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N Krishnakumar
Department of Agroforestry, Forest College and Research Institute, TNAU, Mettupalayam, Tamil Nadu, India

KT Parthiban
Department of Agroforestry, Forest College and Research Institute, TNAU, Mettupalayam, Tamil Nadu, India

Correspondence
N Krishnakumar
Department of Agroforestry, Forest College and Research Institute, TNAU, Mettupalayam, Tamil Nadu, India

Micropropagation (*In vitro*) techniques for sandal wood (*Santalum album* L.)

N Krishnakumar and KT Parthiban

Abstract
Studies on *Santalum album* L. were carried out in the tissue culture laboratory at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, India to standardize the *in vitro* propagation techniques. The results on explant sterilization revealed that 0.01 percent HgCl2 for 5 min and 0.05 percent HgCl2 for 5 min were the best treatments respectively for sterilizing shoot tip from mature tree. Treatments involving 0.01 percent $HgCl_2$ for 5 min and and 0.01 percent NaOCl for 3 min effectively controlled the contaminant respectively in nodal and intermodal segments. Among the explants tested for multiple shoot induction, intermodal segments proved good results. Among the two media viz., MS, White media, the species responded well to MS Medium. The best treatment for obtaining multiple shoot induction was MS + 5.0 mgl^{-1} Kin + 2.0mgl^{-1} BAP for shoot tip explants. For rooting of shoot tip cuttings 3 mgl^{-1} IBA was found to be the best treatment combination for maximum sprouting, rooting of cuttings, shoot length and root length

Keywords: *Santalum album*, sandalwood, *in vitro* propagation, micropropagation

Introduction
Sandal is a commercially and culturally important plant species belonging the family santalaceae. Sandal is considered is as one of the most valuable tree in the world. The sandal is known for its oil which is pronounced as the most famous East Indian sandal wood oil which is produced from the heartwood of sandal on distillation (Krishnakumar *et al.,* 2016) [15]. The sandal wood oil has been known in the perfume industries for several century. Red sander had the prominent importance in the business. Research initiatives are under way to exploit its potential application pharmaceutical industries (Srinivasan *et al*., 1992).
Conventional methods of asexual propagation like grafting, budding, layering etc. for many of the plants and trees are often too slow or fail completely. *In vitro* propagation of sandalwood was attempted as early as 1963 reported mature endosperm with white's medium, but the callus did not proliferate well (Rangaswamy and Rao, 1963) [33]. Micropropagation using tissue culture allows much greater control and manipulation of the development of tissues within the culture tube than conventional methods (Parthiban *et al.,* 2000). Tissue culture techniques help to mass multiply the trees species and the techniques have already revolutionized mass propagation of many hardwoods and softwoods (Bajaj, 1986; Tewari, 1994). Micropropagation is the method of choice for production of huge number of genetically identical plants or cloning of superior genotypes in shorter time span (Chelak and Rogers, 1990).
However, the standard protocols pertaining to in vitro regeneration of *Santalum album* are limited.

Materials and Methods
Methods
The investigations were carried out in the tissue culture laboratory of Forest College and Research Institute, Mettupalayam during the period 2014 to 2015.
Shoot tips and nodal segments were collected from selected *Santalum album* L. tree. The explants of 2.0 – 2.5 cm in length were dissected out from the mother plant and collected in distilled water. After collection, the explants were shaken mildly with water for 30 minutes in a conical flask. A slant cut was given at the base of the explants to expose more surface area for better transport of nutrients. Explants were collected in a conical flask containing sterile distilled water and brought to the lab. The explants were washed thoroughly with running tap water and then disinfected with Tween 20 solution (0.1%) for few minutes and washed thoroughly with running tap water for two hours. The following treatments were imposed on the explants to screen the ideal and optimal concentration of sterilants.

After the sterilization treatment, the explants were rinsed 3-4 times thoroughly in sterilized water to remove the sterilants and were inoculated in MS (Murashige and Skoog, 1962) medium. The following observations were recorded two weeks after inoculation i) Per cent culture response, ii) Per cent contamination and iii) Per cent drying.

Table 1: Treatment details of sterilization of explants

Sterilants	Treatments	Duration
$HgCl_2$ (0.01 %)	1	1 min
	2	3 min
	3	5 min
$HgCl_2$ (0.05 %)	4	1 min
	5	3 min
	6	5 min
NaOCl (1%)	7	1 min
	8	3 min
	9	5 min
NaOCl (3%)	10	1 min
	11	3 min
	12	5 min

Preparation of media for culturing

All stock solutions and final medium were prepared by following the procedure of Bhojwani and Razdan (1983).

