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Microcloning of sandalwood *(Santalum album* Linn.) from cultured leaf discs

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Leaf discs of sandalwood were cultured on different fortifications of MS medium to judge their *in vitro* response. *In vitro* morphogenesis, (somatic embryogenesis and/or organogenesis) leading to plantlet regeneration was influenced considerably by plant growth regulators. Among various medium experimented MSD.5Td (MS + 1.0mg.l^{-1} 2,4-D + 0.5mg.l^{-1} TDZ) supported maximum direct somatic embryogenesis (11.44%), indirect somatic embryogenesis (54.23%) and mean numbers of somatic embryo(s) per explant (160.08), whereas culture medium MS2D.5Td (MS + 2.0mg.l^{-1} 2,4-D + 0.5mg.l^{-1} TDZ) promoted indirect organogenesis (20.38%). Inoculation medium MS2Td.5N (MS + 2.0mg.l^{-1} TDZ + 0.5 mg.l^{-1} NAA) proved superior for direct organogenesis (9.48%) and regeneration of plantlets *via* direct organogenesis (36.69%). MS medium fortified with 2.0 mg.l^{-1} TDZ and 1.0 mg.l^{-1} GA_3 proved superior for plant regeneration *via* somatic embryogenesis (163.63%) while regeneration medium MSTd.5GA.5N (MS +1.0 mg.l^{-1} TDZ+ 0.5 mg.l^{-1} GA_3 +0.5 mg. l^{-1} NAA) regenerated plantlets *via* indirect organogenesis (141.25%). The *in vitro* raised plantlets were acclimatized and established successfully in the field.

Key words: *Santalum album*, leaf disc culture, organogenesis, somatic embryogenesis, plantlet regeneration.

Abbreviations: MS-Murashige and Skoog medium; BA-6-benzylaminopurine; TDZ-Thidizuron; Kn-Kinetin; NAA-α-Naphthalene acetic acid; 2, 4-D- 2, 4-dichlorophenoxyacetic acid; 2, 4, 5-T - 2, 4, 5- trichlorophenoxyacetic acid; IBA-Indole-3-butyric acid; GA_3: Gibberelic acid.

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Introduction

Santalum album L. 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, and also in Agarbathi (incense sticks) industries. *Santalum album* has the highest oil content (about 6%) of the species of the genus *Santalum*. The sandalwood and its oil demand (80%-90%) in the international market have been fulfilled by Indian sandalwood for decades. Production of Sandalwood has fallen sharply over the past decades due to the mushrooming of illegal sandalwood processing units. Conventional breeding of sandalwood for introgression of new genetic information can be an expensive and difficult task because of its long generation time, sexual incompatibility and heterozygous nature (Rugkhla, 1997). *In vitro* regeneration techniques can be used to encounter difficulties of traditional propagation methods by microcloning of superior lines.

In vitro propagation of sandalwood was attempted as early as 1963. Induction of callus from mature endosperm on modified white's medium was reported, but the callus did not proliferate further (Rangaswamy and Rao, 1963). Induction of callus, differentiation of embryoids and subsequent development into plantlets from endosperm (immature seeds) has been reported by Lakshmi Sita *et al*. (1980), which can be used to produce economically superior sandal plants. As yet various explants such as embryo (Rangaswamy and Rao, 1963), hypocotyls (Bapat and Rao, 1979; Lakshmi Sita *et al*., 1979; Rao and Bapat, 1992), shoot tip (Lakshmi Sita and Raghava Ram, 1995), nodal segment (Bapat and Rao, 1979; Lakshmi Sita *et al*., 1979; Rao and Bapat, 1992; Rugkhla and Jones, 1998; Sarangi *et al*., 2000; Sanghamitra and Chandni, 2010), leaf disc (Mujib, 2005), endosperm (Lakshmi Sita *et al*., 1979; Bapat and Rao, 1979; Rao and Bapat, 1992) and cell suspension cultures (Dey, 2001) with varying degree of success have been used for rapid multiplication of sandal trees.

