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ORIGINAL ARTICLE

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Micropropagation of an endangered Indian sandalwood (*Santalum album L.*)

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Abstract *Santalum album* is known as East Indian sandalwood. It is the most economically important tree harvested for heartwood oil, and India is among the chief exporters of sandalwood and its products. Multiple shoots were induced from nodal shoot segments derived from a 50- to 60-year-old candidate plus tree (CPT) on Murashige and Skoog (MS) medium supplemented with 0.53 μM α-naphthaleneacetic acid (NAA) and 11.09 μM 6-benzylaminopurine (BA). In vitro differentiated shoots were multiplied on MS medium with 0.53 μM NAA, 4.44 μM BA, and additives: 283.93 μM ascorbic acid, 118.10 μM citric acid, 104.04 μM cystine, 342.24 μM glutamine, and 10% (v/v) coconut milk. New shoots were harvested repeatedly for up to three subculture passages on fresh medium at 4-week intervals. Microshoots treated with 98.4 μM indole-3-butyric acid (IBA) for 48 h produced roots on growth-regulator-free, quarter-strength MS basal salts medium with vitamin B_5 and 2% sucrose. In vitro root induction was achieved from microshoots pulsed with 1230 μM IBA for 30 min in soilrite rooting medium. The percentage of rooting in soilrite was higher than that for agar medium, and in vitro raised plants were established in the field and showed normal growth.

Key words Chandan · Tissue culture · Mature tree · Axillary shoot proliferation · In vitro and ex vitro rooting

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Introduction

Sandalwood or Chandan belongs to the Santalaceae family, a medium-sized evergreen hemi root parasitic tree, highly valued for its fragrant heartwood, which contains sandal oil that is used in perfumes, cosmetics, medicine, and also in agarbathi (incense sticks) industries (Srinivasan et al. 1992). *Santalum album* has the highest oil content (about 6%) of the species of the genus *Santalum*. The sandalwood and oil demand (80%–90%) in the international market has been fulfilled by Indian sandalwood for decades. However, the production of sandalwood declined from 4000 tons in 1950 to 2000 tons in 1990 and to about 1000 tons in 1999 (Ananthapadmanabha 2000). The acute shortage in supply and the demand imbalance resulted in the closure of several sandalwood-based industries in India and other Asian countries.

Santalum album is recalcitrant to in vivo and in vitro propagation, for which only limited success has been achieved so far (Sanjaya et al. 2003). Natural regeneration and artificial propagation occur mainly by seeds; however, seedlings are extremely heterozygous due to outcrossing. On the other hand, vegetative propagation is accomplished by grafting, air layering, and with root suckers, but the production of clones is inefficient and time consuming (Srimati et al. 1995). Previous reports on in vitro propagation have focused on adventitious bud regeneration from in vitro grown seedling explants: hypocotyl (Bapat and Rao 1978; Rao and Bapat 1992), endosperm (Sita et al. 1979), zygotic embryo (Rai and McComb 2002), and somatic embryogenesis through a callus phase (Sita et al. 1980). Although small numbers of plants were regenerated, the methods are still a long way from being optimized. Indeed, the bottleneck is in vitro rooting, which limits the widespread application of micropropagation techniques in sandalwood.

The prior selection of an elite genotype in the field and shoot multiplication cycles thereof without going through the callus phase more or less guarantees the production of true-to-type planting stock (Ahuja and Libby 1993). The dynamic and expanding knowledge on clonal propagation

of forest trees and application of plant tissue culture-based biotechnology has been employed in many forest trees for quality planting stock production (Mascarenhas and Muralidharan 1998; Sharma and Ramamurthy 2000; Beck and Dunlop 2001). In vitro clonal propagation of candidate plus tree (CPT) of *S. album* through axillary shoot proliferation has not been reported, as far as we are aware. Overexploitation, sandal spike disease (caused by a mycoplasma-like organism), failure of regeneration efforts, and illicit felling have narrowed the gene pool of this heritage species. There is, therefore, a need to develop clonal techniques to produce disease-resistant and high oil-yielding clones of CPTs.

