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RESEARCH ARTICLE

Optimizing *in vitro* micropropagation strategies in *Santalum album* L. through different explants

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Abstract

This study focuses on optimization of *in vitro* propagation protocols for *Santalum album* L. (Indian sandalwood) to address the increasing global demand and to aid in conservation aspects. This investigation evaluated various micropropagation parameters including explant types, basal media, disinfection methods and plant growth regulators (PGR). Murashige and Skoog medium consistently outperformed other media, with shoot tips showing the highest morphogenetic response (66.66%). Surface disinfection with 4% NaOCl for 10 min was the most effective and resulted in the explant survival rate of 86%. For shoot induction, 2 mg/L kinetin in MS medium resulted in the highest number of shoots (3.81) and longest shoots (5.39 cm) after 60 days. An effect was observed when PGR was combined with 5.0 mg/L kinetin + 2.0 mg/L BAP, corresponding to a shoot induction rate of 65.75%. Root induction was recorded at 14.79% after 10 days with the best treatment (MS + 1 mg/L IBA). Callus culture showed limited success as only one treatment (MS + 1 mg/L BAP + 25% coconut water) showed a regenerative response of 7.14%. These results provide a foundation for micropropagation of Sandal while highlighting areas that require further optimization, particularly in root induction and acclimation phases.

Keywords

Indian sandalwood; explants disinfection; micropropagation; plant growth regulators; shoot induction

Introduction

Santalum album L., commonly known as Indian sandalwood and belonging to the Santalaceae family, is one of the world's most precious and aromatic woods. It is a hemi-parasitic tree species of significant economic, cultural and ecological importance (1). This popular tree species is native to the Indian subcontinent, particularly diversified in the forests of southern India, particularly in the states of Karnataka, Tamil Nadu and Kerala. Its natural habitat includes dry deciduous to semi-evergreen forests and thrives in areas with moderate rainfall and dry conditions for part of the year. The tree grows well in red ferruginous clay which is abundant in these regions. The species is classified as endangered by the IUCN due to its limited natural habitat in the Indian subcontinent as well as owing to its aromatic heartwood and oil, which have high value in various cultural, medicinal and economic contexts (2). Nowadays, plantations are being raised all over India extensively and have been introduced in other countries also viz., Australia, Indonesia and the islands of Pacific and Indian oceans. Those regions have a similar climatic condition to the native range of the species in India which permits

successful growth outside its indigenous range (3). Currently, the sandalwood cultivation area in India is approximately 9,000 hectares, with Karnataka and Tamil Nadu accounting for a significant proportion of this area through private and public plantations.

The demand for wood and wood products has increased in India in recent years. This increased demand for wood has led wood-based industries to expect phenomenal increase in the productivity of native tree species alongside new discoveries. Historically, our national forests had been the primary source of timber for wood-based industry. However, the National Forest Policy of 1988 changed this scenario by directing the timber industry to obtain raw materials from sources other than state-owned forests. The Next Generation Planting Platform predicted that global wood demand would increase from 3,401 million m^3 in 2010 to 7,553 million m^3 in 2030 and then to 13,082 million m^3 in 2050 (4). In recent years, the global demand for sandalwood and its derivatives has surged, driven by the expanding perfume, cosmetics and aromatherapy industries. According to a report by Grand View Research (5), the global sandalwood market size was valued at USD 981.2 million m^3in 2020 and is expected to grow at a compound annual growth rate (CAGR) of 6.2% from 2021 to 2028. India as the primary source of natural sandalwood has seen a particularly sharp increase in demand. The Indian sandalwood market alone was estimated at INR 4,000 crores (approximately USD 550 million m^3) in 2019, with projections indicating continued growth (6).

Despite the high market demand, the natural populations of sandal have been severely depleted due to overexploitation, illegal harvesting and habitat loss. In India, the natural sandalwood population has declined by over 75% in the last two decades (7). This decline has led to impose strict regulations on sandalwood harvesting and trade, further exacerbating the supply-demand imbalance. It is important to highlight the importance of sustainable cultivation practices to ensure the long-term availability of sandalwood genetic resources. Efforts to genetically improve sandalwood trees for high yield and disease resistance offer promising solutions to meet the growing demand (8). Genetic selection combined with a successful and viable propagation method is key to tree improvement (9). Clonal propagation has gained prominence which acts as an alternate to traditional seed orchard breeding. This propagation method produces genetically uniform progenies with desired characters, making it vital in tree improvement programmes. Tree improvement through seeds (pure line) faces challenges owing to its long rotation period, out breeding and limited genetic gain (10).

