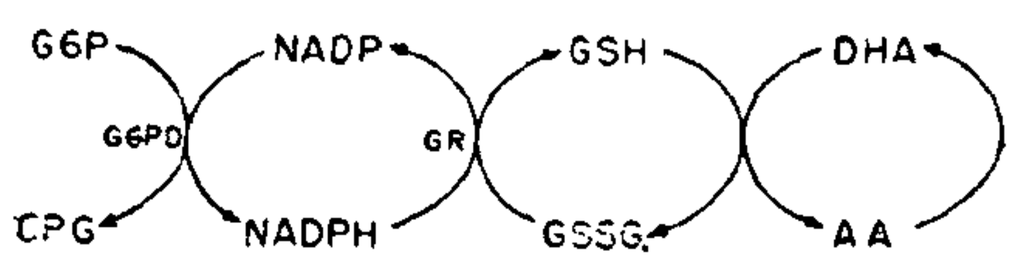
that obtained with 0·25 μ mole GSSG (Fig. 1), there was a decrease in DHA reduction by 47%, 73% and 87% respectively with 0·1 μ mole, 0·05 μ mole and 0·025 μ mole GSSG. Similarly, in comparison with that obtained with 0·025 μ mole NADP (Fig. 1), the DHA reduction was less by 30%, 67% and 83% respectively with 0·01 μ mole, 0·005 μ mole and 0·0025 μ mole NADP. There was no reduction of DHA in the absence of GSSG or NADP.



SCHEME I. Schematic representation of DHA reduction in the guinea pig tissues.