Materials and methods

Explant collection and surface sterilization

A high oil-yielding (4.0%–5.0%) CPT of 50–60 years of age (Fig. 1A), was selected from the Institute of Wood Science and Technology campus, Bangalore, as a source of explants. Some of the branches were pruned periodically to obtain new sprouts. Nodal shoot segments (2.5–3.5 cm in length) with dormant axillary buds were collected from the CPT, dipped in a 0.1% (v/v) liquid detergent (Tween-80, BDH, Mumbai, India) for 5 min and washed four or five times with distilled water. Subsequently, the explants were surface sterilized with 0.075% (w/v) mercuric chloride (Hi-media, Mumbai, India) for 5–6 min and thoroughly washed six to eight times with sterile distilled water. Following surface sterilization, both exposed ends of the explants were trimmed and the remaining segment was inoculated vertically on the culture medium.

Effect of plant growth regulator and season on shoot development

In order to find out the best auxin and cytokinin and their optimum concentration for multiple shoot production and subsequent growth, various concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) were used in Murashige and Skoog (MS) medium (Murashige and Skoog 1962). The pH of the medium was adjusted to 5.8 with 0.1 N HCl or 0.1 N NaOH before autoclaving and before gelling it with 7.0 g l^{-1} agar-agar (Hi-Media, India). Molten medium (20 ml) was dispensed into 25 × 150-mm culture tubes (Borosil, Mumbai, India), which were capped with cotton plugs and autoclaved at 105 kPa and 121°C for 20 min. All the cultures were incubated at 25° ± 1°C with a 16-h photoperiod provided by cool white fluorescent lamps with light intensity of 60 μmol m^{-2} s^{-1} (Philips, Mumbai, India) and 60%–65% relative humidity (RH). Nodal shoot segments were collected from new sprouts on pruned branches of the CPT during the periods of November–January, February–April, May–July, and August–October and were cultured on MS medium with 0.53 μM NAA and 11.09 μM BA (MS1).

Shoot multiplication

Nodal shoot segments isolated from in vitro grown shoots on MS1 medium were subcultured on MS medium supplemented with different combinations of NAA and BA with or without the following additives: 283.93 μM ascorbic acid, 118.10 μM citric acid, 104.04 μM cystine, 342.24 μM glutamine, and 10% (v/v) coconut milk (MS2) for shoot multiplication. After 4 weeks, proliferated shoots higher than 2.0 cm and original explants were subcultured repeatedly up to five times on MS2 medium at 4-week intervals. Data were recorded for each subculture.

Root induction

In vitro raised shoots of 3.0–4.0 cm in length were used for in vitro root induction. Microshoots were pulse treated (0–72 h) with 98.4 μM indole-3-butyric acid (IBA) and subsequently transferred to MS3 medium [one quarter MS basal salts and vitamins of B_5 medium (Gamborg et al. 1968), sucrose 20.0 g l^{-1}, and agar-agar 7.0 g l^{-1}]. Cultures were incubated in the dark for 1 week and subsequently shifted to light at 28° ± 1°C with a 16-h photoperiod provided by cool white fluorescent lamps with light intensity of 60 μmol m^{-2} s^{-1} (Philips, India). Similarly, microshoots were treated with various concentrations of auxins viz: indole-3-acetic acid (IAA), IBA, NAA, and α-naphthoxyacetic acid (NOA) for 30 min and transferred to 400-ml culture bottles (with closures) containing autoclaved moist soilrite medium (50% peatmoss, 25% expanded perlite, and 25% vermiculite; Karnataka Explosives, Bangalore, India), and incubated at 28° ± 1°C with a 16-h photoperiod light. The percentage of root induction, number of roots, and root length were recorded at the end of the eighth week.