Four different basal media *viz.* MS (Murashige and Skoog, 1962), B5 (Gamborg *et al.,* 1968), WP (Lloyd and Mc Cown, 1980) and Whites (White, 1963) were initially used to optimize suitable medium for organogenesis. The prepared media were sterilized at 121°C (15 lbs pressure) for 20 minutes and used for culturing explants.

Screening of explants and basal medium

Two explants *viz*., nodal segments and shoot tips were tested for their organogenic potential using four different basal media *viz*., MS and White's medium and the experiment was conducted with 4 replication, each replication comprised five culture vessels

Table 2: The treatment details of screening explants and basal medium

Treatments	Type of explants	Name of the medium
T_1	Shoot tip	MS
T_2		White's
T_3	Nodal segments	MS
T_4		White's

The observation on per cent cultures with shoot induction was recorded after two weeks of inoculation.

Screening culture medium for shoot induction

The explants were inoculated in MS basal medium supplemented with various concentrations and combinations of BAP and Kinetin. The basal MS medium served as control. The following were the different media combinations used in this study. i) MS (basal), ii) MS + BAP (1 mg l^{-1} to 5 mg l^{-1}), iii) MS + Kin (1 mg l^{-1} to 5 mg l^{-1}) and MS + BAP and Kin (all combinations from 1 mg l^{-1} to 5 mg l^{-1}). The cultures were exposed to a light (approximately 2000 lux) and darkness cycle of 16 h and 8 h respectively. The culture room temperature was maintained at 25 ± 2°C. The following observations were made. i) Per cent cultures with shoot induction, ii) Average number of shoots per explants and Shoot length

Screening culture medium for root induction

Individual shoots were dissected out from the multiple shoots aseptically and cultured on MS medium containing various concentrations and combinations of auxins (IAA and IBA) in order to induce rhizogenesis. The treatment details are given. i) MS basal, ii) MS + IAA (1 mg l^{-1} to 5 mg l^{-1}), iii) MS + IBA (1 mg l^{-1} to 5 mg l^{-1}) and MS + IAA + IBA (1 mg l^{-1} to 3 mg l^{-1} in all combinations). The cultures were exposed to similar conditions extended for shoot induction. The following observations were recorded. i) Per cent cultures with root induction, ii) Number of roots per explants and iii) Root length

Result and Discussion

Sexual reproduction by seed is not dependable since it may result in high degree of heterogeneity among plants. Traditional tree breeding methods are time consuming and expensive especially in tree species, due to long gestation period. Hence, propagation of plants through tissue culture is gaining popularity in forestry to reproduce plant taxa otherwise difficult to propagate conventionally or by vegetative means (Govil and Gupta, 1997). Tissue culture plays an important role in solving the problem through rapid *in vitro* multiplication of novel genotypes and to screen useful variants. Currently successful methodologies have been developed through tissue culture and their possible application in forestry (Bajaj, 1986).

Identification of suitable basal medium and explants

Two different media *viz.*, MS and Whites were used for morphogenic response for each of the explants. Among different media, MS medium recorded higher value (43.12 %) compared to White's media (26.25 %) in terms of per cent morphogenic response. The explants also differed for their per cent morphogenic response. The highest morphogenic response was recorded by shoot tip explants (52.50 %) which differed by nodel segment explants (59.82 %) (Table 3; Figure 1).

Table 3: Screening of basal medium and explants (Values are mean of four replication)

Sl. No.	Media	Morphogenic response (%)		
		Shoot explant	Nodal segments	Mean
1	MS basal medium	63.75	22.50	43.12
2	WHITE'S	41.25	11.25	26.25
Grand mean		52.50	16.88	

