was MS + 2, 4-D 4.0 mg l^{-1}, with a relative growth rate of 2.5. Lakshmi and Vaidhyanathan (1979) reported good callus formation from nodal segments of *S. album* in MS medium with BAP. Callus initiation was observed in nodal segments of sandal in MS medium containing 2, 4-D and BAP (Bapat *et al.* 1985). Callus initiation in this study was observed in the induction medium three weeks after inoculation. The colour of the callus was yellow to light green.

Organogenesis is characterised by the production of unipolar primordium, with the subsequent development of the primordium into vegetative shoots connected to the maternal tissues through precambial strands (Wynman *et al.* 1992). Cytokinins are involved in the development of multiple shoots. The callus turned into compact green upon repeated subculturing. The nodulated clump-like structures further differentiated into shoots. The treatment MS + BAP 4 mg l^{-1} gave 18% organogenesis from the callus cultures (Table 2). Bapat *et al.* (1985) isolated protoplasts from embryogenic cell suspension cultures derived from proliferating shoot segments of a 20-y-old sandal wood tree and produced embryogenic cell aggregates and globular embryos which further developed into plantlets.

To obtain full plantlets the regenerated shoots must be transferred to a rooting medium, which differs from shoot multiplication medium, especially in its hormonal composition. Results showed that shoots subcultured in MS medium with IBA 2.0 mg l^{-1} proved superior (5.6%) in exhibiting single root initials (Table 3). However, rooting was observed in only

Table 1. Effect of growth regulators on callus initiation in sandal nodal explant

Treatments	Number of explants	Relative growth	Callusing percentage
MS + BAP 1 mg l^{-1}	28	1.0^{e}	4.21^{h}
MS + BAP 2 mg l^{-1}	28	1.4^{d}	13.32^{e}
MS + BAP 4 mg l^{-1}	28	3.0^{a}	27.75^{a}
MS + 2, 4-D 1 mg l^{-1}	28	1.0^{e}	8.16^{g}
MS + 2, 4-D 2 mg l^{-1}	28	1.9^{c}	15.65^{d}
MS + 2, 4-D 4 mg l^{-1}	28	2.5^{b}	18.50^{c}
MS + BAP 1 mg l^{-1} + 2, 4-D 1 mg l^{-1}	28	1.2^{e}	11.20^{f}
MS + BAP 2 mg l^{-1} + 2, 4-D 2 mg l^{-1}	28	2.0^{c}	26.75^{b}

Mean values in a column with the same letter are not significantly differe ($p = 0.05$) according to DMRT.

Table 2. Effect of growth regulators on shoot differentiation from callus cultures

Treatment	Number of calli	Per cent cultures forming shoots	No. of shoots	Shoot length (cm)
MS Basal (control)	28	0	0	0
MS + BAP 1 mg l^{-1}	28	0	0	0
MS + BAP 2 mg l^{-1}	28	12^{b}	2.45^{b}	3.65^{b}
MS + BAP 4 mg l^{-1}	28	18^{a}	2.59^{a}	3.85^{a}
MS + IBA 1 mg l^{-1}	28	2^{c}	2.17^{c}	3.21^{c}
MS + IBA 2 mg l^{-1}	28	3^{c}	2.16^{c}	3.32^{c}
MS + IBA 1 mg l^{-1} + BAP 1 mg l^{-1}	28	3^{c}	2.62^{a}	3.92^{a}

Mean values in a column with the same letter are not significantly different ($p = 0.05$) according to DMRT.

Table 3. Effect of growth regulators on rooting from callus cultures

Treatments	Per cent rooting
MS Basal (control)	0
MS + IBA 1 mg l^{-1}	0
MS + IBA 2 mg l^{-1}	5.6
MS + IBA 3 mg l^{-1}	2.1
MS + red gram extract	0

another treatment, MS + IBA 3 mg l^{-1}, which gave 2.1% rooting. Bapat & Rao (1989) also failed to obtain rooting when using mature explants. Hence maturity of the explants may be the reason for not obtaining *in vitro* rooting in the present study. This warrants further investigation.

References

BAPAT, V. A. 1993. Studies on synthetic seeds of sandalwood (*Santalum album* L.) and mulberry (*Morus indica* L.) Pp. 145–155 in Redenbaugh K. (Ed.) *Synseeds. Application of Synthetic Seeds to Crop Improvement.* CRC Press, USA.

BAPAT, V. A., & RAO, P. S. 1989. *In vitro* strategies for sandalwood propagation. Pp. 145–155 in Dhawan, V. (Ed.) *Application of Biotechnology in Forestry and Horticulture.* Plenum Press, London.

BAPAT, V. A., GILL, R. & RAO, P. S. 1985. Regenerations of somatic embryos and plantlets from stem callus protoplasts of sandal wood tree (*Santalum album* L.). *Current Science* 54(19): 978–982.

GOMEZ, K. A. & GOMEZ, A. A. 1984. *Statistical Procedures for Agricultural Research.* John Wiley and Sons, New York.

LAKSHMI S. G. & VAIDHYANATHAN, E. S. 1979. Rapid multiplication of *Eucalyptus* by multiple shoot production. *Current Science* 481: 350–352.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium in rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum,* 15: 473–493

VENKATESAN, K. R., SRIMATHI, R. A. & KULKARNI, H. D. 1995. Survey of sandalwood populations. Pp. 3–53 in Srimathi, R. A., Kulkarni, H. D. & Venkatesan K. A. (Eds.) *Recent Advances in Research and Management of sandal* (Santalum album *L.*) in India. Associated Publishing Company, New Delhi.

WYNMAN, J. N., BRASSARD, D. F. & LALIBERT, S. 1992. Ploidy level stability of callus tissue, axillary and adventitious shoots of Larix × *Eurolepis flenry* regenerated *in vitro. Plant Science* 85: 189–196.