Clonal propagation and tissue culture techniques (11) are vital for maintaining natural genetic diversity, enhancing tree genetics and meeting forestry demands through rapid *in vitro* multiplication and yield improvement (12). The rising demand for *Santalum album L.* (Indian Sandalwood) has highlighted the inadequacy of research and commercial applications in clonal forestry, presenting significant challenges in meeting industrial needs. As a vital genetic resource, Indian Sandalwood offers potential to address the increasing demand for wood raw materials in forest-based sectors. To ensure sustainable utilization and conservation, it is crucial to optimize *in vitro* propagation strategies using different explants. This approach would enhance the clonal forestry potential of Indian Sandalwood. Investment in research and development from both government and industry is essential to refine propagation techniques and meet the growing industrial demand effectively (13).

Under such circumstances, the present study was conducted to optimize *in vitro* propagation strategies in *Santalum album* through different explants.

Materials and Methods

Based on the plus tree selection method (14), the parent source was selected from Marayoor town in Kerala state and raised in the mini mother garden in the research and development nursery at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India (11°19N, 76°56E, 300 meters MSL, Rainfall 800 mm, pH 7.1) during 2023 - 2024 (Fig 1a).

Fig. 1. Sandal tissue culture (**a**) Fiver year old sandal mother garden.
a. Mini Mother Garden(5 year) Clonal Nursery Forest College and Research Institute, Mettupalayam, Tamil Nadu, India.

Selection of explants

Different explants viz., shoot tips, internodes and nodal segments were collected from 5-year-old sandal stock in a mini mother garden during the pre-monsoon season (May to June). The explants of 2 to 3 cm in length were dissected from the mother plant and immersed in distilled water during transport.

Sterilization of explants

The explants were gently shaken and thoroughly washed with running water. After a 10 mins of disinfection with Tween 20 solution, the explants were thoroughly washed using sterile, distilled water for two min. Then the explants were treated with 2% Bavistin and further cleaned with sterile distilled water. Following the surface disinfection procedure with $HgCl_2$ (0.05 - 1%), $CaoCl_2$ (1 & 3%) and NaOCl (0.25 - 4%) each treated for 5,7, 10,15 min at different time intervals, the explants were thoroughly rinsed three to four times with sterilized water to eliminate the sterilant and subjected for shoot induction using basal medium (Fig 1. b & c).

Fig. 1. Sandal tissue culture (**b**) experiment on sterilizing explant, (**c**) Sterlized explent ready for inoculation.

Preparation of media for culturing

The ideal and appropriate medium for organogenesis was optimized using MS media (Murashige and Skoog) and Woody Plant Media (WPM). All stock solutions and final medium were prepared following (15). Various combinations with growth regulators and media were deployed. To culture the explants, the prepared media were sterilized at 121°C (15 lbs pressure) for 20 min. The cultures were maintained under cool white fluorescent light providing a photosynthetic photon flux density (PPFD) of 40-50 $\mu mols^{-1}m^{-2}$. Cultures were incubated at 25 ±2 °C in a light-darkness cycle of 16 hours and 8 hours and the relative humidity was maintained at 60-70% respectively.

The following observations i) Culture response percent ii) Contamination percent iii) Drying percent were recorded ten days after inoculation (16).

Direct organogenesis

For direct organogenesis either by axillary shoot proliferation or by adventitious shoot formation, the explants viz. shoot tip, nodal and inter segments were inoculated in the identified basal medium supplemented with a variety of concentrations and combinations of plant growth regulators to find out the optimum concentration of cytokinins for shoot induction, whereas the control was fundamental basal medium. The treatment schedule was imposed on the screened explants (Table 1). Following observations i) culture response percentage, ii) number of buds developed (visual count was taken 30 days after inoculation), iii) number of multiple shoots explant-1 (visual count was taken 60 days after inoculation), iv) shoot length was recorded. The length of each shoot was calculated by measuring the length of shoots produced by each sprouted explant and expressed in cm (Fig 1. d & e).

