

Somatic embryo irregularities in *in vitro* cloning of sandal (*Santalum album* L.)

A. Ilah¹, M. Z. Abdin² and A. Mujib¹

¹ Department of Botany, ² Centre for Biotechnology, Faculty of Science, Hamdard University, New Delhi-110062, India

Plant tissue culture based propagation (multiple shoot/ somatic embryogenesis) has been employed with conventional methods for the reproduction of Sandalwood seedlings for some time. In somatic embryogenesis, we found an initial high potential of embryo induction was followed by a low, declining conversion frequency of plantlets. In this process of embryo formation, maturation and germination, large-scale abnormalities were noticed. Advanced stage embryos remained quiescent for varied periods before germination. This may be due to dormancy, and further exchange of ideas is needed for rapid synchronous seedling production.

Introduction

The Indian sandalwood is known worldwide because of its unique fragrance in oil and wood. Though native to peninsular India, the plant *Santalum album* L., is cultivated in other parts of India and abroad. Depletion by cutting and the incidence of spike disease are the two major problems in India. In India and Asia Pacific, the plant parts have been proudly used in several traditional incidences including marriage and death – the two crucial events of life.

Owing to the huge importance in domestic and foreign market, rapid deforestation and pilferage has been taking place in India and around. The incidence of spike disease has also made things more complicated. Along with conventional methods, plant tissue culture propagation has also been integrated for some time, in which the somatic embryogenesis pathway has been proven to be very effective for rapid production of propagules (Bapat *et al.* 1990; Mujib *et al.* 1998). However, even after four decades of intensive research, conversion frequency to mature seedlings from embryo is not very high, thus, proper utilization of this technique is not fully enjoyed. The authors have focused on some of these aspects in detail.

Materials and Methods

Locally collected seeds were germinated *in vitro*. The hypocotyl and nodal stem were used as explants for

the establishment of callus. Media supplemented with 2.26 μM 2,4-D and 2.68 μM CPA separately proved to be very effective. Two weeks old callus was immediately transferred to solid media supplemented with 2.70 μM NAA and 2.22 μM BAP to produce embryogenic mass (Figure 1a).

The embryogenic callus was then cultured on both solid and liquid Murashige & Skoong (MS) and McCown media for maturation and development of embryo. All the cultures were incubated in a culture room at a temperature of 24 \pm 20C, 70-80% relative humidity and illumination at 20- $\mu\text{m m}^{-2}\text{S}^{-1}$.

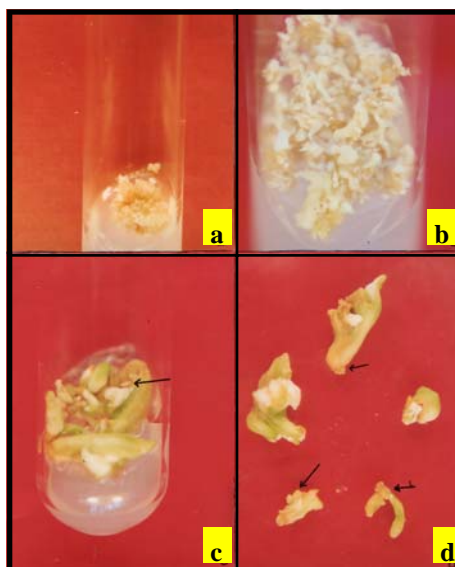


Figure 1
a) Embryogenic callus mass on solid medium, b) somatic embryos with different stages of development, c) advanced stages of embryos, arrowhead shows adventive embryo, d) irregularities on somatic embryos; top arrowhead: less developed root, left arrowhead: secondary callus from embryo, right arrowhead: fused somatic embryos at the base.

Results and Discussion

The induced callus has a strong tendency towards forming somatic embryos, as a large number were immediately induced upon transfer to embryogenic media (Figure 1b). Both the solid and liquid media were equally effective, however, liquid medium at the initial stage was more responsive (Table 1). Better oxygen availability and uptake to growing cell masses in liquid medium may trigger ready induction of proembryo and embryo (Huang *et al* 1992; Jay *et al* 1992).

For embryo maturation, woody plant medium (WPM) which contains inorganic compounds of lesser amounts, performed better in comparison to MS. The maturation and growth of embryo was virtually arrested unless the produced embryos were separated and plated on agar solidified media. This reduced frequency of transformation towards the mature stage is quite obvious as these bipolar somatic embryos received little polar effect when cultivated in agitated liquid medium. All the mature stage embryos were not transformed into truly developed seedlings (Figure 2c).

Abnormality was very high on both solid and liquid media and was manifested by the appearance of several irregularities (Figure 1c, 1d) on embryo which includes aggregation of pro-embryos and embryos, growth arrestation, browning of embryos, embryo with no or ill developed

Growth Medium (MS/MC)	G	H	T	C	Abnormality (%)
Solid					
MS	22.10±3.19	18.10±1.89	14.20±1.56	12.80±0.81	68.40
MC	-----	-----	16.20±1.28	15.20±2.31	54.10
Liquid					
MS	27.50±2.12	24.20±2.22	12.10±2.43	10.20±1.81	56.20
MC	-----	-----	13.10±2.36	9.20±1.01	52.80

G= Globular, H= Heart, T= Torpedo, C= Cotyledonary, MS=Murashige & Skoong, MC=McCown, ----- = data not scored due to insignificant deviation. All the values are expressed as mean ± SD. At least 3 replica per treatment.

