

AN ATTEMPT TO CONSERVE A VULNERABLE TREE SPECIES OF *Santalum album* L. THROUGH MICROPROPAGATION

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AN ATTEMPT TO CONSERVE A VULNERABLE TREE SPECIES OF *Santalum album* L. THROUGH MICROPROPAGATION. A rare kind of tropical plant in the Santalaceae family is *Santalum album*. The active ingredient in *S. album*, santalol, is also referred to as sandalwood oil and is highly prized in the fragrance business for its fixative qualities and pleasant, enduring scent. Out of all the species in the genus *Santalum*, *S. album* has the greatest oil concentration (about 6%). The wild plants are overharvested for their wood, which is used to make santalol, as well as for other uses including woodcarving and traditional medicine. *S. album* is an easily hurt plant. Thus, the creation of an in vitro mass propagation protocol for this valuable species is necessary in order to generate homozygous clones with large yields for the establishment of sandalwood plantations. In this study, a full-strength MS medium supplemented with varying concentrations of BAP and Kn (0.5-2.5 mg/l) was used to cultivate the shoot tip and intermodal portions of *S. album* that were collected from the wild. The maximum shoot development (4.50 ± 0.50) occurred at a BAP concentration of 1.5 mg/l. IBA and IAA were added to the rooting medium along with the developing shoots. IBA (2.0 mg/l) had the highest mean number of roots (4.90 ± 0.25) and root length (5.75 ± 0.47 cm). Shoots that had been successfully rooted were moved to the field to harden. According to the current study, MS medium with 1.5 mg/l of BAP and 2.0 mg/l of IBA is an appropriate technique for micropropagating and conserving *S. album* is fragile tree species.

Keywords: Vulnerable, shoot tip, internode, BAP, IBA, conservation

UPAYA KONSERVASI JENIS POHON RENTAN *Santalum Album* L. MELALUI MIKROPROPAGASI. Jenis tumbuhan tropis yang langka dalam famili Santalaceae adalah *Santalum album*. Bahan aktif dalam *S. album* juga disebut sebagai minyak cendana dan sangat dihargai dalam bisnis wewangian karena kualitas fiksatifnya serta aromanya yang menyenangkan dan tahan lama. Dari semua spesies dalam genus *Santalum*, *S. album* memiliki konsentrasi minyak terbesar (sekitar 6%). Tanaman liar dipanen secara berlebihan untuk diambil kayunya, yang digunakan untuk membuat santalol, serta untuk keperluan lain termasuk ukiran kayu dan obat tradisional. *S. album* merupakan tanaman yang mudah terluka. Oleh karena itu, pembuatan protokol perbanyakan massal in vitro untuk spesies berharga ini diperlukan untuk menghasilkan klon homozigot dengan hasil yang besar untuk pendirian perkebunan cendana. Dalam penelitian ini, media MS berkekuatan penuh yang dilengkapi dengan berbagai konsentrasi BAP dan Kn (0.5-2.5 mg/l) digunakan untuk mengolah pucuk pucuk dan bagian antarmodal *S. album* yang dikumpulkan dari alam. Perkembangan tunas maksimum (4.50 ± 0.50) terjadi pada konsentrasi BAP 1.5 mg/l. IBA dan IAA ditambahkan ke media perakaran bersamaan dengan tunas yang sedang berkembang. IBA (2.0 mg/l) memiliki rata-rata jumlah akar tertinggi (4.90 ± 0.25) dan panjang akar (5.75 ± 0.47 cm). Tunas yang telah berhasil berakar dipindahkan ke lapangan untuk dilakukan pengerasan. Menurut penelitian saat ini, media MS dengan 1.5 mg/l BAP dan 2.0 mg/l IBA merupakan teknik yang tepat untuk perbanyakan mikro dan konservasi *S. album* adalah spesies pohon yang rapuh.