Table 1. Treatment schedule for direct organogenesis

Types of Explants	Plant growth Regulators	Concentration
Shoot tip	6-Benzylaminopurine (BAP)	0.3 mg/L to 5 mg/L
	Kinetin (KIN)	1 mg/L to 5 mg/L
	Naphthalene acetic acid (NAA)	0.3 mg/L to 5 mg/L
	Gibberellic acid (GA3)	0.3 mg/L to 5 mg/L
Nodal segments	6-Benzylaminopurine (BAP)	1 mg/L to 2 mg/L
	Kinetin (KIN)	1 mg/L to 2 mg/L
	Naphthalene acetic acid (NAA)	0.5 mg/L to 1 mg/L
Inter nodal segments	6-Benzylaminopurine (BAP)	1 mg/L to 2 mg/L
	Kinetin (KIN)	1 mg/L to 2 mg/L
	Naphthalene acetic acid (NAA)	0.5 mg/L to 1 mg/L
	Gibberellic acid (GA3)	1 mg/L to 2 mg/L

Indirect organogenesis

Callus was initiated from the nodal segment with the following auxins viz., 2.4-D, IAA, IBA and BAP. Following observations i) days taken for callus initiation, ii) culture response percentage, iii) relative growth of callus was recorded (Fig 1. f & g).

Callus regeneration

Thirty days after callus initiation, callus clumps were transferred to regeneration medium. The regeneration medium consisted of MS basal medium supplemented with BAP, KIN, NAA, GA, activated charcoal and coconut water.

Fig. 1. Sandal tissue culture (**d**) & (**e**) Stage between initiation and development.

Fig. 1. Sandal tissue culture (**f**) & (**g**) Callus initiation and shoot growth.

Rhizogenesis

To stimulate rhizogenesis, individual shoots were dissected and subcultured in an aseptic manner on the identified basal media supplemented with different amounts and combinations of auxins (IAA and IBA). Similar environmental conditions were also extended as in the case of shoot induction. Following observations i) days taken for root initiation, ii) percentage of culture rooted, iii) number of roots per explant, iv) root length (visual count was taken and recorded) were recorded.

Statistical analysis

The analysis was done using R software version 4.4.0 (ggplot2 package) and the Statistical Package for Social Sciences (SPSS) program, version 16.0 for Windows (SPSS, Chicago, IL, USA). Significant differences between treatments were estimated using Duncans multiple-range tests, with the statistical significance level fixed at P<0.05.

Results and Discussion

Optimal basal medium and explants morphogenic response

Selection and disinfection of explants are crucial steps in plant tissue culture techniques. Compared to herbaceous species, woody plants generally exhibit limited response and greater challenges in micropropagation. The age of the explant significantly impacts the development of vegetative buds; explants collected from 16 to18 year-old wild trees often show poor responses or high contamination rates. Contamination poses a substantial challenge, as establishing aseptic cultures from field-grown plants carries a high risk of internal and external contamination (17, 18). Only a limited number of explants are suitable for reliably demonstrating rapid direct and indirect shoot organogenesis. However, it was found that the meristematic tip was the most effective for regeneration compared to the adventitious tip (19). In the current study, 1 cm dissected explants such as nodes, internodes, leaves and cotyledons were collected from a mature, pruned tree during the winter season and inoculated. Conversely, another study demonstrated that cotyledonary nodes derived from seeds and immature seeds of a 6-year-old tree were appropriate for callus culture (20). Nodal explants have been shown to facilitate a high rate of shoot multiplication in *Syzygium travancoricum* (21) and shoot tips from mature trees in *Syzygium francissi* (22).

The analysis of variance (ANOVA) revealed significant differences ($p < 0.05$) in morphogenic response among different media and explant types (Fig. 2). MS media showed the highest mean morphogenic response (32.25%), significantly outperforming

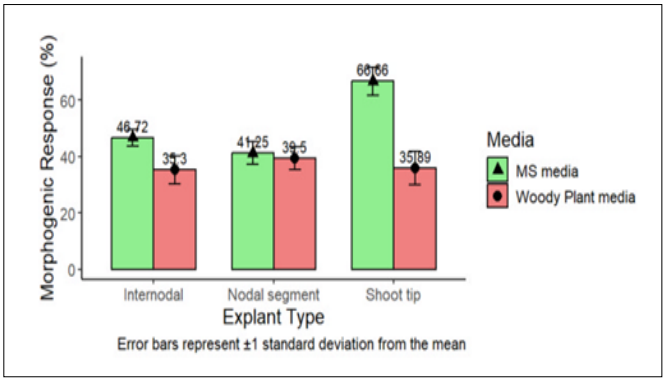

Fig. 2. Screening of basal medium and explants.

other media types. Among explant types, nodal segments exhibited the highest mean response (36.13%), followed by internodal segments (32.63%) and shoot tips (23.80%). Among the explant types, shoot tips showed the highest morphogenic response (66.66%) in MS medium, followed by internodes (46.72%) and nodal segments (41.25%). This indicates that shoot tips are the most responsive explant type for sandal micropropagation, likely due to their meristematic nature and high regenerative potential.