Table 1: No. of embryos/10mg or ml of embryogenic callus mass.

root, and root with less developed shoot axis (Figure 2a).

Many irregularities were common but in the solid medium, embryo development was further plagued by swelling of embryos, callusing with further embryogenesis, large-scale adventive or secondary embryo formation and root degeneration.

Induction of woody plant medium, which was very active on embryo induction, was found to be less efficient. Root formation was sometimes complemented with excised *in vitro* shoots (Figure 2b) however, frequency, number and growth of roots were limited (Table 2). Although the use of bioreactor was useful in some sense (Das *et al.* 1999), a sizable abnormality frequency still exists. Mujib *et al.* (1998) earlier indicated that *in vitro* raised somatic embryo of direct and indirect origin exhibited dormancy of varied nature and required time for germination.

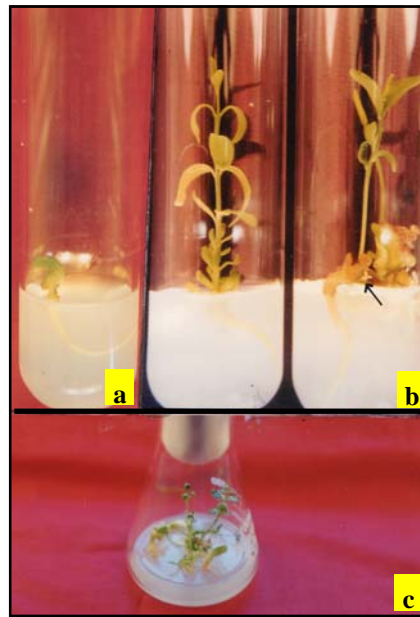


Figure 2.

- Somatic embryo with root, less developed shoot.
- Root regeneration from excised shoot tips, secondary embryo (arrowhead)
- Non-synchronous somatic seedlings, germinated *in vitro*.

Plant Growth Regulator (PGR)	Concentration. (µM)	Number of Roots	Percentage of Root Frequency (%)	Length of Roots (cm)
NAA	5.4	2.5±0.81	60	0.75±0.21
NAA	10.8	3.1±1.51	66.6	0.9±0.32
NAA	21.6	2.1±0.83	44.4	0.92±0.41
IAA	5.71	2.75±1.78	83.3	1.25±1.01
IAA	11.42	3.6±1.81	33.3	1.8±0.36
IAA	22.84	2.3±0.81	42.8	0.75±0.23
IBA	4.90	2.2±1.94	37.4	0.45±0.21
IBA	9.80	2.61±1.98	32.1	0.85±0.23
IBA	19.60	-----	-----	-----

Table 2: Root induction at various hormonal treatments. Data scored after 45 days of incubation. All values are expressed as Mean ± SD.

As the *in vitro* embryos do not have any seed coat the inhibitor(s) or physiological barrier(s) may lie within the embryo itself.

The authors call for participation from a range of scientists, including plant physiologists, to take up this challenge for rapid uniform, maturation and conversion of all or nearly every somatic embryo into seedlings to be useful in future planting programme.

References

- Bapat, V. A., Fulzele, D. P., Heble, M. R. & Rao, P. S. (1990). Production of somatic embryos in bioreactors. *Current science*, 59:746-748.
- Das, S., Das S., Pal, S., Mujib, A., Sahoo, S., Dey, S., Ponde, N. R. & Dasgupta, S. (1999). A novel process for rapid mass propagation of *Santalum album* L. in liquid media and bioreactors. *Acta Hort.*, 50: 281-288.
- Huang, L. C., Vits, H., Staba, E. J., Cooke, T. J. & Hu, W. S. (1992). Effect of cultivated age and embryo size on specific oxygen uptake rate in developing somatic embryo of *Daucus carota* L. *Biotechnology Letters*, 14: 701-706
- Jay, V., Genestier, S. & Courduroux, J. C. (1992). Bioreactor studies on the effect of dissolved oxygen concentration on growth and differentiation of carrot (*Daucus carota* L) cell cultures. *Plant Cell Report*, 11:605-608
- Mujib, A., Bandyopadhyay, S., Jana, B. K. & Ghosh, P. D. (1998). Direct embryogenesis and *in vitro* plant regeneration in *Hippeastrum hybridum*, *Plant Tissue Culture*, 8(1)19-25
- Mujib, A., Das, S., Dey, S. & Bhattacharya, B. (1995). Influence of agitation in *in vitro* cultivation of *Catharanthus roseus* (L.). G. Don multiple shoot. *Phytomorphology*, 45:239-245.
- Mujib, A., Das, S., Das, S., Pal, S. & Dey, S. (1998). Biotechnological routes of mass propagation of *Santalum album* L. I Khan I. A. & Khan A. (eds): *Role of Biotechnology in Medicinal & Aromatic Plants*, Ukaaz publication, Hyderabad, pp 83-94.