Kata kunci: Rentan, pucuk pucuk, ruas, BAP, IBA, konservasi

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I. INTRODUCTION

The eastern Himalayas and the Western Ghats, two of the 38 global biodiversity hotspots, are located in India, one of the most populous countries in the world. Along with countless endemic and Rare Endangered Threatened plant species, these places are home to some of the most important gene pools of medicinal plants, wild cultivable crop variants, and other species of commercial relevance (Roy et al., 2012). A number of endemic plant species, including 5,725 Angiosperm species, 10 Gymnosperm species, 193 Pteridophytes, 678 Bryophytes, 466 Lichens, 3,500 Fungi, and 1,924 Algae, are found in India's diverse flora (Sanjappa, 2005). One of the only remaining areas of great plant biodiversity are tropical forests, which are home to valuable, uncommon, and endangered commercial and medicinal tree species that must be immediately conserved for future generations and sustainable use.

The foundation of traditional medicine, medicinal plants, has been the focus of extensive pharmacological research in recent years. The recognition of the importance of medicinal plants as possible sources of novel compounds with therapeutic potential and as sources of novel compounds for drug development has led to this. Medicinal plants are employed for their antiviral, antibacterial, and antifungal properties in various parts of the world. Drugs generated from plants are used as a prototype for safer and more effective pharmaceutical products (Manikandan et al., 2017, 2019, & 2021). For millennia, plants have been a vital source of medicinal materials. The usage of plants to treat numerous diseases dates back to prehistory and people of all continents has this historical tradition (Manikandan et al., 2020). Since plant-derived medications have greatly improved human health and well-being, plants have served as an inspiration for new medicinal molecules. They serve as a phytomedicine that may be utilised to treat illnesses as well as a source of essential chemical structure for the creation of novel antibacterial

medications (Manikandan & Ramasubbu, 2020; Manikandan et al., 2022). They promote excessive collection of therapeutic plants, which results in extractive exploitation and puts the species in danger of going extinct. Because medicinal plants can be propagated in vitro to replace the supply chain for medications and other pharmaceutical items, there is evidently growing interest in this process. In vitro culture techniques provide extremely useful resources for conserving germplasm and mass-producing several endangered plant species (Pan et al., 2003).

One valuable tropical plant species that is a member of the Santalaceae family is *Santalum album* L., also known as Indian sandalwood (Rai, 1990). There are 29 genus in this family and nineteen of the nearly 400 species in this family are unique to the *Santalum* genus (Fox, 2000; Harbaugh, 2007; Harbaugh & Baldwin, 2007; Harbaugh et al., 2010; Butaud, 2015). The sandal oil found in the aromatic heartwood of *S. album*, an evergreen, hemi root parasitic tree of medium size, is highly prized in the fragrance, cosmetics, medical, and agarbatti (incense sticks) sectors (Srinivasan et al., 1992). It is indigenous to the subcontinent of India. The active ingredient in *S. album*, santalol, is also referred to as sandalwood oil and is highly prized in the fragrance business for its fixative qualities and pleasant, enduring scent (Jain et al., 2003). Of the species in the genus *Santalum*, *Santalum album* has the greatest oil content (6%) (Srinivasan et al., 1992).

Due to centuries of overexploitation, several species in the genus *Santalum* have been extinct, and three more are considered threatened. Due to the industrial exploitation of sandalwood's aromatic, oil-rich heartwood, the species' biodiversity has decreased. The IUCN designated *S. album* as vulnerable in 1998 due to its rapidly diminishing status (Awasthi, 2007). Biotechnology offers a viable and efficient technique of propagating members of this species in order to preserve the existing germplasm. Using clonal material

with a comparable genetic background throughout cultivation is crucial for improving the homogeneity of essential oils and for producing economically significant metabolites in large quantities (Teixeira da Silva et al., 2016).