Sterilant Effects on Explant

Effective surface disinfection is crucial for establishing aseptic cultures. The study compared three common sterilants *viz.* mercuric chloride ($HgCl_2$), sodium hypochlorite (NaOCl) and calcium hypochlorite (Ca $(OCl)_2$) at various concentrations and durations. The explants were then rinsed twice with sterile distilled water, followed by three washes with autoclaved distilled water. This disinfection process demonstrated that using a combination of two or more sterilizing agents yielded better results compared to previous methods (23). Higher shooting responses were observed in node and cotyledon explants when an appropriate surface disinfection process was applied. Utilizing a single sterilizing agent was not effective in controlling microbial contamination, which is a significant issue during surface disinfection as it can affect the explants' multiplication potential. Disinfectants such as ethanol and mercuric chloride, along with a few drops of Tween 20, were highly effective against fungal and bacterial contamination in the nodal parts of *Manihot esculenta* (24, 25).

The results indicate a significant effect of sterilant concentration and treatment duration on explant survival, contamination and drying rates. The optimal treatment was found to be 1% $HgCl_2$ for 7 min, which resulted in 44% survival, 42% contamination and 14% drying. Higher concentrations (1%) and longer duration (10 min) led to 100% drying, indicating

phytotoxicity. Lower concentrations (0.05%) were less effective in controlling contamination with rates up to 91% (Fig. 3). The best treatment was 4% NaOCl for 10 min, resulting in 86% survival, 9% contamination and 3% drying. Higher concentrations (4%) and longer duration (15 min) led to increased drying (67%), indicating potential tissue damage. Lower concentrations (0.25%) were less effective in controlling contamination, with rates up to 74% (Fig. 4). Overall, $CaOCl_2$ was less effective than $HgCl_2$or NaOCl in sterilizing explants. The best results were obtained with 3% $CaOCl_2$ for 10 min yielding 20% survival, 54% contamination and 26% drying. Lower concentrations (1%) and shorter durations were ineffective in promoting explant survival as mentioned in (Fig. 5).

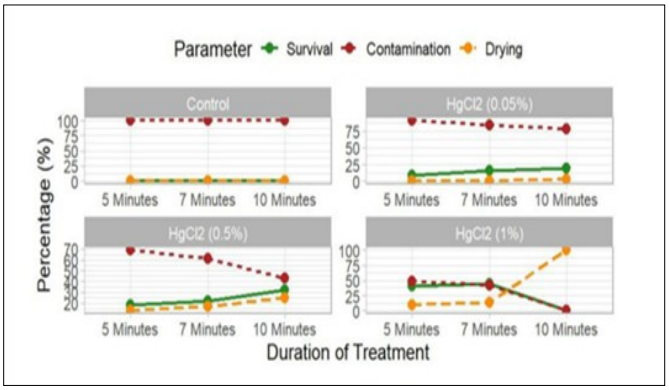

Fig. 3. $HgCl_2$ disinfection for sandal explants.

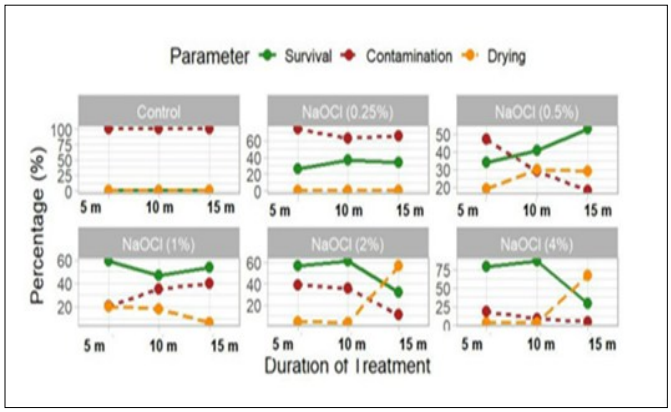

Fig. 4. NaOCl disinfection for sandal explants.