There is only one published report on shoot bud formation directly from *in vitro* cultured leaves for sandal (Mujib, 2005). In woody species relatively little information is available on shoots formed directly on leaves without a callus phase (Preece *et al*., 1993). Leaves obtained from *in vitro* grown plants provide a useful source of explants which eliminate the risk of contamination. Furthermore, simultaneous direct shoot bud formation on such leaves will reduce the possibilities of genetic variation common in plants regenerated from cultured cells or tissues (Mujib, 2005). However, a systematic study on the effects of combinations of plant growth regulators on morphogenesis is still insufficient, which may overlook the potential combinations of certain plant growth regulators that are more suitable. Present study has been undertaken to

develop a potential system of *in vitro* regeneration from leaf explant *via* somatic embryogenesis and to understand the possible role of plant growth regulators in deciding mrophogenic pathway.

Materials and methods

To begin with a preliminary experiment, leaf discs of sandalwood were cultured on basal MS media (Murashige and Skoog, 1962) fortified with three different auxins, *namely*: 2,4-D, NAA and 2,4,5 T and three cytokinins *viz*: BAP, kinetin and TDZ in varying concentrations to find out better *in vitro* response. During preliminary experiments, it was observed that an auxin or a cytokinin alone was not adequate for inducing morphogenesis in higher frequencies (data not presented). Consequently, for concluding experiment basal MS medium was amended with different concentrations of BAP, TDZ and Kn in combination with NAA and 2,4-D in varying concentrations. Apart from MS basal macro and micro salts, vitamins, all the media were supplemented with 30 $g.l^{-1}$ sucrose, 7.5 $g.l^{-1}$ agar and pH was adjusted to 5.8±0.1. Readymade MS basal medium, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

Leaf discs were obtained from *in vitro* grown regenerants (3-4-weeks-old germinated seed) as well as plants propagated in field. For germination, the seed were surface sterilized in 0.1% $HgCl_2$ for 4 min, and rinsed 3 times in sterile double distilled water. All the cultures were grown on MS basal medium supplemented with 0.8% agar powder. For *in vitro* grown regenerants, no surface sterilization was applied; whereas for sticks collected from orchard an intensive surface sterilization was adopted. For this purpose tender shoots were excised from mother plant and first and second leaves of tender shoots were collected in distill water and washed under running tap water for 30 minutes. Then leaves were placed into double distilled water containing 2% Tween 20 (v/v) for 20 minutes to remove the adhering fine particles. The cleaned leaves were then treated with 1% of Bavistin® (BASF, Germany) for 10 minutes in horizontal shaker at 30 rpm. The cleaned leaves were then treated with 70% (v/v) ethanol for 1 minute followed by a treatment of aqueous solution of 0.2% $HgCl_2$ for 10 minutes with initial vacuum of 100 psi. Finally, the leaves were subjected for 4-5 rinsing with sterile double distilled water and were cut into small square pieces (5-8 mm) with sharp-edged scalpel and cultured abaxial (i.e. the smooth ventral side) in petridishes containing culture media. In each petridish, 7-8 pieces of leaf discs were plated. Petridishes containing cultures sealed with Lab film (Parafilm®) were incubated under complete darkness at 25±2°C for one week. Later *in vitro* cultured explants were subjected to

temperature regime of 25 ± 2 ^{o}C in a growth room and were provided with cool, white fluorescent light with a 16-h photoperiod.

After 4-5 weeks of initial culturing, somatic embryoids and calli were transferred to MS regeneration medium fortified with different concentrations and combinations of plant growth regulators (BAP, TDZ, Kn and NAA in varying concentrations in combination with GA_3), 20 g. l^{-1} sucrose and 7.5 g.l^{-1} agar. However, in case, cultures forming organ directly on explant surface were sub-cultured on same initial medium for regeneration of plantlets. Cultures were kept at 25±2^{o}C and 12 hr photoperiod. Once root formation was not attained on regeneration medium, plantlets were subsequently transferred to MS rooting medium supplemented with different concentrations of IBA, NAA, BA, Kn and GA_3 (alone as well as in combinations), 15.0 g. l^{-1} sucrose and 7.5 g. l^{-1} agar. For regeneration and rooting, reduced level of sucrose was used on the basis of work conducted by various scientists as well as preliminary experiments in this laboratory. Plants uprooted from cultures and thoroughly washed with running tap water to remove the adhering agar were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were placed in Environmental Growth Chamber under 30±2°C and 65±5% RH for 15-20 days for acclimatization. Acclimatized plants were then transferred to Green House for 30 days for hardening before transplanting to the field. The experiment was laid out in Completely Randomized Design with two replications. Per replication approximately 100-120 leaf discs were excised and cultured on each media. The arc-sine transformation was made before the analysis of data, since all data were in percentage. In cases, where values exceeded more than 100% *Log* transformation was adopted.