Hardening and transfer of plantlets to the containers

In vitro rooted shoots were carefully removed from the culture medium and transferred into 400-ml glass bottles containing autoclaved moist soilrite. The transplanted plantlets were maintained at 28° ± 1°C and 60 μmol m^{-2} s^{-1} light intensity for a 16-h photoperiod. The relative humidity in the containers with rooted plants was reduced gradually, and after 2 months the plantlets (8–10 cm in shoot length) were transplanted into 450-ml single-cell root trainers with potting medium (compost 50%, sieved sand 40%, garden soil 10%). The plants were sprayed with one quarter MS macro and microelements at 15-day intervals to boost the growth at hardening. Six-month-old in vitro rooted plants were planted in the field; overall procedures and different stages involved in micropropagation are shown in Fig. 2.

Data collection and statistical analysis

A completely randomized block design was followed for all experiments. Each treatment consisted of 12 replications and each experiment was repeated twice, in order to con-

firm the results. The data were analyzed by one-way analysis of variance (ANOVA) by using Sigmastat statistical software (Jindal, New Delhi, India), and the mean values were compared using the critical difference (CD).

Results

Shoot initiation

The combined use of BA and NAA significantly affected the shoot length. The nodal shoot segments exhibited maximum bud break and the highest number of shoots (Fig. 1B) when cultured on MS1 medium with 0.53 μM NAA and 11.09 μM BA (Table 1). Higher concentrations of BA yielded more shoots; however, these shoots were dwarfed and retarded in growth. The nodal shoot segments cultured during November–January yielded maximum bud break (91.67%) and the highest number of shoots per explant (Fig. 3). Poor shoot development was observed when explants were cultured in May–July on optimum shoot-induction medium.

Shoot multiplication

As shown in Table 2, the addition of additives in the medium significantly enhanced the shoot number per shoot segment on MS2 medium supplemented with 0.53 μM NAA and 4.44 μM BA (Fig. 1C), as compared with medium without additives. Indeed, shoots cultured on additive-rich medium were more stabilized in growth than those cultured on additive-deficient medium. Although higher concentrations of BA in the medium increased the shoot number, the shoot length was drastically reduced. Most of these shoots were unable to promote shoot multiplication or rooting. Subculturing within 4 weeks was essential to maintain healthy shoot growth; moreover, repeated subculture of the original explant (nodal shoot segment) every 4 weeks on fresh MS2 medium produced a crop of shoots (Fig. 1D). Enhanced rate of shoot multiplication was observed initially up to the third subculture, and gradual decline was recorded in the fourth and fifth subcultures (Fig. 4).

In vitro rooting

The duration of pulse treatment had significant effect on rooting capacity; the best results were obtained with