According to Sanjaya et al. (2003), *Santalum album* is resistant to both in vivo and in vitro multiplication, with only patchy results to yet. The primary means of artificial and natural multiplication are seeds. Conversely, grafting, air layering, and root suckers are methods used in vegetative multiplication; however, clone creation is a laborious and insufficient process (Srimati et al., 1995). Sandalwood is spread via seeds in the wild. But the germination success rate of seeds is quite low (Viswanath et al., 2009). After storage, seeds lose their viability in six to nine months. As per the IUCN status (2021.1), *S. album* is considered to be a vulnerable tree species. Thus, it's critical to create an in vitro strategy for the mass multiplication of this valuable species in order to generate homozygous clones with large yields for the establishment of sandalwood plantations. This work has been designed to support the conservation efforts on the fragile *S. album* L. (Indian Sandalwood) tree by micropropagation investigations, based on the foregoes.

II. MATERIAL AND METHOD

S. album, a 20-year-old tree, was harvested for its explants (shoot tips and nodal portions) from the Sirumalai hills in Dindigul, Tamil Nadu, India (1200 m asl; 10°11'05.8"N 78°01'04.7"E). After being submerged in running water for half an hour, the explants were treated with a commercial liquid detergent called Tween 80 (1%) and surface sterilised for five minutes using 0.1% Mercuric chloride. Afterwards, rinse with distilled sterile water. The explants were once again sterilised for three minutes using 4% sodium hypochlorite, and then they were washed with sterile distilled water. After a final three minutes of surface sterilisation with 70% ethanol, the material was cleaned with sterile distilled water, and the explants used a

sterile blade to cut the tip end (Ramasubbu et al., 2015&2016; Manikandan et al., 2017; Thiri Bhuvaneswari et al., 2020).

The explants were infused with varying concentrations (0.5 - 2.5 mg/l) of BAP and kinetin (0.5 - 2.5 mg/l) in the MS medium (Murashige & Skoog, 1962). The culture rack in the room held the inoculated glass tubes at a suitable temperature of $25 \pm 2^\circ\text{C}$. The light source was a cool white fluorescent lamp with an average brightness of 2000 lux, and the photoperiod was 16 hours of day and 8 hours of night. Following 30 to 45 days, the number and length of shoots that emerged from the explants were also carefully monitored, along with the development of the shoots from the culture. Then, auxillary shoots that were grown in culture with cytokinin were typically rootless. To get whole plant, grown shoots were transferred to rooting medium supplemented with varying concentrations (0.5 - 2.5 mg/ml) of IAA and IBA. Half strength of MS medium is best for rooting shoots in vast number of plants

To prevent excessive water loss, rooted shoots with four to five completely developed leaves were planted in plastic pots filled with a 3:1:1 v/v mixture of sand, soil, and vermin compost. The pots were covered with polyethylene bags and watered twice a week during the first fifteen to twenty days of growth. For three to four weeks, plantlets were housed at 25°C ($\pm 2^\circ\text{C}$) in artificial light (16 hours photoperiod) supplied by white fluorescent light. After that, the pots were moved outside into the sun. Plantlets that had successfully established themselves were then moved to field conditions after three to four months.

III. RESULT AND DISCUSSION

A. Induction and Multiplication

Explants with a predetermined meristem, like nodes or shoot tips, are typically used in the in vitro propagation of plant species. These explants can come from mature trees or from in vitro or ex vitro germinated seedlings; other

explants, like internodes, stem segments, or leaves of leaf discs, are typically used to induce adventitious shoots or somatic embryos. The explants employed for sandalwood included nodes and shoot tips from seedlings or old trees (Pearis & Senarath, 2015; Radhakrishnan et al., 2001; Singh et al., 2015; Ilah et al., 2002; Primawati, 2006; Revathy & Arumugam, 2011). The author of the current work employed the shoot tip and the internodal portion to induce the shoot and root of *S. album*.