Results and discussions

In the present study, leaves measuring 0.5-1.5 cm in length wounded with all sides, showed higher *in vitro* responses. Longer leaves without wounding showed a total loss of morophogenic potential. It was also noted that leaf discs obtained from the apical meristematic region showed better *in vito* response as compared to explants obtained from remaining leaf lamina. Similar results have also been addressed by Mujib (2005) in sandalwood leaf disc culture. Furthermore, orientation and positioning of the leaf explant on nutrient media had a significant collision on shoot bud development. In present investigation, leaf discs cultured horizontally with rough surface in contact with culture medium showed a higher *in vitro* response as compared to vertically with smooth surface placed in culture medium. This may be due to differential physiological gradients especially the endogenous levels of plant growth

regulators were the most important, existing in different regions of the leaf are probably involved in such discrepancies morphogenetic responses (Wernicke and Milkovits, 1986; Mujib *et al*., 1996; Mujib, 2005).

Leaf discs of sandal were cultured on different fortifications of MS medium. Although, shoots and roots or shoots, roots and somatic embryos have been observed on the same tissue, it is generally considered that the process leading to their appearance are mutually exclusive *i.e*. cells either committed to the organogenic or to the embryogenic pathway. In present investigations, most of the cultures preferred indirect mode of regeneration (indirect somatic embryogenesis and /or organogenesis) followed by direct mode (direct somatic embryogenesis/organogenesis). This phenomenon was completely decided by relative concentrations and proportions of an auxin to cytokinin present in culture medium. The first response of all the leaf discs cultures was similar after 3-5 days independent from culture media. All explants became swollen and no callus proliferation was evident during first few days. After 7-10 days of culture, callus formation was usually observed from the cut edges. Embryoid formation started after approximately 15 days from initial culturing (Fig. 1 A). The embryoid like structures were rounded with irregular outlines usually appeared in clusters (Fig. 1 A). Such somatic embryos germinated after transfer into regeneration medium (Fig. 1 B-C). In direct organogenesis, adventitious structures developed on explant surface (Fig. 1 D-E). Gradually, these adventitious structures developed into multiple shoots. In indirect organogenesis, shootlets shaped from the protuberances or nodular structures arising on the surface of the callus (Fig. 1 F). Shoot formation started about 10 days from initial culturing (Fig. 1 G-H). In cases where there was no root formation, shoots were transferred to the rooting medium for induction of *in vitro* rooting (Fig. 1 I). Various shoot forming calli were able to produce one or many plantlets at a time. Complete plantlets regenerated *via* embryogenesis and shoots developed *via* organogenesis were also counted as regenerated plantlets since they gave rise to complete plantlets after rhizogenesis on rooting medium. Rooted plantlets were elongated and transferred (Fig. 1 J) for hardening in Green House. Later these regenerants were transferred to the Net House for 25-30 days (Fig. 1 K) and field (Fig. 1 L) for acclimatization. The plants, after survival in the net house conditions, were evaluated visually on the basis of their appearance. Although the traits were not scored quantitatively, regenerated plants were found phenotypically normal and true to the type.