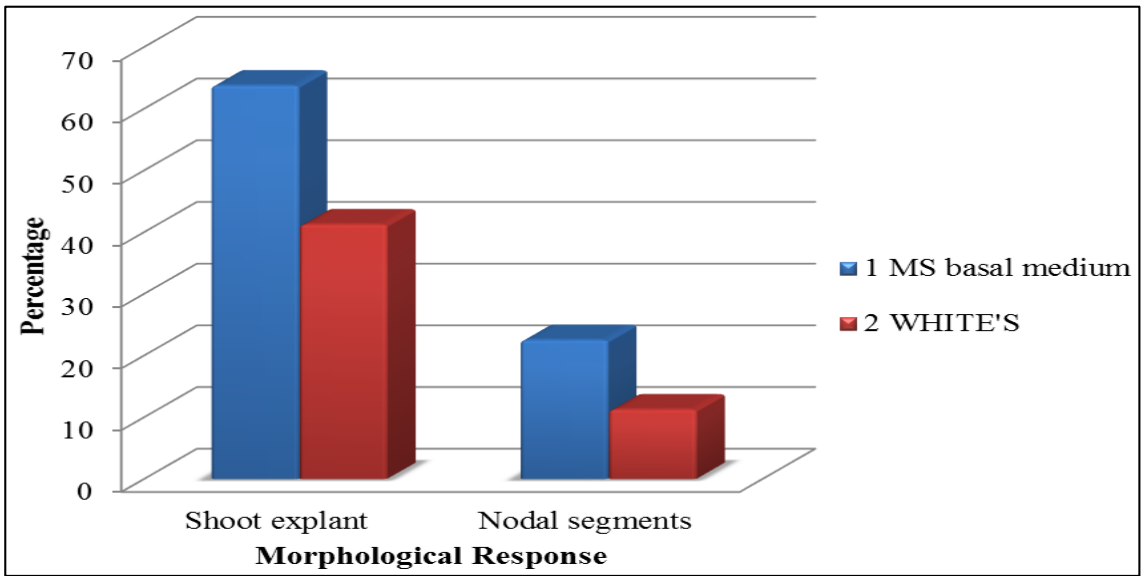

Fig 1: Screening of basal medium and explants

Out of two basal media *viz*., MS and white's investigated, MS medium (43.12 %) performed significantly well over the rest in terms of morphogenic response (Table 3). The superiority of MS medium compared to others had also been well documented earlier by several species *viz*., *Melia dubia* (Kumar, 2011); thronless bamboo (Kamala, 2009) [12]; *Dendrocalamus brandisi* (Mukunthakumar *et al*., 1999); *Acacia mangium* (Rajesh, 1999 and Senthilkumar, 1996); neem (Indu George and Kulkarni, 1997 and Sarita Arya and Inder Dev Arya, 1998); *Eucalyptus tereticornis* (Parthiban *et al*., 1999) [26]; *Eucalyptus* hybrid FRI-4 (Chauhan *et al*., 1996); *Robinia pseudoacacia* (Kamlesh Kanwar *et al*., 1995); *Simarouba glauca* (Rout and Das, 1996; Sekar, 2003 and Geethanjali, 2004) and *Madhuca latifolia* (Manmohan Jegatram *et al*., 2003). A number of woody species have been successfully grown on WPM (Lloyd and McCown, 1980). The superiority of MS medium in the present investigation might be due to higher ionic concentration which lend support to the earlier findings of Rajkumar (1999) and Pattanaik, (1995). The use of modified MS medium for successful *in vitro* propagation is also reported in *Dalbergia sissoo* (Tewari *et al*., 1993) and *Eucalyptus* hybrid (Prabha Bisht *et al*., 2000b). In *Dalbergia sissoo*, out of the two nutrient media tried, MS medium was found to be the best, which gave high rate of shoot proliferation as compared to B5 medium (Joshi *et al*., 2003a). Hence the superiority of MS medium in the present is also in tune with the earlier findings.

Screening of suitable sterilants

Two common sterilants *viz*., sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) at various concentrations and durations were used in standardizing sterilization procedure for obtaining contamination free cultures. The sterilants at various concentration and durations used were presented in the Table 4.

Table 4: Screening optimal concentration of $HgCl_2$ and NaOCl sterilant

Treatments	Duration	Survival (%)	Contamination (%)	Drying (%)
$HgCl_2$ (0.01 %)	1 min.	60.50	23.00	10.50
	3 min.	59.25	24.50	9.25
	5 min.	65.75	19.00	1.25
$HgCl_2$ (0.05 %)	1 min.	50.50	32.00	10.50
	3 min.	45.00	31.00	14.00
	5 min.	46.50	29.50	15.00
NaOCl (1%)	1 min.	54.75	24.50	13.75
	3 min.	65.25	21.00	7.75
	5 min.	55.00	23.75	10.25
NaOCl (3 %)	1 min.	60.75	20.75	11.50
	3 min.	49.50	27.25	14.25
	5 min.	47.25	31.00	11.75
	Grand mean	55.00	25.60	10.81