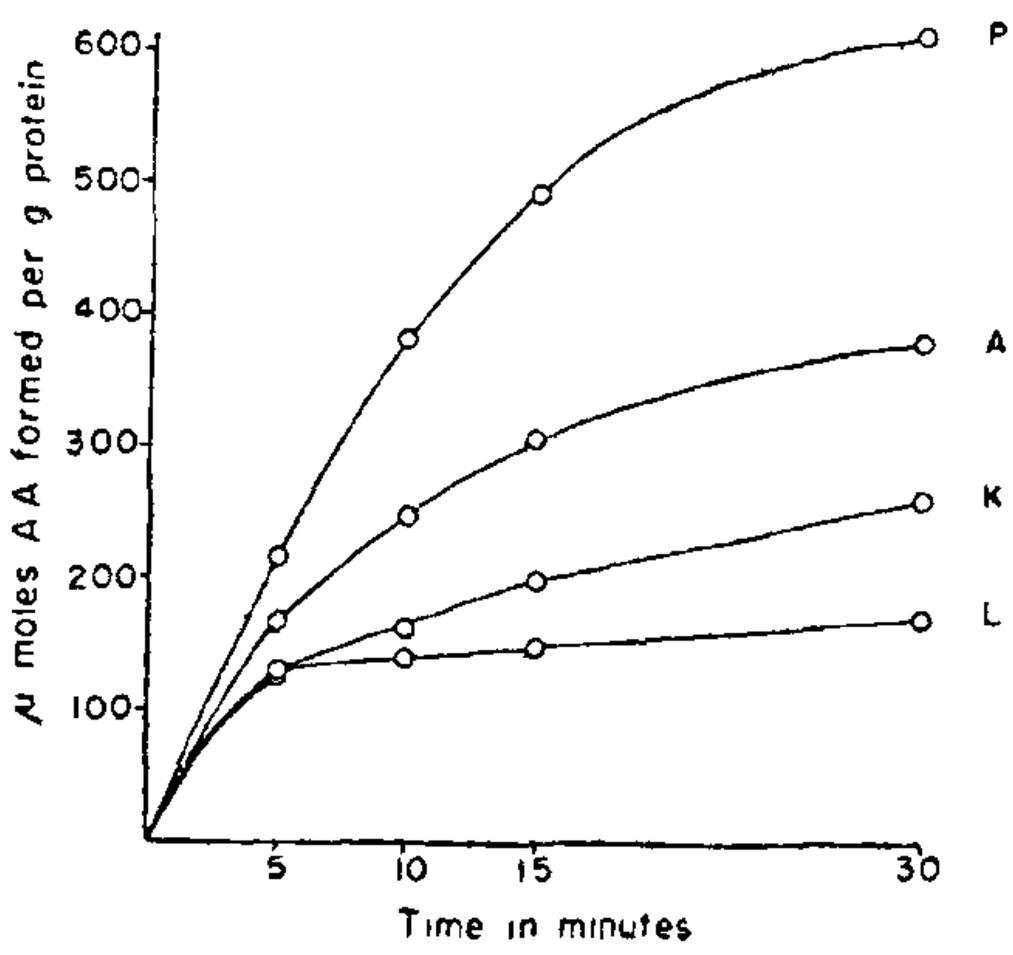


FIG. 1. DHA reduction in the guinea pig tissue soluble supernatant using GSH regenerating system. L, liver; K, kidney; P, pancreas and A, adrenal. The experimental procedure is described in the text

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1. Hughes, R. E. *Nature*, 1964, **203**, 1068.
2. — and Kilpatrich, G. S., *Clin. Chim. Acta*, 1964. **9**, 241.
3. Patterson, J. W., *J. Biol. Chem.*, 1950, **183**, 81.
4. Beutler, E., Duron, O. and Kelly, B. M., *J. Lab. Clin. Med.*, 1963, **61**, 882.
5. Richterich, R., *Clinical Chemistry*, Academic Press, New York, 1969, pp. 360 and 355.
6. Chan, P. C., Becker, R. R. and King, C. G., *J. Biol. Chem.*, 1958, **231**, 231.
7. Mckerns, K. W. and Kalcita, E., *Biochem. Biophys. Res. Comm.*, 1960, **2**, 344.

REGENERATION OF WHOLE PLANTS BY EMBRYOGENESIS FROM CELL SUSPENSION CULTURES OF SANDALWOOD

SANDALWOOD (*Santalum album* L.) is one of the many forest trees difficult to propagate vegetatively by the usual methods. There is a growing demand for sandalwood and its products in the International market. However, there is a considerable reduction in the production from 1974 onwards. Several factors have contributed for the low production[1]. One of the main problems is the spike disease caused by mycoplasma. Plants from 3 years onwards are susceptible to the disease resulting in death. To meet the growing demand, trees of improved quality and disease resistance are needed. Present-day tree improvement programmes are not adequate to meet the growing demand. In recent years, the technique of plant tissue culture has been hailed as the answer to these problems. It can, in principle, produce untold numbers of plants all genetically identical. Until recently application of tissue and cell cultures for propagation is confined mostly to herbaceous plants[2]. The success in tree tissue cultures is comparatively limited. The most pressing problem currently confronting the forest researchers is, to develop an economically efficient technique of propagation from callus and cell cultures. The technique must be suitable to be applied to both hardwood and softwood trees, that are difficult to propagate vegetatively by the usual methods of grafting, or by the rooting of branch cuttings. Regeneration in the form of embryos or plantlets is reported for other forest trees[3–5]. However, except for a few, the frequencies are low and not yet of any commercial interest. Most of these studies are confined to seed embryos or seedling explants. We have earlier reported[6] the somatic embryogenesis in sandalwood from mature trees. For large scale commercial application, the only practical method in the long run is embryogenesis in suspension cultures. We report here embryogenesis in suspension cultures leading to plantlet formation.

Sandalwood shoot callus was isolated as previously reported[6]. Murashige and Skoog's[7] (MS) and White's[8] (WM) media were used as basal media. Basal media were supplemented with auxins, cytokinins and gibberellic acid in different experiments either singly or in combinations. Suspension cultures were maintained in 250 ml conical or nipple flasks at pH 5·2. Conical

flasks were shaken at 70 rpm on a platform shaker and nipple flasks at 1 rpm on rotating steward apparatus. Cultures were incubated at 26 ± 1°C. All the experiments were repeated more than three times.