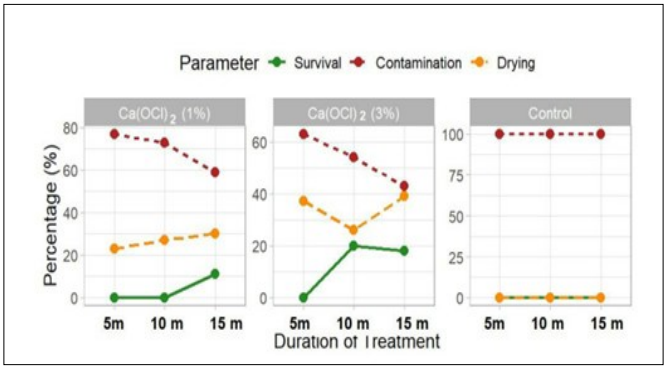

Fig. 5. $Ca(OCl)_2$ disinfection for sandal explants.

In this study, preliminary experiments resulted in a high percentage of explant loss due to microbial contamination. To assess the culture response, a proper disinfection procedure was conducted using NaOCl at 4% concentration for 10 min emerged as the most effective treatment, resulting in the highest survival percentage (86%), lowest contamination (9%) and minimal explant drying (3%). This protocol strikes an optimal balance between disinfection efficacy and explant viability, which is crucial for successful culture initiation. In contrast, using 0.1% mercuric chloride for 3-5 min produced favourable results in nodal explants of *S. cumini* and the use of $HgCl_2$ for surface disinfection of seeds of *S. alternifolium*, followed by rinsing with sterilized distilled water (26).

Multiple shoot induction and callus initiation

In shoot tip culture, kinetin at 1 mg/L and 2 mg/L showed the best results, with 64% and 54% culture response respectively. These treatments also produced the highest number of primary buds (3.58 and 3.87) and shoots (3.57 and 3.81). GA_3 treatments were generally less effective, with lower culture response and fewer shoots (Table 2). In nodal culture**,** Kinetin at 1 mg/L and 2 mg/L again showed the best results, with 48% culture response for both concentrations. These treatments also produced the highest number of primary buds (3.69 and 3.79) and shoots (3.68 and 3.78). BAP treatments were moderately effective, while NAA and GA_3 were less successful in promoting shoot induction (Table 3). In internodal culture, kinetin at 2 mg/L showed the best

Table 2. Effect of growth regulators on multiple shoot induction from sandal shoot tip

Treatment	Culture response (%)	Average number of primary buds (30days)	Average number of shoots (60days)	Average length of shoots (cm)
MS basal control	11	1.98^{bc}	1.91^{c}	3.65^{b}
MS + BAP 1 mg/L	25	2.14^{b}	2.14^{bc}	3.20^{c}
MS + BAP 2 mg/L	21	2.14^{b}	2.14^{bc}	3.26^{c}
MS + BAP 5 mg/L	21	1.89^{c}	1.83^{c}	2.07^{d}
MS + Kin 0.3 mg/L	46	2.53^{a}	2.36^{b}	3.66^{b}
MS + Kin 1 mg/L	64	3.58^{a}	3.57^{a}	4.06^{ab}
MS + Kin 2 mg/L	54	3.87^{a}	3.81^{a}	5.39^{a}
MS + Kin 5 mg/L	43	2.13^{b}	1.88^{c}	3.72^{b}
MS + NAA 0.3 mg/L	21	2.00^{bc}	1.97^{c}	3.55^{b}
MS + NAA 1 mg/L	29	2.00^{bc}	1.97^{c}	3.39^{c}
MS + NAA 2 mg/L	14	1.91^{bc}	1.54^{cd}	3.43^{c}
MS + NAA 5 mg/L	14	1.38^{cd}	1.36^{cd}	2.99^{d}
MS + GA_3 0.3 mg/L	14	1.05^{d}	1.30^{d}	2.93^{d}
MS + GA_3 1 mg/L	14	1.00^{d}	1.00^{d}	3.05^{c}
MS + GA_3 2 mg/L	12	1.11^{d}	0.92^{e}	3.24^{c}
MS + GA_3 5 mg/L	12	1.19^{d}	1.07^{ed}	3.26^{c}
Mean	**25.93**	**1.99**	**1.92**	**3.42**
SE	**1.10**	**0.10**	**0.09**	**0.13**
CD (p=0.05)	**2.21**	**0.21**	**0.19**	**0.28**

Table 3. Effect of growth regulators on multiple shoot induction from sandal nodal segments

