Micropropagation of *Sapindus mukorossi* Gaertn

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Bud break and multiple shoots were induced in apical and axillary meristems derived from one month old seedlings of *S. mukorossi* on Murashige and Skoog (MS) medium supplemented with benzylamino purine (BAP) 0.4 μM or 0.8 μM alone. A combination of BAP and gibberellic acid (GA3) 0.4 μM and 2.8 μM produced elongated multiple shoots from both types of explants. Excised shoots were rooted on MS medium respectively with indole-3-butyric acid (IBA) 3.4 μM or 2.4 μM. The regenerated plantlets were successfully acclimatized and transferred to soil.

Forest trees in general have proved to be difficult to mass propagate by tissue culture. Some success however has been achieved in a few woody tree species. Importance of plant tissue culture for mass propagation of forest trees like Eucalyptus1, Sandal wood2 and Rose wood3 has already been demonstrated. Micropropagation by the method of organogenesis and by multiple shoot production of axillary meristems of seedling explants have been reported in Leucaena4, Albizia5 and Acacia6. Shoot tip cultures were established from germinated seedlings in Red sanders7 and Teak8. So far there are very few reports of Sapindaceae like *Sapindus trifoliatus*9 established by tissue culture.

*Sapindus mukorossi* Gaertn. or *Sapindus detergens* Roxb, soapnut is a perennial tree belonging to the family Sapindaceae, indigenous to northern India. Oil from the seed kernel of soapnut is of interest to the soap industry. The oil is quite useful industrially because of its most valuable phytochemicals like saponins or triglycerides10. The exhausted cake is used as a filler and fertilizer and the shells for making lignin based adhesives or boards11. Vegetative propagation of soapnut did not yield satisfactory results and propagation through seed is unreliable because the per cent survival of the seedlings proved to be meagre due to heavy incidence of mortality at seedling stage in the natural habitat2. Micropropagation of soapnut tree is at a stage of infancy in forest tree species which has great importance in the soap industry and social forestry programmes. In this communication for the first time an *in vitro* micropropagation method for *Sapindus mukorossi* tree using apical and axillary meristem explants has been presented.

**Materials and Methods**

Seeds of soapnut were obtained from Biotechnology Research Centre for Tree Improvement (BIOTRIM), Andhra Pradesh Forest Department Nursery, Tirupati and soaked in conc. H2SO4 for 90 min and washed thoroughly with running tap water. The seeds were surface sterilized with 0.1% HgCl2 for 15 min and rinsed several times with sterile distilled water. Agar water medium (0.8%) without growth regulators was used for seed germination. Seeds inoculated on this medium were incubated at 24°C ± 2°C in the dark or light at a photon flux density of 15 μEm-2 S of white fluorescent tubes for thirty days after which seedlings were used for apical and axillary meristem dissection. The apical and axillary meristems (2-4 mm) were collected from one month old aseptic seedlings and cultured on Murashige and Skoog13 (MS) basal medium containing 2% sucrose and lower concentrations of BAP or KIN ranging from 0.4, 0.8, 1.7 and 2.6 μM, higher concentrations ranging from 4.4, 8.8 and 13.2 μM, combination of BAP and KIN 0.4 or 0.4 μM, 13.2 and 13.2 μM and combination of BAP or KIN with auxin naphthalene acetic acid (NAA) 0.4 and 0.4 μM to 13.2 and 13.2 μM were used for inducing multiple shoots. For multiplication and elongation of established shoots different combinations of GA3 (0.5 and 2.8 μM) alone, combination of BAP and GA3 (0.4 and 0.5 μM, 0.8 and 0.5 μM, 0.4 and 2.8 μM, 0.8 and 2.8 μM) were tried. For root induction, 2-3 cm long shoots were transferred to MS medium with IBA or NAA (0.4, 2.4, 3.4 and 4.9 μM). Media were sterilized at 15,

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lb/sq inch for 20 min. Twenty explants were cultured at 25° ± 2° C in the light (16hr photoperiod). Rooted plantlets were acclimatized gradually in a greenhouse. Results are mean of three culture cycles with 20 replicates per experiment.

