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IN VITRO AND *IN VIVO* MICROGRAFTING OF *SANTALUM ALBUM* SHOOT TIPS

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Indian sandalwood (*Santalum album*) is valued for its fragrant heartwood, which yields an oil widely use in cosmetics, medicines and perfumes. Depletion of existing stands, failure to establish new stocks, and diseases suggest that mature trees are likely to be in short supply over the next decade. Since the natural regeneration of this tree is inadequate, there is a need to develop a reliable method of propagation.

Micrografting has been developed that consists of grafting under aseptic conditions of the miniaturized scion onto a grown rootstock (Jonard 1986). Micrografting has been successfully achieved in both fruit and forest trees (Oiyama 1992, Ewald & Kretzchmar 1996).

***In vivo* condition:** Freshly collected seeds of *S. album* were decoated, washed five times in water and treated with 2711.0 µM GA_3 (gibberellic acid) overnight. These seeds were used to germinate seedlings for rootstock.

***In vitro* condition:** The GA_3 treated seeds were thoroughly washed with distilled water, surface sterilized in 0.15% $HgCl_2$ for 8 min and washed with sterile distilled water under aseptic condition. These seeds were cultured on MS basal medium (Murashige & Skoog 1962), supplemented with 3% sucrose and 0.6% agar-agar (Glaxo-Brand). The cultures were maintained at 25±2 °C under 16-h photoperiod of light with an intensity of 40 µmol $m^2 s^{-1}$. *In vitro* grown 45-day-old seedlings were used as rootstock for *in vitro* micrografting.

Preparation of scions: Lateral branches (10 to 12 cm long) containing preformed apical and axillary buds were collected from 50- to 60-y-old candidate plus trees (CPTs). Two- to three-cm long shoots were isolated, treated with 0.1% fungicide (Bavistin) for 5 min, and washed thoroughly with distilled water. These treated shoots were used for *in vivo* micrografting as scions.

Soft nodal segments (3 to 4 cm long) containing preformed axillary buds were collected from 50- to 60-y-old CPTs, treated with detergent solution 0.1% Tween-20 for 5 min, followed by 0.1% fungicide (Bavistin) for 5 min and washed thoroughly with distilled water. Under aseptic condition, the nodal segments were treated with 70% alcohol for 45 s, sterilized with 0.05% $HgCl_2$ for 5 min and washed with sterile water. These nodal segments were cultured on MS basal medium supplemented with 11.12 µM BAP (benzylaminopurine).

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Cultures were incubated at 25±2 °C under 16 h daily duration of light with an intensity of 40 μmol m^{-2} s^{-1}. Within 4 weeks, 3 to 4 shoots proliferated from the axillary region of nodal segments. *In vitro* regenerated 1- to 2- cm long shoots were used as scions for *in vitro* micrografting.

***In vivo* micrografting:** Incision of 0.5 to 1.0 cm was made on the decapitated 45-day-old greenhouse grown seedling using a surgical blade. A drop of 1% DIECA (sodium diethyl-dithiocabamate) was placed on the wound. Two- to three- cm scion (shoot apex) collected from a CPT was inserted into the incision and elastic strip or paper bandage was applied to cover the grafted zone.

***In vitro* micrografting:** Under aseptic condition, 0.5- to 1-cm split was made on the top of the decapitated rootstock of the 45-day-old seedling using a sharp surgical blade. One- to two-cm long shoot apex scion derived *in vitro* was inserted into the stock incision. It was cultured on liquid half-strength MS medium with 3% sucrose. The radical of the above plant was pushed through the hole in the filter paper bridge as a support.

Maintenance of grafted plantlets: *In vivo* micrografted plants were covered with polythene bags and kept at 21±1 °C with continuous illumination and observed periodically to evaluate the survival of scion and rootstock. Elongation of scion was determined after eight weeks of grafting.

In vitro micrografted cultures were incubated in a culture chamber at 21±1 °C with a continuous illumination and observed periodically to evaluate the survival of scion and rootstock. The percentage of survival and the number of roots per shoot were determined after eight weeks of grafting. Adventitious shoots arising from rootstocks were removed aseptically with surgical scissors.

Hardening and transplantation: After six weeks of *in vitro* grafting, plants with at least two to three expanded leaves grown from scion were transplanted to root-trainers containing sterilized soil-rite (Keltech Energies Ltd. India). Polythene bags covered the top portion of the root-trainers and grafted plants were kept in the growth chamber of a polytunnel for two months. Well-established *in vitro* shoot apex grafted plants with two to four expanded leaves with axillary buds were transferred to the nursery.

The frequency of success in shoot apex grafting of *S. album* was highest in rootstock and shoots derived from *in vitro* method (Table 1). The probability of successful graft unions of both *in vitro* and *in vivo* methods increased with the length of scion material (Tables 1 & 2). Union between stock and scion tended to increase when the scion material had more leaf primordia along with the apical dome. However, the success of grafting declined as the age of the seedling increased, since it was observed that the hypocotyl region became narrower and hard. The *in vitro* and *in vivo* micrografting procedures developed here are simple and efficient. Using these techniques a large number of desired propagules or clones could be produced in a short time.

Table 1 Influence of length of explants on *in vitro* micrografting
(Data recorded at the end of eight weeks)

Component	Number of grafts	Length of scion (cm)	Number of successful grafts
Apical dome	40	0.4–0.5	4
Apical dome & two-leaf primordial	40	1.0–1.5	14
Apical dome & two- to four-leaf primordia	40	1.0–2.0	24

Table 2 Influence of length of explants on *in vivo* micrografting (Data recorded at the end of eight weeks)

Component	Number of grafts	Length of scion (cm)	Number of successful grafts
Apical dome	40	0.8–1.0	0
Apical dome & two-leaf primordia	40	1.0–1.5	0
Apical dome & four- to six-leaf primordia	40	1.0–2.0	12
Apical dome & above six-leaf primordia	40	2.5–3.0	18

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