Treatment	Culture response (%)	Average number of primary buds (30days)	Average number of shoots (60days)	Average length of shoots (cm)
MS basal control	21^{c}	2.06^{c}	1.95^{b}	3.73^{b}
MS + BAP 1 mg/L	21^{c}	2.47^{ab}	2.55^{a}	3.18^{bc}
MS + BAP 2 mg/L	21^{c}	2.59^{a}	2.56^{a}	3.25^{bc}
MS + Kin 1 mg/L	48^{a}	3.69^{a}	3.68^{a}	4.06^{a}
MS + Kin 2 mg/L	48^{a}	3.79^{a}	3.78^{a}	5.39^{a}
MS + NAA 0.5 mg/L	21^{c}	2.33^{b}	1.37^{c}	3.41^{b}
MS + NAA 1 mg/L	21^{c}	2.14^{bc}	1.22^{c}	3.44^{b}
MS + GA_3 1 mg/L	24^{b}	0.57^{d}	0.46^{d}	2.44^{c}
Mean	**28.12**	**2.45**	**2.19**	**3.61**
SE	**1.76**	**0.13**	**0.08**	**0.15**
CD (p=0.05)	**3.74**	**0.27**	**0.18**	**0.33**

results, with 30% culture response, 7.95 primary buds and 4.73 shoots. BAP treatments were moderately effective, while NAA and GA3 showed poor results for shoot induction from internodal segments (Table 4).

The study extensively examined the effects of various plant growth regulators (PGRs) on multiple shoot induction from different explant types. Kinetin (Kin) consistently outperformed other PGRs in promoting shoot induction and proliferation. For shoot tip explants, MS medium supplemented with 2 mg/L Kin produced the highest number of shoots (3.81) and the longest shoots (5.39 cm) after 60 days. Similar results were observed for nodal segments and internodal explants, with 2 mg/L Kin consistently yielding the best results. The superior performance of Kin over other cytokinins like BAP and growth regulators like GA_3 suggests that Sandalwood has a specific cytokinin preference for shoot induction and elongation. This finding is valuable for optimizing micropropagation protocols for this species. Similar results have been reported in other studies where Kin, in combination with other growth regulators, optimized shoot induction in various plant species, including medicinal orchids and woody perennials (27).

The best results were obtained with MS + 5 mg/L Kinetin + 2 mg/L BAP combination, showing 65.75% shoot induction, 4.50 shoots per explant and 4.00 cm shoot length. Generally, higher concentrations of Kinetin (4-5 mg/L) combined with moderate levels of BAP (1-3 mg/L) showed better results (Table 5). When BAP and Kinetin were used in combination, the study observed enhanced shoot induction and proliferation. The treatment of MS + 5.0 mg/L Kin + 2.0 mg/L BAP resulted in the highest shoot induction rate (65.75%) and average shoot length (4.00 cm). This synergistic effect demonstrates the potential benefits of using multiple PGRs to fine-tune the micropropagation protocol when compared to the effect of auxin and cytokinin on shoot amplification in *Albizia lebbek* (28). Similarly, the nodal segments of *Melia azedarach* responded well when cultured in MS medium supplemented with IAA and BAP along with IAA and Kin (29).

In callus initiation, BAP at 4 mg/L showed the best results, with a callus index of 70.42 and 35.21% callusing. 2,4-D treatments were moderately effective with higher concentrations (2-3 mg/L) performing better than lower ones. IAA, IBA and coconut water treatments were ineffective in inducing callus (Fig. 6).

Statistical analysis showed minimal differences among treatments, with most showing no response. Only MS + BAP 1 mg/L + CW 25% showed culture response (7.14%). No treatment resulted in the shoot or root formation from callus. Relative growth of callus and regeneration in sandal was generally poor when compared to eucalyptus (30). The present study encountered difficulties in root induction from multiple shoot explants, with no response observed across different induction media. This highlights a significant challenge in completing the micropropagation cycle for sandalwood and suggests that further research is needed to overcome this hurdle. Attempts at indirect organogenesis through callus culture showed limited success. While callus initiation was achieved using various PGR combination, only one treatment (MS + BAP 1mg/L + CW 25%)

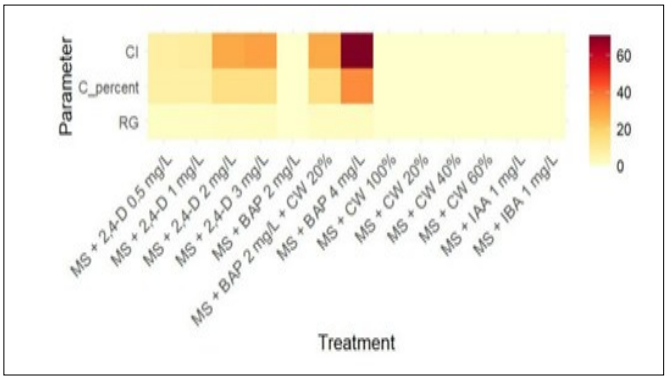

Fig. 6. Effect of growth regulators on callus initiation in different sandal explant (RG- Relative growth, C%-Callusing percentage, CI - Callus index).