Maximum survival of 65.75 per cent was noticed with 0.01 % $HgCl_2$ for 5 min followed by 65.25 per cent in 1% NaOCl for 3 min. Per cent contamination was also least with these two treatments 0.01 % $HgCl_2$ for 5 min (19.00 %) and 1% NaOCl for 3 min (21 %). In all other treatments the contamination observed was ranged between 20.00 and 32.00 per cent. With regard to drying of explants, the treatment 0.01 % $HgCl_2$ for 5 min expressed least value (1.25 %). In all other treatments, the drying was ranged between 7.75 per cent and 15.00 per cent Table 4; Figure 2.

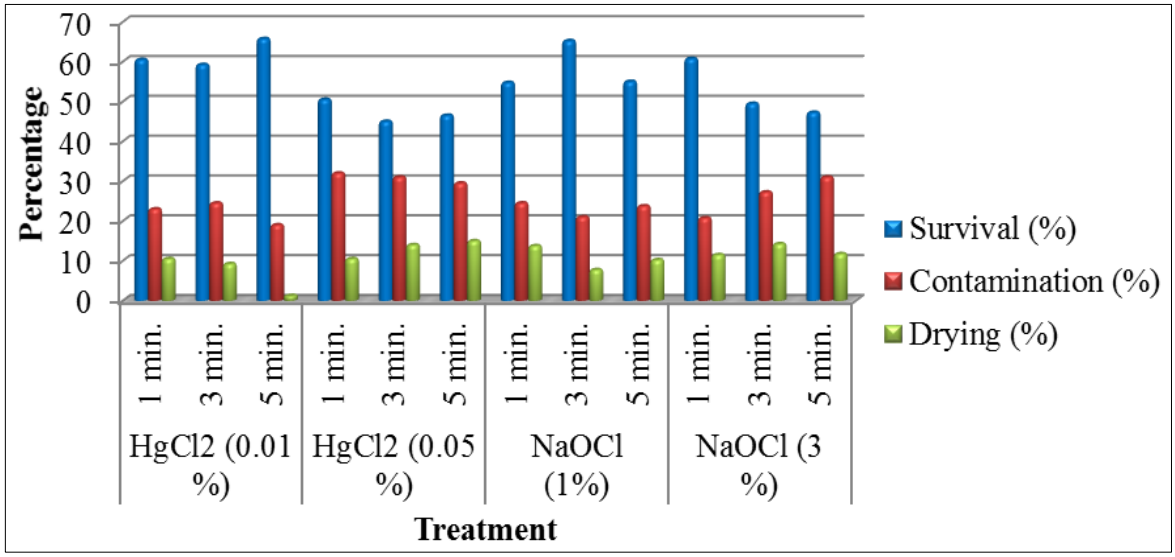

Fig 2: Screening optimal concentration of $HgCl_2$ and NaOCl sterilant

In the present investigation, 0.01 per cent $HgCl_2$ for five minutes was found to be the best treatment for sterilization (Table 4). The same treatment showed minimum percentage of drying (1.25%). Increasing the concentration of the sterilants *viz.*, $HgCl_2$ concomitantly increased the drying percentage. Similar reports were earlier evident in *Madhuca longifolia* (Rout and Das, 1993); *Azadirachta indica* (Sarita Arya and Inder Dev Arya, 1998) [37]; *Acacia mangium* (Sumana *et al*., 1998) [37]; *Pterocarpus marsupium* (Rajkumar, 1999); *Simarouba glauca* (Rout and Das, 1996; Sekar, 2003 and Geethanjali, 2004) [36, 4, 4] and Eucalyptus (Vennila, 2009). The contamination percentage was 19 at 0.1 per cent $HgCl_2$ and survival percentage was only 65.75 per cent. This may be the reason for poor survival at higher concentration. The concentration of the NaOCl sterilants showed no drying but the rate of contamination was higher. However the treatment with 2.0 per cent sodium hyphochloride resulted contamination free cultures in *Eucalyptus tereticornis* (Parthiban *et al*., 1999) and Eucalyptus clones (Vennila, 2009) [47]. Surface sterilization of explants with nascent chlorine, mercuric chloride and hydrogen peroxide may be relatively easy, but the systemic infection/endophytic contamination may be relatively difficult to illuminate (Thorpe *et al*., 1991) [46]. This could be the probable reason for 19 per cent contamination in the treatment i.e., 0.1 per cent $HgCl_2$. 0.2 per cent $HgCl_2$ was employed for surface sterilization of *Populus alba* and *P. nigra* (Kapusta and Skibinska, 1985) [13]. In sandal explants, soaking in Bavistin (0.1- 0.2 %) followed by washing with 0.1 per cent $HgCl_2$ was effective treatment for the elimination of fungal contamination but not for bacterial contamination (Reddy and Sukanya Subramanian, 1998).