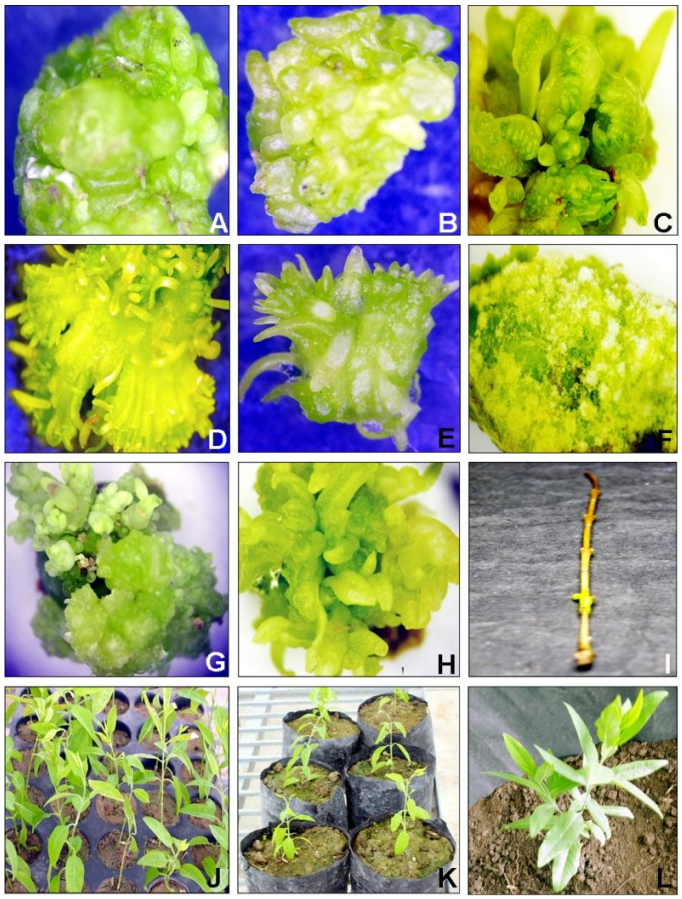

Fig. 1. Plant regeneration from leaf disc culture of sandalwood: A. Initiation of globular somatic embryos; B-C Germination of somatic embryo; D-E. Multiple shoot induction *via* direct organogenesis; F. Initiation of protuberances on surface of callus mass; G-H. Multiple shoot initiation *via* indirect organogenesis; I. *In vitro* rooting of regenerants; J. Hardening of regenerants in Green House; K. Hardening of regenerants in Net House and .L. Plants transferred in Field.

The analysis of variance (Table 1-2) revealed highly significant ($p<0.01$) differences between the response of nutrient media combinations in terms of overall direct and indirect somatic embryogenesis, average number of somatic embryo formation, direct and indirect organogenesis and plantlet regeneration. It indicates the presence of the considerable amount of variability amongst different culture media combinations.

Effects of different culture media combinations on somatic embryogenesis are presented in Table 1. Results revealed that auxin 2,4-D induced callus in higher frequencies at concentration ranging from 1.0-3.0 $mg.l^{-1}$. The size of callus enlarged with an increased level of 2,4-D up to 4.0 $mg.l^{-1}$.

Beyond this concentration the calli were turned into dark black colour and cell mortality was observed from cultured tissues. This finding is in accordance with the findings of Rugkhla and Jones (1998). Direct and indirect somatic embryogenesis and average number of somatic embryo per explant in higher proportion were attained on culture medium containing relatively higher proportion of 2,4-D (1.0-2.0 $mg.l^{-1}$) in combination with a lower proportion of TDZ (0.5 $mg.l^{-1}$). This revealed that a combination of higher proportion of 2,4-D with TDZ is necessarily required for induction of somatic embryogenesis. Similar finding have also been addressed by Rugkhla and Jones (1998) in sandalwood, Lu (1993) and Huetteman and Preece (1993) in watermelon, muskmelon, geranium and grape. In sandalwood, the requirement of TDZ for somatic embryo induction was quite low compared to other species. TDZ at low concentrations has been reported to be more efficient in inducing somatic embryogenesis than other cytokinins, particularly in recalcitrant woody species (Huetteman and Preece, 1993). The reasons for the high activity of TDZ in woody species have not been investigated at the physiological or molecular level. A carbon isotope study showed that TDZ was very stable in the culture media and persistent in plant tissue (Mok and Mok, 1985). It may be suggested that TDZ stabilized the internal optimum balance of cytokinin and auxin required for induction and expression of somatic embryogenesis (Saxena *et al.,* 1992; Lu, 1993). Furthermore, although TDZ may induce somatic embryogenesis solely but this is more effective when applied with an auxin 2,4-D.Howerver, other auxins NAA and 2,4,5-T was not found as effective as 2,4-D for inducing somatic embryogenesis in sandal. This is in accordance to findings of Rugkhla and Jones (1998).