Table 4. Effect of growth regulators on multiple shoot induction from sandal internodal segment

Treatment	Culture response (%)	Average number of primary buds (30days)	Average number of shoots (60days)	Average length of shoots (cm)
MS basal control	19^{d}	1.71^{d}	1.07^{c}	3.59^{b}
MS + BAP 1 mg/L	23^{c}	2.07^{c}	1.38^{c}	3.25^{b}
MS + BAP 2 mg/L	23^{c}	2.19^{c}	1.16^{c}	3.61^{b}
MS + Kin 1 mg/L	28^{b}	5.73^{b}	3.28^{b}	4.12^{a}
MS + Kin 2 mg/L	30^{a}	7.95^{a}	4.73^{a}	4.53^{a}
MS + NAA 0.5 mg/L	11^{ed}	0.74^{e}	0.31^{d}	2.48^{cd}
MS + NAA 1 mg/L	14^{e}	0.76^{e}	0.19^{e}	2.72^{c}
MS + GA_3 1 mg/L	11^{ed}	0.86^{e}	0.31^{d}	3.74^{b}
MS + GA_3 2mg/L	10^{ed}	0.71^{e}	0.31^{d}	3.18^{b}
Mean	**18.77**	**2.52**	**1.41**	**3.46**
SE	**0.95**	**0.20**	**0.07**	**0.13**
CD (p=0.05)	**2.00**	**0.42**	**0.16**	**0.28**

Table 5. Combination of BAP and Kinetin on multiple shoot induction

Treatment	Shoot Induction (%)	Number of shoots(cm)	Shoot length (cm)
MS + 1 mgl^{-1} Kinetin + 1 mgl^{-1} BAP	37.50^{de}	1.68^{d}	2.83^{c}
MS + 1 mgl^{-1} Kinetin + 2 mgl^{-1}BAP	42.00^{bcd}	1.26^{d}	2.76^{c}
MS + 1 mgl^{-1} Kinetin + 3 mgl^{-1}BAP	45.50^{bc}	1.50^{d}	3.68^{b}
MS + 1 mgl^{-1} Kinetin + 4 mgl^{-1}BAP	48.75^{bc}	2.40^{c}	3.28^{b}
MS + 1 mgl^{-1} Kinetin + 5 mgl^{-1}BAP	51.25^{bc}	2.33^{c}	3.95^{b}
MS + 2 mgl^{-1} Kinetin + 1 mgl^{-1} BAP	40.00^{cd}	1.58^{d}	1.85^{d}
MS + 2 mgl^{-1} Kinetin + 2 mgl^{-1}BAP	45.05^{bc}	1.93^{d}	3.89^{b}
MS + 2 mgl^{-1} Kinetin + 3 mgl^{-1}BAP	42.50^{bcd}	1.26^{d}	1.92^{d}
MS + 2 mgl^{-1} Kinetin + 4 mgl^{-1}BAP	47.75^{bc}	2.56^{c}	2.09^{c}
MS + 2 mgl^{-1} Kinetin + 5 mgl^{-1}BAP	49.00^{bc}	1.89^{d}	2.50^{c}
MS + 3 mgl^{-1} Kinetin + 1 mgl^{-1} BAP	33.75^{ef}	1.63^{d}	2.96^{c}
MS + 3 mgl^{-1} Kinetin + 2 mgl^{-1}BAP	32.50^{f}	2.03^{c}	1.56^{d}
MS + 3 mgl^{-1} Kinetin + 3 mgl^{-1}BAP	47.50^{bc}	2.13^{c}	2.92^{c}
MS + 3 mgl^{-1} Kinetin + 4 mgl^{-1}BAP	41.25^{cd}	3.34^{b}	3.61^{b}
MS + 3 mgl^{-1} Kinetin + 5 mgl^{-1}BAP	42.25^{cd}	3.41^{b}	2.82^{c}
MS + 4 mgl^{-1} Kinetin + 1 mgl^{-1} BAP	45.00^{bc}	1.88^{d}	1.98^{d}
MS + 4 mgl^{-1} Kinetin + 2 mgl^{-1}BAP	46.25^{bc}	2.14^{c}	2.43^{c}
MS + 4 mgl^{-1} Kinetin + 3 mgl^{-1}BAP	48.25^{bc}	3.23^{b}	3.11^{b}
MS + 4 mgl^{-1} Kinetin + 4 mgl^{-1}BAP	51.50^{bc}	2.74^{c}	2.92^{c}
MS + 4 mgl^{-1} Kinetin + 5 mgl^{-1}BAP	47.75^{bcd}	2.71^{c}	3.45^{b}
MS + 5 mgl^{-1} Kinetin + 1 mgl^{-1} BAP	54.25^{abc}	3.10^{b}	3.10^{b}
MS + 5 mgl^{-1} Kinetin + 2 mgl^{-1}BAP	65.75^{a}	4.50^{a}	4.00^{a}
MS + 5 mgl^{-1} Kinetin + 3 mgl^{-1}BAP	44.50^{bc}	3.50^{b}	3.05^{b}
MS + 5 mgl^{-1} Kinetin + 4 mgl^{-1}BAP	62.25^{ab}	4.25^{a}	2.75^{c}
MS + 5 mgl^{-1} Kinetin + 5 mgl^{-1}BAP	55.50^{abc}	3.75^{b}	3.40^{b}
Grand mean	**46.702**	**2.509**	**2.912**
SE	**1.526**	**0.183**	**0.135**
CD (p=0.05)	**3.205**	**0.384**	**0.284**