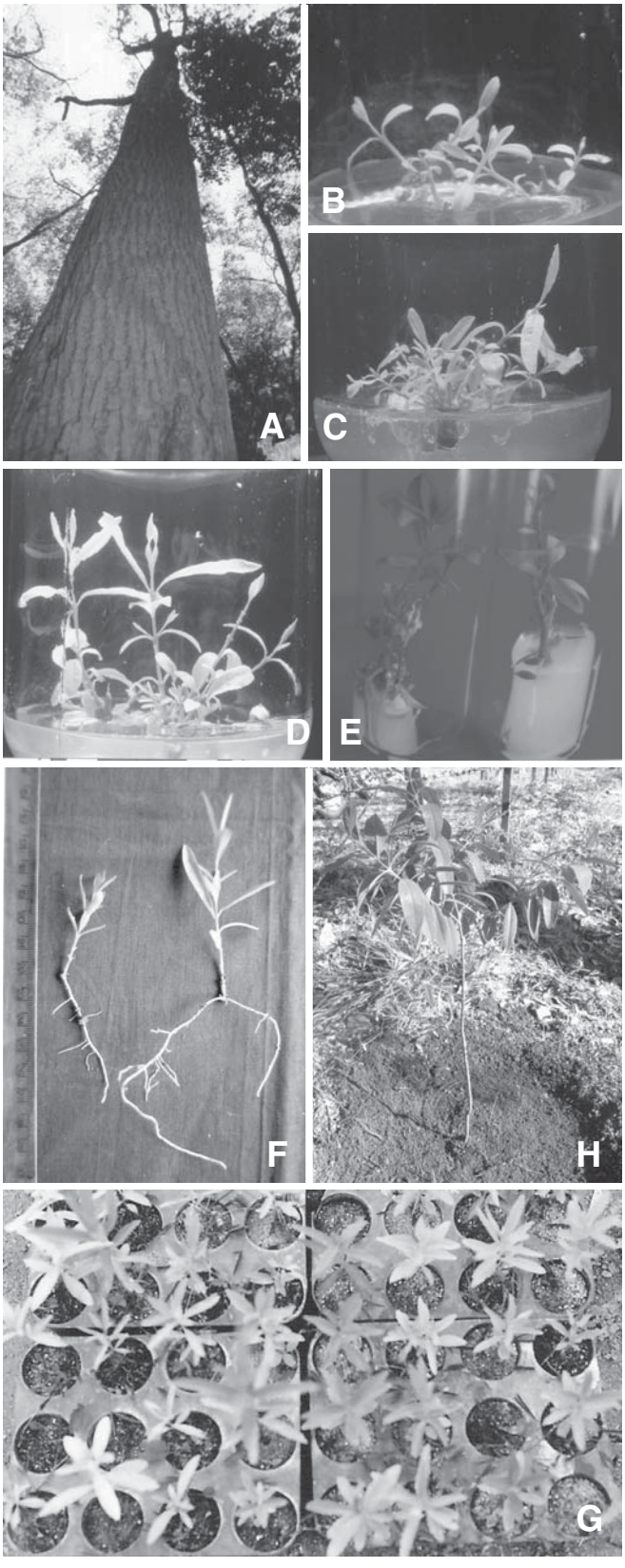

Fig. 1A–H. Different stages of in vitro propagation of sandalwood (*Santalum album* L.). **A** High oil-yielding (4.0%–5.0%) CPT of 50–60 years of age. **B** Development of multiple shoots from nodal shoot segments on MS1 medium. **C** Further shoot multiplication during subculture on MS2 medium. **D** Crop of shoots from repeated subculture of original explant on MS2 medium at interval of 4 weeks. **E** In vitro rooting of pulse treated shoots with 98.4 μM IBA for 48 h on MS3 medium after 8 weeks. **F** In vitro rooted microshoots in soilrite medium after pulse treatment with 1230 μM IBA (for 30 min). **G** Six-month-old plants maintained in root trainers at nursery. **H** One-year-old in vitro raised plant in the field

Table 1. Effect of different concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) in Murashige and Skoog (MS) medium on the shoot induction of *Santalum album*

Treatments (μM)		Number of shoots	Shoot length (cm)
NAA	BA		
0.00	0.00	0.42 ± 0.25^{a}	0.35 ± 0.20^{a}
0.00	2.21	0.67 ± 0.49^{ab}	0.59 ± 0.42^{ab}
0.53	2.21	0.88 ± 0.64^{abc}	0.74 ± 0.46^{bc}
1.07	2.21	0.75 ± 0.54^{abc}	0.53 ± 0.29^{ab}
2.68	2.21	0.71 ± 0.48^{abc}	0.48 ± 0.26^{ab}
0.00	4.44	1.04 ± 0.39^{bc}	0.98 ± 0.37^{cd}
0.53	4.44	1.25 ± 0.46^{c}	1.23 ± 0.42^{def}
1.07	4.44	1.17 ± 0.32^{bc}	1.00 ± 0.30^{cd}
2.68	4.44	1.13 ± 0.38^{bc}	1.06 ± 0.31^{de}
0.00	11.09	2.92 ± 0.47^{d}	1.73 ± 0.13^{hi}
0.53	11.09	4.92 ± 0.12^{j}	3.23 ± 0.06^{l}
1.07	11.09	3.46 ± 0.22^{def}	2.10 ± 0.18^{k}
2.68	11.09	3.33 ± 0.28^{de}	2.02 ± 0.08^{j}
0.00	22.18	4.63 ± 1.29^{ij}	1.31 ± 0.09^{efg}
0.53	22.18	4.42 ± 1.12^{j}	1.81 ± 0.08^{i}
1.07	22.18	4.58 ± 1.21^{hij}	1.60 ± 0.15^{hi}
2.68	22.18	4.33 ± 1.28^{hi}	1.17 ± 0.06^{def}
0.00	44.36	3.92 ± 1.56^{fgh}	1.13 ± 0.04^{def}
0.53	44.36	4.25 ± 1.85^{ghi}	1.54 ± 0.03^{gh}
1.07	44.36	3.83 ± 1.01^{efgh}	1.33 ± 0.06^{fg}
2.68	44.36	3.75 ± 0.80^{efg}	0.99 ± 0.15^{cd}
		CD = 0.55	CD = 0.26