Effects of different culture media combinations on organogenesis are presented in Table 1. Higher direct organogenesis and plantlet realization from direct organogenesis in the range of 32.13% - 36.69 % was achieved with culture medium supplemented with 2.0 $mg.l^{-1}$ TDZ/BA and 0.5-1.0 $mg.l^{-1}$ NAA. However, addition of higher concentration of kinetin in culture medium drastically reduced the plantlet regeneration to as low as 4.20%. In earlier studies both adenine (BA and Kn) and phenyl urea derivatives (TDZ) were used for sandalwood tissue culture. Regeneration of multiple shoots directly has been reported earlier in response to BA (Lakshmi Sita *et al.,* 1979; Rao and Bapat, 1992; Mujib, 2005), kinetin (Lakshmi Sita *et al*., 1979; Rao and Bapat, 1992) and TDZ (Rugkhla and Jones, 1998). However, the requirement for exogenous auxin and cytokinin in the process varies with the tissue system, apparently depending on the endogenous levels of the hormones present in the tissue (Norstrog, 1970). Moreover, the formations of adventitious organs depend on the reactivation of genes concerned with the organogenic phase of

development. Culture medium amended with higher proportion of 2,4-D in association with lower concentration of TDZ supported formation of organogenic calli from callus mass.

When cultures did not form plantlets on induction medium, they were subsequently transferred to regeneration medium (Table 2). Regeneration medium MS2TdGA proved to be the most responsive for the regeneration of plantlets from somatic embryos (163.61%). The system described here required different hormonal combinations and concentrations from those previously reported to obtain the most normal somatic embryos and their best germination. Rao and Bapat (1995) used media containing. IAA and BAP for multiplication and conversion to plantlets and noted the problem of low conversion frequency. In this study, mature somatic embryos needed to be germinated in media containing GA_3, otherwise many abnormal somatic embryos were obtained and this resulted in a low conversion to plantlets. Regeneration medium MSTd.5GA.5N performed well for plantlet regeneration from organogenic calli (141.25%). In such cases, both the cut ends of leaf discs are so profused that the entire surface of the cutting has covered with shoot buds. Similar response was reported by Mujib *et al*. (2005) for leaf culture of sandalwood. Maximum plantlets regeneration of somatic embryos on media supplemented with higher concentration of cytokinin TDZ in combination with 1.0 $mg.l^{-1}$ GA_3 revealed that two-fold cytokinin and GA_3 were required as compared to plantlets regenerated *via* indirect organogenesis. However, supplementation of 0.5 $mg.l^{-1}$ NAA is required additionally for better regeneration ability of organogenic calli. Media fortified in combination of an auxin, cytokinin and GA_3 performed well as compared to other media with a growth regulator in isolation which suggests that plantlet formation is determined by quantitative interaction, i.e. ratios rather than absolute concentration of substances participating in growth and development.

Regenerated shoots, 3-4 months old, with 3-4 whorls of leaves and 1-2 inches tall were excised from the mother tissue and subcultured on various fortifications of MS medium for rooting (Table 3). Different hormonal combinations were tried as supplement to MS agar gelled and liquid media. Surprisingly, in none of the formulated media, the excised shoot developed roots despite the prolonged period under different culture conditions. The excised shootlets were implanted on MS agar gelled medium with different concentrations of NAA, IBA, and Kn alone as well as IBA in combinations with BA, Kn NAA and GA_3. IBA in moderate and higher concentration, favored healthy growth of shootlets for long periods but root formation take place in lower frequency (1-3%), whereas, NAA was not at all suitable and the shoots could not sustain for a long. Combination of IBA with kinetin induced

formation of white friable calli at the cut end; however, the calli did not organize into root. Combinations of IBA with BA rather induced adventitious shoot formation instead of rooting. Agar gelled medium with different concentrations of IBA in combination of BA/ Kn and GA_3 also did not induce root formation in the excised shoots. These findings have close agreement with Sarangi *et al*. (2000) who also failed inducing *in vitro* rooting in sandalwood despite of a long and repetitive effort.

The system described here required different hormonal combinations and concentrations from those previously reported by Rao and Bapat (1995) who used media containing IAA and BAP to obtain the most normal somatic embryos, multiplication, germination and conversion into plantlets and noted the problem of low conversion frequency. In the present study, TDZ /BA with 2,4-D can spontaneously induce embryogenesis at a higher frequency and with greater reproducibility. This finding is similar to finding of Rugkhla and Jones (1998) who also reported 100% somatic embryo induction with application of TDZ alone as well in combination with 2,4-D. However in this study, 2,4-D performed better than NAA. Mature somatic embryos needed supplementation of GA_3 for germination, conversion and elongation of plantlets, otherwise many abnormal somatic embryos were obtained and this resulted in a low conversion into plantlets.