Standardizing suitable medium for shoot induction

Effect of BAP on multiple shoot induction

Among the various treatments, the treatment MS + 3.0 mgl^{-1} BAP (50.75 %) followed by MS + 4.0 mgl^{-1} BAP (50.25 %) recorded higher per cent shoot induction compared to MS basal medium (14.50 %). All other treatments showed increased shoot induction which ranged between 33.25 % (MS + 5.0 mgl^{-1} BAP) and 40.25 % (MS + 1.0 mgl^{-1} BAP) over that of MS basal medium (14.50 %) (Table 5). The two treatment viz., MS + 3.0 mgl^{-1} BAP (3.05) and MS + 2.0 mgl^{-1} BAP (2.81) recorded higher number of shoots per explant over MS basal medium (1.63). The average shoot length was 4.25, however the treatment MS + 3.0 mgl^{-1} BAP (5.08 cm) proved superior but on par with MS + 4.0 mgl^{-1} BAP (4.99 cm) (Table 5). Thus, from the overall perspective the treatment MS + 3.0 mgl^{-1} BAP was brought into sharp focus for shoot multiplication.

Table 5: Effect of BAP on multiple shoot induction

S.No.	Treatments	Per cent cultures with shoot induction	Average number of shoots/explant	Length of shoots (cm)
1	MS Basal	14.50	1.63	2.96
2	MS + 1.0 mgl^{-1} BAP	40.25	2.68	3.47
3	MS + 2.0 mgl^{-1} BAP	42.25	2.81	4.05
4	MS + 3.0 mgl^{-1} BAP	50.75	3.05	5.08
5	MS + 4.0 mgl^{-1} BAP	50.25	2.53	4.99
6	MS + 5.0 mgl^{-1} BAP	33.25	1.75	4.93
	Grand Mean	40.21	2.41	4.25

Effect of kinetin on multiple shoot induction

Among the different concentration of kinetin evaluated, the treatment MS + 3.0 mgl^{-1} Kinetin (66.00 %) recorded higher value for per cent cultures with shoot induction (Table 6). Considering average number of shoots per explants' similar trend was noticed. With regard to average shoot length, the following treatments viz., MS + 3.0 mgl^{-1} Kinetin (4.43 cm), MS + 4.0 mgl^{-1} Kinetin (3.72 cm), MS + 2.0 mgl^{-1} Kinetin (3.37 cm) and MS + 5.0 mgl^{-1} Kinetin (3.08 cm) recorded higher values compared to MS basal media (Table 6). Considering the per cent cultures with shoot induction, average number of shoots and average shoot length per explant, the treatment MS + 3.0 mgl-1 Kinetin was found to be superior.

Table 6: Effect of Kinetin on multiple shoot induction

S.No.	Treatments	Per cent cultures with shoot induction	Average number of shoots/explant	Length of shoots (cm)
1	MS Basal	12.50	1.63	1.96
2	MS + 1.0 mgl^{-1} Kinetin	40.00	2.10	2.51
3	MS + 2.0 mgl^{-1} Kinetin	40.25	1.40	3.37
4	MS + 3.0 mgl^{-1} Kinetin	66.00	2.11	4.43
5	MS + 4.0 mgl^{-1} Kinetin	50.50	1.99	3.72
6	MS + 5.0 mgl^{-1} Kinetin	27.50	1.34	3.08
	Grand Mean	39.46	1.76	3.18

Effect of conjoint addition of BAP and Kinetin on multiple shoot induction

Significant differences were obtained among various treatments with BAP and Kinetin conjointly; however three treatments *viz.*, MS + 5.0mgl^{-1} Kin + 2.0mgl^{-1} BAP (70 %) and MS + 5.0mgl^{-1} Kin + 4.0mgl^{-1} BAP (62.50 %) recorded higher per cent cultures with shoot induction (Table 7).