Callus obtained was routinely subcultured on MS basal medium supplemented with 1 mg/l of 2,4-Dichlorophenoxyacetic acid. Actively growing callus was subcultured to the same medium without agar. Cultures formed a good suspension within four weeks. Suspensions after two passages were used to induce embryogenesis. Basing on the earlier work, about 15 combinations were used successfully (Table I). The degree of embryogenesis varied with different growth regulators.

TABLE I

Effect of growth regulators on suspension cultures

Sl. No.	Medium composition	Embryogenesis
1.	MSB + 1 mg/l GA	++++
2.	MSB + 2 mg/l GA	+++
3.	MSB + 0·3 mg/l K + 1 mg/l GA	++++
4.	MSB + 0·5 mg/l K + 1 mg/l GA	+++
5.	MSB + 1 mg/l K + 1 mg/l GA	+++
6.	MSB + 2 mg/l K + 1 mg/l GA	+++
7.	MSB + 0·3 mg/l BAP + 1 mg/l GA	++++
8.	MSB + 0·5 mg/l BAP + 1 mg/l GA	++
9.	MSB + 1 mg/l BAP + 1 mg/l GA	++
10.	MSB + 0·3 mg/l BAP + 1 mg/l IAA	+++
11.	MSB + 0·3 mg/l BAP + 1 mg/l NAA	++
12.	MSB + 0·3 mg/l BAP + 2mg/l NAA	++
13.	MSB + 0·5 mg/l K + 0·5 mg/l IAA	+++
14.	MSB + 0·5 mg/l K + 1 mg/l IAA	+++
15.	MSB + 0·5 mg/l K + 2 mg/l IAA	+++

K—Kinetin; BAP—Benzyl ammo purine; IAA—Indole acetic acid; NAA—Naphthalen eacetic acid; GA—Gibberellic acid. ++ moderate, +++ High, ++++ intense.

In four weeks time, proembryonal stages are formed. Under appropriate experimental conditions, embryo formation is a continuous process and various embryonal stages occur side by side. Direct microscopic observations showed groups of parenchymatous single cells, these produced a multicellular aggregates before the embryo could be initiated. Embryogenesis starts with unequal divisions and the formation of a complex of small cells developing into the early stages of heart-shaped embryo. Figures. 1–8 show different stages of embryogenesis and plantlet formation. Embryoid suspensions were subcultured to same media after four weeks. By the end of 8–10 weeks the embryoids are visible to the naked eye. At this stage these are transferred to the solid medium. MS supplemented with 0·3 mg/l K + 1 mg/l GA was found to be superior for further growth. When the embryoids are showing the clear dark green shoot portions and white root portions the individual embryoids are subcultured to WM supplemented with 0·5 mg/l IAA for further plant growth.

FIGS. 1–5. Different stages of embryonal development in four week old suspensions. Figs. 1, 2. multicellular aggregates; Fig. 3. Free floating globular mass; Fig. 4. Globular mass and a developing heart-shaped embryo; Fig. 5. Free floating heart-shaped embryo. Figs. 6–8. Development of a plantlet on solid medium.

The events of embryo formation in the cell and the callus cultures occur in many widely differing families of angiosperms[9], thus showing that the capacity for embryogenesis is widely distributed and possibly is a property of somatic cells. However, to date, no tree species has been reproduced directly from cells or aggregates growing in a liquid suspension culture[5]. Embryogenesis seems to proceed in the manner shown by Reinert *et al.*[10], *i.e.*, it starts with an unequal division

of cells and the formation of a complex of small cells, gradually developing into an heart-shaped embryo. When these grow bigger in size, they detach themselves and are found free floating in the liquid medium as shown in the figure. Although, it was assumed by Steward *et al.*[11], that embryoids come from single cells in carrot, later, it was shown experimentally by several workers that Steward's hypothesis cannot be generalized,[12–14] and embryos are formed from multicellular aggregates.