Treatments followed by the same letters within the columns are not significantly different. Data recorded at the end of the fourth week; Number of replicates for each treatment: 24
CD, critical difference

Table 2. Effect of different concentrations of NAA and BA with additives in MS medium on shoot multiplication during subculture in *Santalum album*

Treatments (μM)		Number of shoots without additives	Number of shoots with additives	Mean shoot length without additives (cm)	Mean shoot length with additives (cm)
NAA	BA				
0.00	0.00	0.54 ± 0.26^{a}	0.67 ± 0.23^{a}	0.30 ± 0.07^{a}	0.35 ± 0.07^{a}
0.00	0.44	0.79 ± 0.17^{ab}	0.92 ± 0.08^{ab}	0.40 ± 0.03^{abc}	0.45 ± 0.02^{ab}
0.26	0.44	1.08 ± 0.34^{abc}	1.25 ± 0.20^{bc}	0.53 ± 0.06^{abcd}	0.60 ± 0.02^{bc}
0.53	0.44	1.17 ± 0.41^{abc}	1.38 ± 0.24^{cd}	0.64 ± 0.08^{bcd}	0.68 ± 0.05^{c}
0.00	2.21	1.21 ± 0.43^{bc}	1.42 ± 0.25^{cd}	0.39 ± 0.03^{ab}	0.46 ± 0.01^{ab}
0.26	2.21	1.38 ± 0.51^{bc}	1.58 ± 0.25^{cd}	1.18 ± 0.30^{e}	1.36 ± 0.10^{ef}
0.53	2.21	1.54 ± 0.52^{c}	1.75 ± 0.20^{d}	1.29 ± 0.49^{ef}	1.50 ± 0.25^{fg}
0.00	4.44	2.46 ± 1.82^{d}	2.92 ± 0.95^{e}	1.08 ± 0.29^{e}	1.28 ± 0.14^{de}
0.26	4.44	2.50 ± 0.17^{d}	3.04 ± 1.00^{e}	1.54 ± 0.44^{f}	1.79 ± 0.12^{h}
0.53	4.44	2.83 ± 0.41^{d}	4.63 ± 0.59^{h}	2.04 ± 0.75^{g}	2.61 ± 0.10^{i}
0.00	11.09	2.75 ± 0.20^{d}	3.42 ± 0.60^{fg}	1.07 ± 0.18^{e}	1.18 ± 0.07^{d}
0.26	11.09	2.88 ± 0.38^{d}	3.58 ± 0.59^{g}	1.17 ± 0.27^{e}	1.35 ± 0.16^{def}
0.53	11.09	2.92 ± 0.73^{d}	3.33 ± 0.93^{efg}	1.50 ± 0.12^{f}	1.63 ± 0.10^{gh}
0.00	22.18	2.50 ± 1.74^{d}	2.92 ± 0.95^{e}	0.66 ± 0.08^{cd}	0.70 ± 0.06^{c}
0.26	22.18	2.67 ± 0.80^{d}	3.08 ± 0.86^{e}	0.67 ± 0.11^{d}	0.73 ± 0.09^{c}
0.53	22.18	2.75 ± 0.93^{d}	3.17 ± 0.93^{ef}	0.66 ± 0.07^{cd}	0.75 ± 0.06^{c}
		CD = 0.65	CD = 0.43	CD = 0.26	CD = 0.17