**Results and Discussion**

Experiments on explant type, shoot tips and axillary meristems for multiple shoot formation were tested. In all the BAP concentrations tested, 0.4 and 0.8μM concentrations were more effective for inducing 6 to 8 multiple shoots within a month from axillary meristems. However on the same medium shoot tips were proliferated and 4 to 6 shoots were formed in 4-5 weeks. Addition of higher concentration of BAP (8.8 and 13.2 μM) to MS medium induced more callus formation in both the explants within a week from the cut surface, and along the surface from the apical to basal part of the explants. Initiation of callus was faster in all the higher concentrations of BAP. In order to test the passaging on shoot multiplication, the shoots obtained

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*Fig. 1*- Formation of multiple shoots regenerated from axillary buds of *S. mukorossi* after 30 day of culture; *Fig. 2*- Rooting of shoots on MS + 3.4 μM IBA + 2% sucrose after 15 days of culture; *Fig. 3*- *In vitro* raised *S. mukorossi* plant 30 days after transplanting to soil.
from apical and axillary meristems were separated and recultured on to the same shoot multiplication media (MS with 0.4 or 0.8 μM BAP) and shoot multiplication was determined after second and third subcultures. Highest number of shoots (8-10) were recorded from a single explant within three weeks (Fig. 1). No increase in shoot multiplication was observed by prolonging the culture period beyond sixth subculture.

Shoots obtained by this method were divided into 5-8 mm nodal explants with single axillary bud for further proliferation to increase the number of shoots. These buds proliferated into 5 to 8 multiple shoots in 4 weeks on MS medium with BAP 0.4 and 0.8 μM individually. Experiments conducted with BAP in combination of kinetin and auxin showed single shoots with callus formation. Of the two cytokinins BAP was most effective for inducing bud break and shoot proliferation in apical and axillary meristem (Table 1). Similar results were reported in Madhuca latifolia\(^4\).

Within 8 weeks of culture, the regenerated shoots elongated up to 2-3 cm in height. Prolonged culture on the same medium did not increase the shoot length. For shoot elongation the shoots were separated and grown on MS medium with GA\(_3\) (0.5, 2.8 μM) in combination with BAP (0.4, 0.8 μM) treatments. The shoots were elongated up to 5 to 7 cm in 4 weeks in all the BAP and GA\(_3\) treatments. Lower concentrations of BAP (0.4 or 0.8 μM) were favorable for bud proliferation and application of GA\(_3\) to these shoots increased their length. For elongated multiple shoot formation with a combination of BAP (0.4 μM) and GA\(_3\) (2.8 μM) was optimum. In the present study combination of GA\(_3\) with a cytokinin was effective in inducing shoot elongation. Different concentrations of GA\(_3\) alone failed to increase the shoot elongation however when GA\(_3\) was applied in combination with BAP effectiveness of gibberellic acid was improved in causing shoot elongation. Application of GA\(_3\) to in vitro regenerated shoots increased their length in Azadirachta indica\(^5\).

The regenerated shoots were transferred to MS medium with IBA and NAA of different concentrations for rooting. Among these concentrations the regenerated shoots were rooted in IBA (3.4 and 4.9 μM)
in 15 days of culture (Fig. 2). IBA and NAA (0.4 and 2.4µM) induced callus at the base of the shoots with poor rooting after 30 days of incubation (Table 2). A combination of IBA with NAA inhibited roots formation and showed only callus at the basal cut ends.

Regenerated plantlets were transferred to plastic containers filled with vermiculite. During first week the potted plantlets were covered with polythene bags to provide high humidity. Transplantation success was 60% (Fig. 3). Plantlets were subsequently transferred to larger pots and gradually acclimatized to outdoor conditions. In the present study multiplication by multiple shoot methods from shoot tip or axillary meristems was developed for successful in vitro propagation of *Sapindus mukorossi* an economically important tree.

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