In conclusion, the present results substantiate the fact that leaf disc is an excellent explant source and is available throughout the year especially in sandal where choice of explants is limited due to short availability of quality seeds as seeds are prone to seed borer insect, short viability and dormancy of seeds. The present study shows that leaf disc explants of sandalwood carry a high potential for rapid multiple shoot regeneration and subsequent micropropagation. This protocol provides a successful and rapid technique that can be used for mass *in vitro* propagation of elite species. Furthermore, these techniques may be used for raising embryogenic cell suspension cultures for production of secondary metabolite products for exploiting these in pharmaceutical industries. The multiplication of friable embryogenic tissue or repetitive embryogenesis was more accessible as starting materials for *Agrobacterium-mediated* transformation.

Table 1. *In vitro* morphogenesis in cultured leaf discs on different fortification of MS media

Culture Media	Direct somatic embryogenesis	Indirect somatic embryogenesis	**Average no. of somatic embryos	Direct organogenesis	Indirect organogenesis	Plantlet regeneration *via* direct organogenesis
MS.5D.5Td	7.48^{e}	31.18^{d}	124.35^{k}	2.51^{ef}	18.95^{a}	8.32^{h}
MSD.5Td	11.44^{a}	54.23^{a}	160.08^{a}	5.51^{cd}	19.04^{a}	20.06^{e}
MS2D.5Td	10.54^{b}	45.63^{b}	151.69^{b}	1.95^{f}	20.38^{a}	4.72^{i}
MS3D.5Td	9.29^{d}	13.45^{i}	132.67^{j}	1.24^{f}	11.36^{e}	4.49^{i}
MS4D.5Td	6.77^{e}	9.49^{j}	102.82^{t}	2.78^{e}	3.94h^{i}	8.29^{h}
MS5D.5Td	2.47^{h}	6.85^{k}	71.05^{w}	2.13^{f}	3.34^{j}	8.28^{h}
MS.5B.5N	7.02^{e}	27.38^{f}	109.26^{s}	1.18^{f}	6.04^{gh}	4.38^{i}
MSB.5N	10.33^{bc}	30.91^{d}	119.17^{p}	2.18^{f}	12.27^{cd}	8.13^{h}
MS2B.5N	9.42^{cd}	42.76^{c}	139.49^{g}	8.15^{b}	16.53^{b}	32.67^{b}
MS3B.5N	8.63^{d}	31.13^{d}	120.84^{n}	8.18^{b}	18.24^{b}	32.13^{b}
MS4B.5N	6.44^{ef}	9.49^{j}	95.55^{u}	6.81^{bc}	14.12^{c}	24.34^{d}
MS5B.5N	2.12^{hi}	6.85^{k}	65.84^{x}	1.08^{f}	3.51^{ij}	4.37^{i}
MS.5Td.5N	7.04^{e}	27.46^{f}	77.28^{v}	4.05^{de}	16.44^{b}	16.76^{f}
MSTd.5N	7.48^{e}	31.96^{d}	144.63^{d}	7.34^{b}	18.64^{ab}	28.58^{c}
MS2Td.5N	5.65^{f}	27.76^{ef}	149.13^{c}	9.48^{a}	19.25^{a}	36.69^{a}
MS3Td.5N	1.78^{i}	21.69^{g}	141.62^{f}	4.17^{d}	9.41^{f}	16.17^{f}
MS4Td.5N	1.35^{i}	13.77^{i}	132.93^{i}	3.35^{e}	2.55^{j}	12.43^{g}
MS5Td.5N	1.10^{i}	7.03^{k}	124.12^{l}	1.43^{f}	1.85^{j}	4.48^{i}
MS.5Kn.5N	4.44^{g}	13.41^{i}	113.31^{q}	3.38^{e}	9.44^{f}	12.17^{g}
MSKn.5N	5.65^{f}	28.74^{e}	122.83^{m}	5.25^{d}	9.51^{ef}	20.18^{e}
MS2Kn.5N	4.65^{g}	31.18^{d}	142.80^{e}	3.02^{e}	11.52^{de}	12.46^{g}
MS3Kn.5N	1.48^{i}	22.86^{g}	137.44^{h}	3.59^{e}	9.36^{f}	12.61^{g}
MS4Kn.5N	1.30^{i}	19.68^{h}	119.71^{o}	2.84^{e}	7.32f^{g}	8.42^{h}
MS5Kn.5N	1.06^{i}	9.28^{j}	112.57^{r}	1.78^{f}	1.57^{j}	4.20^{i}
Mean	5.62	23.50	121.29	3.89	11.02	14.38
CD (0.5%)	1.108#	1.798#	0.027*	1.620#	2.220#	1.629#

Arc-sine transformation).
*Log transformation.
Values within column followed by different letters are significantly differed at 5% probability level.