Treatments followed by the same letters within the columns are not significantly different. Data recorded at the end of the fourth week. Number of replicates for each treatment: 24. Additives: ascorbic acid (283.93 μM), citric acid (118.10 μM), cystine (104.04 μM), glutamine (342.24 μM), and coconut milk 10% (v/v)

microshoots treated with 98.4 μM IBA for 48 h and subsequently cultured on MS3 medium (Fig. 1E, Table 3). However, shoots exposed to prolonged pulse treatment leads to necrosis, and no root induction was observed in the control. Incubation of auxin-treated shoots in the dark for 1 week at $28° \pm 1°C$ favored rooting more so than the same conditions with light (data not shown). Out of the different auxins (IBA, NAA, NOA, and IAA) tested, the microshoots treated with 1230 μM IBA (for 30 min) showed maximum rooting (50%) in soilrite medium (Fig. 1F), and no root induction was noted for IAA and in the control without auxin treatment (Table 4). These results suggest that rooting ability and recovery of plants were easier and quicker in soilrite medium than in agar medium.

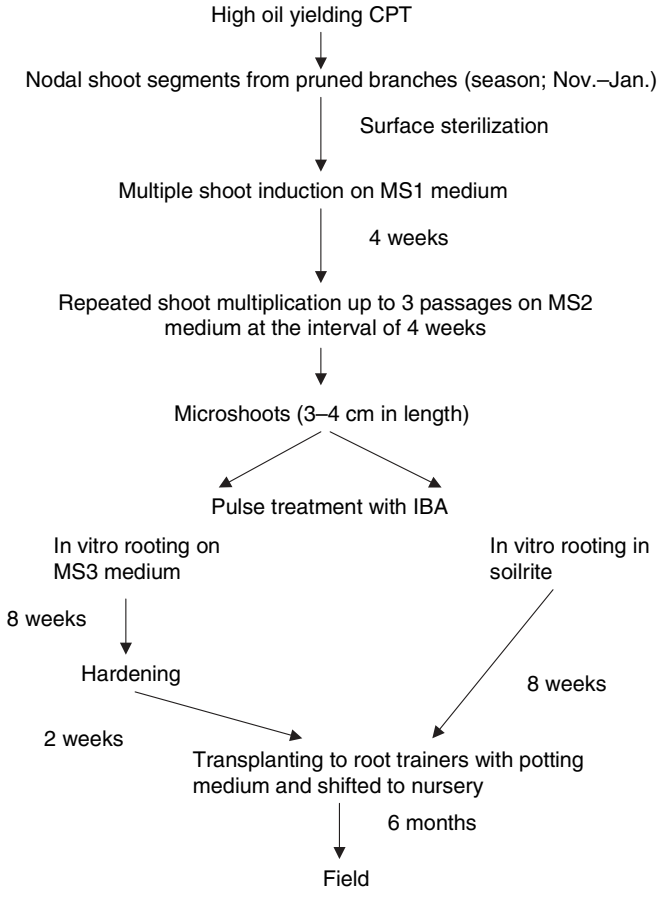

Fig. 2. A schematic representation of the different steps in micropropagation of *Santalum album* L. *CPT,* candidate plus tree; *IBA*, indole-3-butyric acid

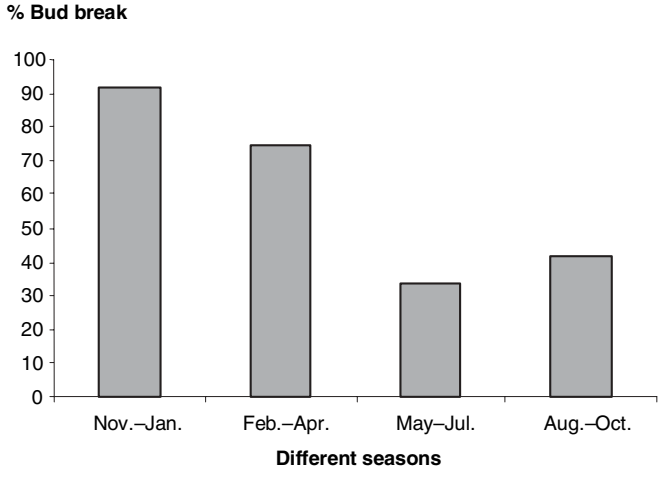

Fig. 3. Effect of explant harvest period (season) on multiple shoot induction on MS medium supplemented with 0.53 μM α-naphthaleneacetic acid (NAA) and 11.09 μM 6-benzylaminopurine (BA) in *Santalum album*. Data recorded at the end of the fourth week; number of replicates for each treatment: 24. The data were analyzed by one-way analysis of variance (ANOVA), and the mean values were compared using the critical difference