Regeneration in the form of embryo or plantlet has been reported for other trees. In almost all the cases the frequencies are very low and of no commercial interest yet. Among conifers, the most common material used for propagation studies was seed embryo or seedling explants, which quickly give good results. Except for limited use, the seed cloning offers very little to the tree breeder who wishes to propagate elite trees and not their progeny. Only recently several labs have switched onto propagation studies using 4–5 years old material, old enough to be classified elite. In the present investigation callus is initiated from shoot pieces collected from mature sandal trees of about 20–25 years age and this callus is used to initiate suspension cultures. This is the first time for any tree species to develop complete plantlets from suspension cultures.

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1. Jayappa, V., In *International Seminar on Essential Oils*, 1979, Oct. 29th–Nov. 1st, Bangalore.
2. Murashige, T., *Ann. Rev. Plant Physiol.*, 1974, **25**, 135.
3. Banga, J., In *Plant Cell Tissue and Organ Culutre*, Eds. J. Reinert and Y. P. S. Bajaj, Springer–Verlag, Berlin, Heidelberg, New York, 1977, p. 398.
4. Durzan, D. and Campbell, R. A., *Canad. J. For. Res.*, 1974, **4**, 151.
5. Winton, L. and Huhtinen, O., In *Modern Methods in Forset Genetics*, Eds. J. P. Micksche, Springer–Verlag, Berlin, Heidelberg, New York, 1976, p. 243.
6. Lakshmi Sita, G., Raghava Ram, N. V. and Vaidyanathan, C. S., *Plant Science Letters*, 1979, **15**, 265.
7. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 437.
8. White, P. R., *The Cultivation of Animal and Plant Cells*, 2nd edn., Ronald Press, New York, 1963.
9. Raghavan, V., *Experimental Embryogenesis in Vascular Plants*, Acad. Press, London, 1976.
10. Reinert, J., Backs-Huseman, D. and Zerban, H., In *Les cultures de tissues de plants*, Colloques Internetsonanx du CNRS, Paris, 1971, No. 193, 261.
11. Steward, F. C., Kent, A. E. and Mapes, M. O., In *Current Topics in Developmental Biology*, Eds. A. A Moscona and A. Monroy, Acad. Press, New York, London, 1966, **1**, 113.
12. Sussex, I. M., *Phytomorphology*, 1972, **22**, 50.
13. Jones, L. H., *Ann. Bot. N.S.*, 1974, **38**, 1077.
14. McWilliams, A. A., Smith, S. M. and Street, H. E., *Ibid.*, 1974, **38**, 243.

CHEMICAL CONSTITUENTS OF THE BARK OF *SANTALUM ALBUM* LINN.

THE chemical constituents of the sandal bark have not been so far subjected to a systematic chemical examination. The present communication gives a brief account of the chemical constituents of the benzene extract of sandal bark. The sandal bark was also found to contain 14% of tannins.

The air-dried powdered bark was subjected to sequential extraction with petroleum ether (60–80°), benzene, chloroform, acetone and methanol.

While the petroleum ether extract gave a waxy material from which no pure compound could be isolated, the benzene extract (2–3% of yield) gave on repeated chromatography of its petroleum ether elute over basic alumina, a crystalline solid (0·1–0·3% of yield) m.p. 115–116° (EtOAc), $[\alpha]_D^{24°} + 20°$ ($CHCl_3$) identified (IR, NMR and MS) as urs-12en-3β yl palmitate[1], which was found to exhibit a phenomenal insect growth inhibition and chemosterilant activities[2]. Topical application of this compound in microdoses on freshly formed pupae of some forest insects (*viz.*, *Atteva fabriciella, Eligma narcissus, Eupterote geminata*, etc.) produced morphologically defective adults with crumpled wings and shorter abdomen; these did not successfully mate and lay eggs. Such compounds, of late, are becoming popular as "third generation pesticides"[3] in controlling forest pests without the bad side effects of the common organic pesticides.

The benzene elute furnished a viscous yellow liquid ester, which on saponification and purification of the alcoholic product gave a crystalline solid m.p. 131° (MeOH), $[\alpha]_D^{25°} - 41°$ ($CHCl_3$) in a yield, of 0·1%, responds positive to Liebermann–Burchard test for sterol (pink → violet → green), characterised as β-sitosterol by m.p., Co-TLC., Co-IR and it had