Table 2. Effect of different plant growth regulators on plantlet regeneration from cultured leave discs *via* indirect somatic embryogenesis and organogenesis

Culture media	Plant regeneration *via* somatic embryogenesis	Plant regeneration *via* indirect organogenesis
MS.5B.5GA	141.92[i]	102.92[p]
MSB.5GA	144.67[g]	110.44[m]
MS2B.5GA	149.71[e]	121.66[j]
MS.5BGA	115.23[t]	105.34[o]
MSBGA	131.19[n]	129.03[g]
MS2B.5GA	155.35[d]	134.18[c]
MS.5Td.5GA	133.25[k]	101.25[q]
MSTd.5GA	142.59[h]	111.10[l]
MS2Td.5GA	159.51[b]	130.54[f]
MS.5TdGA	133.53[j]	128.27[h]
MSTdGA	146.37[f]	132.74[e]
MS2TdGA	163.61[a]	138.37[b]
MS.5Kn.5GA	108.33[u]	42.09[w]
MSKn.5GA	117.64[r]	53.15[u]
MS2Kn.5GA	131.27[m]	69.76[s]
MS.5KnGA	85.54[x]	50.32[v]
MSKnGA	96.48[w]	68.44[t]
MS2KnGA	105.82[v]	78.74[r]
MS.5B.5GA.5N	115.92[s]	107.21[n]
MS.5Td.5GA.5N	124.45[o]	111.10[l]
MS.5Kn.5GA.5N	118.23[q]	123.70[i]
MSB.5GA.5N	159.28[c]	115.82[k]
MSTd.5GA.5N	132.78[l]	141.25[a]
MSKn.5GA.5N	121.30[p]	132.78[d]
Mean	130.58	105.84
CD (0.05%)	0.021	0.0308

Arc-sine transformation).
*Log transformation.
Values within column followed by different letters are significantly differed at 5% probability level.

Table 3. Response of regenerated shootlets on MS basal medium with different concentrations of plant growth regulators tried for induction of *in vitro* rooting in sandalwood

Culture media combinations	In vitro rooting response
MS.1N	Healthy for 1 month, no rooting, dried.
MS.5N	As above
MSN	Growth + healthy for 2 months, no rooting
MS5N	As above
MS10N	Dried within one month, no rooting
MS.1IB	Healthy for 1 month, no rooting, dried
MS.5IB	As above
MSIB	Healthy for 3 month, no rooting
MS5IB	Healthy for 4 months, no rooting
MS10IB	Growth + healthy for 6 months, no rooting
MS.1Kn	Growth + callusing at the cut end, no root formation
MS.5Kn	Adventitious shoots formed, no rooting
MSKn	Hairy roots
MS5Kn	Hairy roots
MS10Kn	Hairy roots
MS.1IB.5B	Growth + healthy for 4 months, adventitious shoots formed, no rooting
MS.5IB.5B	As above
MSIB.5B	As above
MS5IB.5B	Healthy for 4 months, no rooting
MS10IB.5B	Growth + healthy for 6 months, no rooting
MS.1IB.5Kn	No growth, healthy for 1 month, no rooting
MS.5IB.5Kn	As above
MSIB.5Kn	As above
MS5IB.5Kn	Healthy for 4 months, no rooting
MS10IB.5Kn	Growth + healthy for 6 months, no rooting
MS.1IB.5Kn.5G	Healthy for 1 month, no rooting
MS.5IB.5Kn.5G	As above
MSIB.5Kn.5G	Healthy for 3 months, no rooting
MS5IB.5Kn.5G	Healthy for 4 months, no rooting
MS10IB.5Kn.5G	Growth + healthy for 6 months, no rooting

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