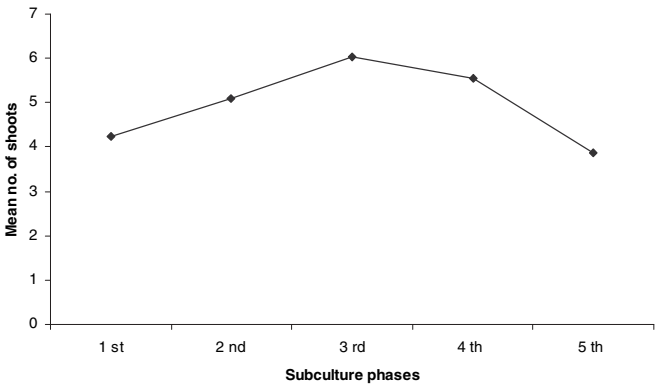

Fig. 4. Effect of repeated subculture of original explant on MS medium with 0.53 μM NAA, 4.44 μM BA and additives in *Santalum album*. Additives: ascorbic acid (283.93 μM), citric acid (118.10 μM), cystine (104.04 μM), glutamine (342.24 μM), and coconut milk 10% (v/v). Data recorded at the end of the fourth week; number of replicates for each treatment: 24. The data were analyzed by one-way ANOVA, and the mean values were compared using the critical difference

Acclimatization and establishment of plants

In vitro root induction and hardening were accomplished simultaneously in soilrite, whereas in vitro rooted shoots in agar-based medium were transferred into soilrite and new growth was observed within 2–3 weeks. It was observed that semicontrolled conditions of temperature (30° ± 5°C) and humidity (70%–80% RH) was necessary at hardening. No plantlet mortality was observed after transplanting to containers for both rooting methods (Fig. 1G). In the field, all tissue culture-derived plants as well as seedlings (from seeds) exhibited a 100% survival rate and normal growth. No significant difference in the height and stem girth of tissue culture-raised plants and seed-raised seedlings was observed within 1 year (Fig. 1H).

Discussion

The use of preexisting buds for propagation reduces the possibility of variation among the progeny and therefore can be safely applied for rapid propagation of field-grown CPTs of sandalwood. We optimized shoot multiplication conditions and novel rooting techniques for mass clonal propagation without interference of callus. This method is quite common for the propagation of *Fragaria indica* (Indre and Dhar 2000) and *Acacia mearnsii* (Marguerite et al. 2001). Addition of a low concentration of NAA promoted shoot growth by counteracting the inhibitory effect of BA on shoot elongation. The combined use of BA and NAA was emphasized in micropropagation of *Ficus carica* (Kumar et al. 1998) and *Syzygium travancorium* (Ajith et al. 1999). The season of explant collection also influenced shoot development from individual explants; this fact may be due to long flowering and seed setting habit of trees. These observations are in concordance with Sharma et al.

Table 3. Effect of duration of pulse treatment of indole-3-butyric acid (IBA) (98.4 μM) on in vitro root induction from microshoots of *Santalum album* on one quarter MS medium

Pulse treatment (h)	Root induction (%)	Number of roots ± SE (cm)	Root length ± SE (cm)
Control (water)	00.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1	00.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5	04.16	0.17 ± 0.32^{a}	0.15 ± 0.25^{a}
10	08.33	0.71 ± 0.17^{c}	0.62 ± 0.99^{b}
24	33.34	0.58 ± 0.69^{bc}	0.53 ± 0.56^{b}
48	41.67	2.18 ± 0.06^{d}	3.32 ± 0.67^{c}
72	08.16	0.46 ± 0.78^{ab}	0.47 ± 0.87^{a}
		CD = 0.48	CD = 0.50