Hartini and Endang (2016) state that the Woody Plant Medium is a type of medium that is commonly used for the *in vitro* cultivation of hardwood plant species, whereas MS media is most commonly utilised for the *in vitro* culture of callus and shoots induction for all plants. Since MS medium was higher in mineral

salts and N compounds like ammonium and nitrate than Woody Plant Medium medium, it was more effective at inducing shoots in the propagation of sandalwood (Indrianti, 2003). These elements stimulated the growth of sandalwood. According to Bhargava et al. (2018), Murashige and Skoog's (MS) medium was previously used to propagate plants using *S. album* nodal explants.

In the current study, explants of shoot tip developed the highest number of shoots (4.50 ± 0.50) and shoot length (4.12 ± 0.50 cm), while explants of internodal at 1.5 mg/l concentration of BAP and 2.0 mg/l concentration of Kn formed 5.00 ± 0.20 shoots and 4.70 ± 0.35 cm length (Fig. 1 & 2; Plate 1 & 2). When compared to the Kn, the 1.5 mg/l concentration of BAP had noteworthy

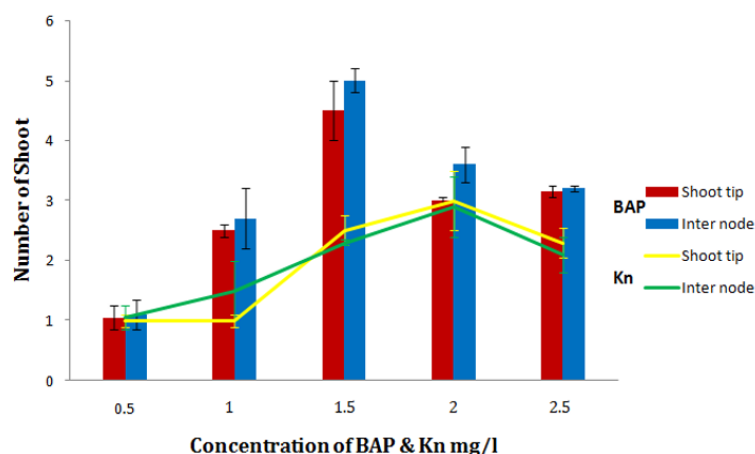


Figure 1. Number of shoot induction in various concentrations of BAP & Kn

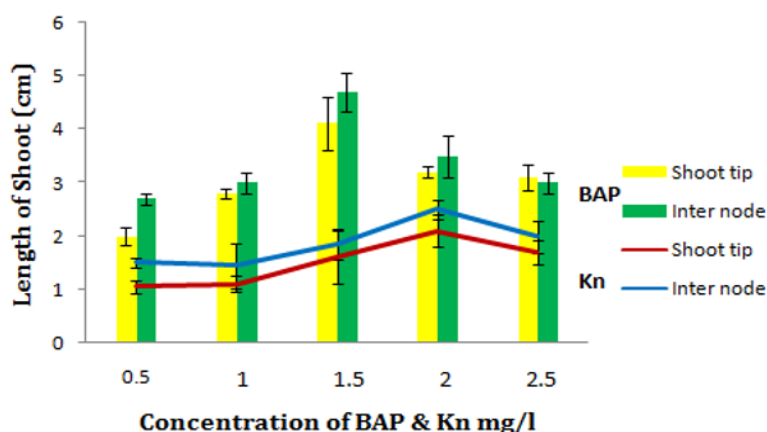


Figure 2. Length of shoot induction in various concentrations of BAP & Kn

outcomes. According to earlier research by Hartini and Endang (2016), MS medium enhanced with 2 mg/l BAP was the most effective medium for inducing shoots from Sandalwood. The 1.5 cm shoot with four leaves formed in MS0 medium. One hypocotyl explant produced twelve shoots after two milligram's per litre of BAP was added to the MS medium. The *S. album* explants that were examined

for multiple shoot induction and intermodal segments yielded positive findings. The species reacted favourably to MS Medium out of the two media—MS and White media. The optimal combination of MS with 5.0 mg/l of Kn and 2.0 mg/l of BAP was found to be effective in inducing numerous shoots in shoot tip explants (Krishnakumar & Parthiban, 2018).