Similar trend was also observed for average number of shoots per explant. Considering the average shoot length, no significant difference was observed among various treatments with BAP and Kinetin conjointly; however MS + 5.0mgl^{-1} Kin + 2.0mgl^{-1} BAP (5.35 cm) recorded higher value compared to other treatments.

Table 7: Effect of conjoint addition of BAP and Kinetin on multiple shoot induction

S.No	Treatments	Per cent cultures with shoot induction	Average number of shoots/explant	Length of shoots (cm)
1	MS + 1.0mgl^{-1} Kin + 1.0mgl^{-1} BAP	47.50	2.54	3.53
2	MS + 1.0mgl^{-1} Kin + 2.0mgl^{-1} BAP	52.50	2.26	3.26
3	MS + 1.0mgl^{-1} Kin + 3.0mgl^{-1} BAP	55.00	2.56	4.28
4	MS + 1.0mgl^{-1} Kin + 4.0mgl^{-1} BAP	48.75	3.40	4.27
5	MS + 1.0mgl^{-1} Kin + 5.0mgl^{-1} BAP	51.25	2.33	3.98
6	MS + 2.0mgl^{-1} Kin + 1.0mgl^{-1} BAP	50.00	2.58	2.89
7	MS + 2.0mgl^{-1} Kin + 2.0mgl^{-1} BAP	56.25	2.93	4.25
8	MS + 2.0mgl^{-1} Kin + 3.0mgl^{-1} BAP	52.50	3.26	2.92
9	MS + 2.0mgl^{-1} Kin + 4.0mgl^{-1} BAP	48.75	2.96	3.32
10	MS + 2.0mgl^{-1} Kin + 5.0mgl^{-1} BAP	50.00	3.09	3.00
11	MS + 3.0mgl^{-1} Kin + 1.0mgl^{-1} BAP	43.75	2.63	3.26
12	MS + 3.0mgl^{-1} Kin + 2.0mgl^{-1} BAP	42.50	3.30	2.98
13	MS + 3.0mgl^{-1} Kin + 3.0mgl^{-1} BAP	57.50	3.24	3.89
14	MS + 3.0mgl^{-1} Kin + 4.0mgl^{-1} BAP	51.25	3.09	4.22
15	MS + 3.0mgl^{-1} Kin + 5.0mgl^{-1} BAP	51.25	4.09	3.33
16	MS + 4.0mgl^{-1} Kin + 1.0mgl^{-1} BAP	55.00	2.88	2.98
17	MS + 4.0mgl^{-1} Kin + 2.0mgl^{-1} BAP	46.25	3.14	3.43
18	MS + 4.0mgl^{-1} Kin + 3.0mgl^{-1} BAP	51.25	4.03	4.11
19	MS + 4.0mgl^{-1} Kin + 4.0mgl^{-1} BAP	57.50	3.74	3.86
20	MS + 4.0mgl^{-1} Kin + 5.0mgl^{-1} BAP	48.75	3.71	4.27
21	MS + 5.0mgl^{-1} Kin + 1.0mgl^{-1} BAP	56.25	4.10	4.11
22	MS + 5.0mgl^{-1} Kin + 2.0mgl^{-1} BAP	70.00	5.35	5.00
23	MS + 5.0mgl^{-1} Kin + 3.0mgl^{-1} BAP	47.50	3.86	3.53
24	MS + 5.0mgl^{-1} Kin + 4.0mgl^{-1} BAP	62.50	4.81	2.56
25	MS + 5.0mgl^{-1} Kin + 5.0mgl^{-1} BAP	57.50	3.99	3.41
	Grand Mean	52.45	3.35	3.63

In the present investigation, compared to MS basal medium, superiority in terms of per cent cultures with shoot induction and average number of shoots per explant, was evident with cytokinins (BAP, Kinetin, BAP plus Kinetin) added medium either singly or conjointly (Table. 5, 6 and 7). Among six treatments attempted, MS + 3.0 mgl^{-1} BAP proved to be ideal for shoot induction for nodal and shoot tip explants. Compared to individual effects of cytokinins, conjoint addition of two cytokinins at a time augmented the per cent cultures with shoot induction. Cytokinins were known to promote cell division and exerted other growth regulatory functions (Skoog *et al.,* 1964; Mothes, 1967) [42 24]. Cytokinins had the greatest effect on bud and shoot initiation and also in tissue culture studies (Boe *et al.,* 1972) [3]. Similar effects were observed in different bamboo species (Huang *et al.,* 1989 [8]; Rajapakse, 1991 [31]; Prutpongse and Govinlertvatana, 1992 [28]; Saxena and Bhojwani, 1993 [39]; Jullien and Van Tran, 1994 [10]; Das and Rout, 1994 and Hirimburegama and Gamage, 1995) [7].