showed a regeneration response, wherein embryoid-like structures failed to develop further. This indicates that indirect regeneration may not be an efficient approach for Sandalwood micropropagation.

IBA at 1 mg/L showed the best results, with 14.29% rooting, 2.50 roots per shoot and 0.83 cm root length. IAA treatments were ineffective in inducing rooting. Higher concentrations of IBA (3 mg/L) also failed to induce rooting, suggesting an optimal concentration around 1-2 mg/L (Fig. 7).

The study's final experiment on rooting micro propagated shoots showed limited success. The best results were obtained with MS + IBA 1mg/L, which induced rooting in 14.79% of shoots after 10 days. However, the overall root growth was poor and further shoot growth was arrested. This suggests that further optimization of the rooting protocol is necessary to improve the efficiency of the micropropagation process.

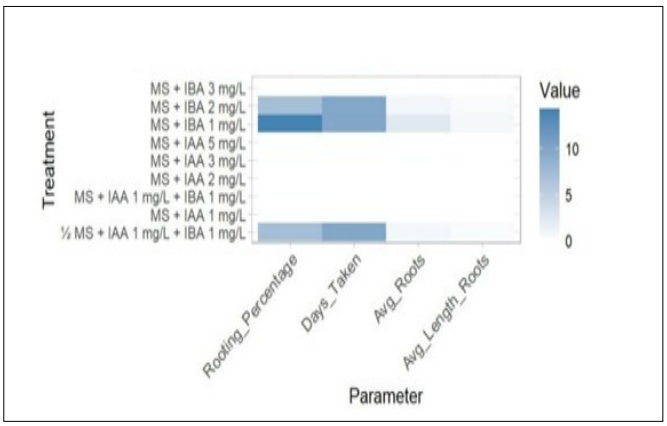

Fig. 7. Effect of IAA and IBA on rooting of micro propagated shoot of sandal.

Conclusion and future perspectives

This study provides a comprehensive analysis of optimizing *in vitro* propagation protocols for *Santalum album* L. (Indian sandalwood). Key findings include the superiority of Murashige and Skoog (MS) medium for shoot induction, with 2 mg/L kinetin in MS yielding the highest number of shoots and longest shoot length. Surface disinfection using 4% NaOCl for 10 min effectively ensured high explant survival rates, minimizing contamination and drying. Despite these advancements, challenges remain, particularly in root induction and acclimatization phases.

Future research should focus on enhancing root induction techniques, exploring novel combinations of plant growth regulators (PGRs) and developing efficient acclimatization protocols to improve the success rate of micropropagation. Additionally, investigations into optimizing medium composition for indirect organogenesis, such as callus culture, are crucial for expanding the clonal propagation potential of sandalwood. Continuous refinement of *in vitro* techniques will be essential to meet the increasing global demand for sandalwood while ensuring sustainable conservation of this valuable species.

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Authors' contributions

The conceptualization of the research was carried out by MG, JS and HP. The formal analysis was conducted collaboratively by MG, MM, JS, HP, RR and SPP. Funding acquisition was secured through the efforts of MG, JS, HP, RR, KTP, MK and VK. The methodology was developed by JS, HP, IS, MG, MM and RR. Finally, the supervision and validation of the work were overseen by MG, HP and JS.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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