Treatments followed by the same letters within the columns are not significantly different. Data recorded at the end of the eighth week; number of replicates for each treatment: 24. After pulse treatment, microshoots were cultured on hormone-free quarter-MS basal medium (B_5 vitamins, sucrose 2%)

Table 4. Effect of auxin type and their concentrations on in vitro rooting of microshoots of *Santalum album* in soilrite as rooting medium

Treatments (μM)	Root induction (%)	Number of roots ± SE	Root length ± SE (cm)
Control	–	$-^{a}$	$-^{a}$
IBA			
246	16.67	0.42 ± 0.95^{bc}	0.44 ± 0.05^{abc}
492	33.34	0.79 ± 0.39^{c}	0.88 ± 0.08^{c}
1230	50.00	2.46 ± 0.42^{d}	3.58 ± 0.05^{d}
2460	16.67	0.29 ± 0.08^{ab}	0.41 ± 0.04^{abc}
NAA			
268.5	–	$-^{a}$	$-^{a}$
537	–	$-^{a}$	$-^{a}$
1342.5	–	0.21 ± 0.06^{ab}	0.38 ± 0.09^{ab}
2685	8.33	0.13 ± 0.20^{ab}	0.24 ± 0.04^{ab}
NOA			
247	–	$-^{a}$	$-^{a}$
494	8.33	0.17 ± 0.02^{ab}	0.20 ± 0.07^{ab}
1235	25.00	0.38 ± 0.51^{b}	0.66 ± 0.04^{bc}
2470	16.67	0.25 ± 0.07^{ab}	0.45 ± 0.03^{abc}
IAA			
253.5	–	$-^{a}$	$-^{a}$
507	–	$-^{a}$	$-^{a}$
1267.5	–	$-^{a}$	$-^{a}$
2535	–	$-^{a}$	$-^{a}$
		CD = 0.37	CD = 0.48

Treatments followed by the same letters within the columns are not significantly different. Data recorded at the end of the eighth week; number of replicates for each treatment: 24. Duration of auxin treatment: 30 min

NOA, α-naphthoxyacetic acid; IAA, indole-3-acetic acid

(2003) in *Crataeva adansoni*, where shoot initiation is highly influenced by season. Incorporation of additives in shoot multiplication medium improved the quality and had auxiliary effect on shoot multiplication, suggesting that the parasitic nature may have complex nutrition requirements. However, the exact role of additives on enhanced shoot growth in relation to the parasitic nature of sandalwood is poorly understood. The use of additives in the medium for the development of quality shoots has been emphasized elsewhere (Rathore et al. 1993; Conklin 2001).

Maturation of sandalwood tree is usually accompanied by a decline in vegetative vigor. Adult trees require rejuvenation treatments for recovering morphological and physiological conditions favorable to rooting (Von Aderkas and Bonga 2000). In the present investigation, the rejuvenation of shoots was achieved under in vitro conditions by repeated subculture, and rejuvenated shoots responded better under both rooting conditions tested. In *Prosopis cineraria,* Shekawat et al. (1993) demonstrated the effect of rejuvenation on in vitro rooting by using subcultured shoots. The application of IBA by a pulse treatment was used in adventitious rooting of *Maytenus emarginata* (Rathore et al. 1992) and *Tectona grandis* (Siril and Tewari 1999). Although rooting was achieved in both methods, the percentage of rooting was higher in soilrite medium; it is likely that better aeration, high concentration of IBA, and short exposure might have triggered the genes responsible for the production of proteins required for adventitious root formation. Further detailed studies are necessary to dissect parasitism and the role of auxin requirement in rooting at molecular level. The present investigation is another step forward demonstrating the application of root-trainer tech-

nology and compost as a major potting medium ingredient for quality plant production; finally it helps in steady establishment of in vitro raised plants in field conditions. Because *Santalum album* is a slow-growing species it is too early to evaluate the superiority of in vitro raised plants; some noticeable difference in overall performance may arise in the future. In summary, in vitro rooting in soilrite is a cost-effective, innovative method for the rapid clonal propagation of *S. album* and other tree species.

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