Kinetin is a common natural cytokinin widely used in plant tissue culture (Lakshmi Sita *et al.,* 1980) [17]. However, BAP is the cheapest and one of the most effective cytokinens used in many tissue culture investigations. The favourable effect of kinetin on bud break had been reported in many tree species *viz.*, *Eucalyptus tereticornis*, *E. globulus* (Gupta and Mascarenhas, 1987) [5]. The favourable effect of BAP was also evident in many tree species *viz.*, *Azadirachta indica* (Su *et al.,* 1997; Mallika Vanangamudi *et al.,* 1997) [21]; *Albizia procera* (Majumdar *et al.,* 1998) [18]; *Acacia mangium* (Ahmed, 1991) [1], *Hopea odorata* (Scott *et al.,* 1995) [40] and *Santalum album* (Radhakrishnan *et al.,* 2001) [30] at the concentrations ranging from 0.1 mg per litre to 5 mg per litre. However in the present study, MS medium with 3 mg per litre each of BAP and Kinetin found to induce higher shoot bud organogenesis. In many cases, the cocktails of cytokinins were known to induce higher shoot bud organogenesis than their contribution when added individually in teak (Gupta *et al.,* 1980 [6]; Mascarenhas *et al.,* 1993) [22] and in sandalwood

(Parthiban *et al.*, 1998) [26] which thus lend support to the current investigation.

Micro propagation studies in *Santalum album* L. indicated that MS + 3.0 mgl^{-1} Kinetin is the best treatment for shoot induction (Kalpataru Dutta Mudoi and Mina Borthakaur, 2009). The shoot bud induction was also observed in same treatment in the current study.

Standardizing suitable medium for root induction

Effect of IAA and IBA on root induction

All the treatments recorded significantly higher rooting per cent due to treatment with various concentrations of auxins. Among the treatments, the treatment MS + 3.0 mgl^{-1} IAA (60.50 %) proved superior from the stand point of per cent cultures with root induction (Table 8). Similar trend was also observed in number of roots per explants wherein the all auxin added treatments expressed superiority. Among the treatments, MS medium supplemented with 3.0 mgl^{-1} IBA reigned superior in terms of number of roots per explants. Considering per cent cultures with root induction along with average number of roots and average root length per explant, the treatment MS + 3.0 mgl^{-1} IBA was found to be optimal for root induction.

Table 8: Effect of IAA and IBA on root induction

S.No.	Treatments	Per cent cultures with root induction	Average number of roots/explant	Length of roots (cm)
1	MS Basal	0.00	0.00	0.00
2	MS + 1.0 mgl^{-1} IAA	16.50	1.39	3.25
3	MS + 2.0 mgl^{-1} IAA	55.50	2.18	4.67
4	MS + 3.0 mgl^{-1} IAA	60.50	3.52	5.80
5	MS + 4.0 mgl^{-1} IAA	43.75	3.18	4.71
6	MS + 5.0 mgl^{-1} IAA	25.25	1.86	4.28
7	MS + 1.0 mgl^{-1} IBA	20.50	1.00	2.91
8	MS + 2.0 mgl^{-1} IBA	66.25	4.89	4.72
9	MS + 3.0 mgl^{-1} IBA	50.75	2.43	4.71
10	MS + 4.0 mgl^{-1} IBA	33.75	2.07	4.12
11	MS + 5.0 mgl^{-1} IBA	31.75	2.23	3.39
	Grand Mean	36.77	2.25	3.87

Effect of conjoint addition of IAA and IBA on root induction

The conjoint addition of two auxins *viz.*, IAA and IBA have recorded significantly improved results towards percent culture with root induction, average number of roots per explants and the length of roots compared to the MS basal medium which has not recorded any root induction. Among the treatments the MS medium supplemented with MS + 3.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA recorded higher root induction (63.75 %) followed by MS medium supplemented with MS + 3.0 mgl^{-1} IAA + 1.0 mgl^{-1} IBA (55.00 %) and these two treatments expressed parity with each other. In case of average number of roots per explants the MS medium supplemented with MS + 3.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA recorded higher value (5.07) and similar trend was also observed in the length of the roots (6.93). However four treatments for average number of roots per explant *viz.*, MS + 2.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA (4.06), MS + 2.0 mgl^{-1} IAA + 3.0 mgl^{-1} IBA (3.83),MS + 3.0 mgl^{-1} IAA + 1.0 mgl^{-1} IBA (4.73), MS + 3.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA (5.07) and three treatments in case of length of roots *viz.*, MS + 2.0 mgl^{-1} IAA +1.0 mgl^{-1} IBA (6.18 cm), MS + 3.0 mgl^{-1} IAA + 1.0 mgl^{-1} IBA (6.75 cm) and MS + 3.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA (6.93 cm) recorded significantly higher values and hence these treatments could be used for rhizogenesis towards *invitro* rooting of *Santalum album* L. micro roots (Table 9).

Table 9: Effect of conjoint addition of IAA and IBA on root induction

S.No.	Treatments	Per cent cultures with root induction	Average number of roots/explant	Length of roots (cm)
1	MS Basal	0.00	0.00	0.00
2	MS + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} IBA	32.50	2.40	3.82
3	MS + 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA	43.75	2.15	4.39
4	MS + 1.0 mgl^{-1} IAA + 3.0 mgl^{-1} IBA	40.00	2.34	4.58
5	MS + 2.0 mgl^{-1} IAA + 1.0 mgl^{-1} IBA	37.50	3.14	6.18
6	MS + 2.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA	42.50	4.06	5.45
7	MS + 2.0 mgl^{-1} IAA + 3.0 mgl^{-1} IBA	43.75	3.83	6.02
8	MS + 3.0 mgl^{-1} IAA + 1.0 mgl^{-1} IBA	55.00	4.73	6.75
9	MS + 3.0 mgl^{-1} IAA + 2.0 mgl^{-1} IBA	63.75	5.07	6.93
10	MS + 3.0 mgl^{-1} IAA + 3.0 mgl^{-1} IBA	40.00	3.51	5.72
	Grand Mean	39.88	3.12	4.98

In the present study, rhizogenesis was obtained due to addition of auxin. The treatment, MS + 2.0 mgl^{-1} IBA (66.25 %) proved better for root induction and also higher average number of roots per shoot. Kalpataru Dutta Mudoi and Mina Borthakur (2009) reported the maximum root induction in MS + BAP 1.0 mg l^{-1} + NAA 3.0 mg l^{-1}.

Best rooting was obtained in Bamboos using NAA (Sarita Arya and Sushma Sharma, 1998) [28] in *Bambusa balcooa* (Malay Das, 2005) [20] and IBA in *Dendrocalamus asper* (Inder Dev Arya *et al*., 2002) [9]. Similarly Malay Das and Pal (2002) reported effectiveness of IBA for root initiation in *Pterocarpus santalinus* which also lend support to the current investigation.

Auxins were known to induce rooting in many woody plants (Bhatnagar *et al.*, 1968) [2]. The positive effect of IBA on rooting of *in vitro* propagated plants had been established in many tree species *viz.*, *Prosopis tamaruga* (Nandwani and Ramawat, 1992) [25], *Albizia procera* (Majumdar *et al.*, 1998) [18]; teak (Sunitibala Devi *et al.*, 1994; Khatri *et al*., 2001) [45, 14]; *Sterculia urens* (Purohit and Dave, 1996) [29] and *Zizyphus*

mauritiana (Mathur *et al.*, 1995) [23] which are consistent with the results of present findings. But the concentration of auxin is often critical to provide sufficient stimulus to initiate roots while preventing the excessive formation of callus (Yeoman, 1986) [48]. However in the present study IBA alone added medium proved higher rooting success.

Conclusion

Among the media tested, MS medium was found to be optimal and ideal compared to other media. For sterilizing of explants, 0.01 per cent $HgCl_2$ for five minutes was found to be the best treatment and yielded maximum survival percentage. Compared to MS basal medium, treatment with BAP and Kinetin either singly or conjointly proved superior. The optimal treatment for shoot induction was MS medium supplemented with MS + 5.0mgl^{-1} Kin + 2.0mgl^{-1} BAP for shoots tip explants. For rhizogenesis, treatment with IBA alone-added medium proved higher rooting success as well as number of roots per shoot. Among the various treatments, MS + 3 mgl^{-1} IBA proved better in terms of root induction and higher average number of roots per shoot.

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