Figure 3. Shoot induction in various concentrations of BAP & Kn

B. Rooting

IBA and IAA were added to the rooting medium along with the developing shoots. IBA (2.0 mg/l) had the effective mean number of roots (4.90 ± 0.25) and length of root (5.75 ± 0.47 cm). In IAA (2.0 mg/l), the greatest mean number of roots (3.10 ± 0.19) and root length (2.80 ± 0.25) were found (Fig. 4; Fig. 5). When compared to IAA, the 2.0 mg/l concentration of IBA produced noteworthy outcomes. The current study was corroborated by a prior study, which found that there was a strong rooting response on MS medium with IBA (Janarthanam & Sumathi, 2011).

The most effective treatment for optimising sprouting, cutting rooting, shoot length, and root length was to root shoot tip cuttings of *S. album* 3 mg/L IBA (Krishnakumar & Parthiban, 2018). On woody plant media with 1.5 mg/l BAP and 1.5 mg/l IBA, nodal explants of *Syzygium densiflorum* produced the greatest number of shoots (7.7 ± 0.08). In half strength woody plant media supplemented with 0.5 mg/l IBA, the maximum number of roots (3.83 ± 0.53) and root length (3.4 ± 0.05 cm) were obtained (Ramasubbu & Divya, 2000). The 0.5 strength MS medium supplemented with 1.48 gM IBA produced the highest root induction (75%) and survival (80%) in *Holostemma annulare* (Sudha et al., 1998).

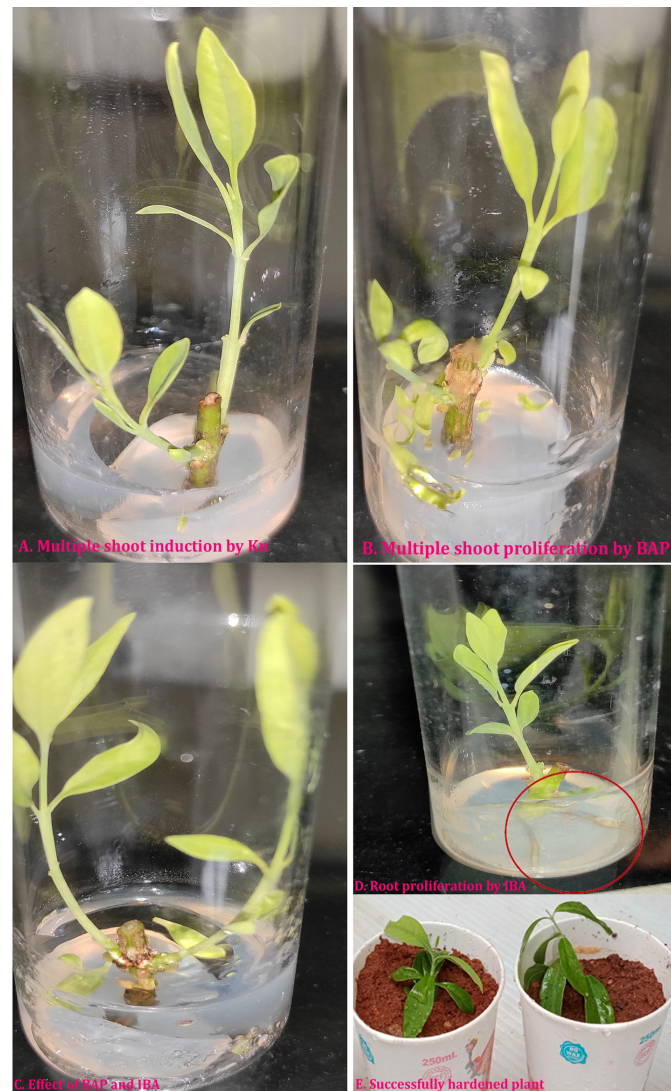
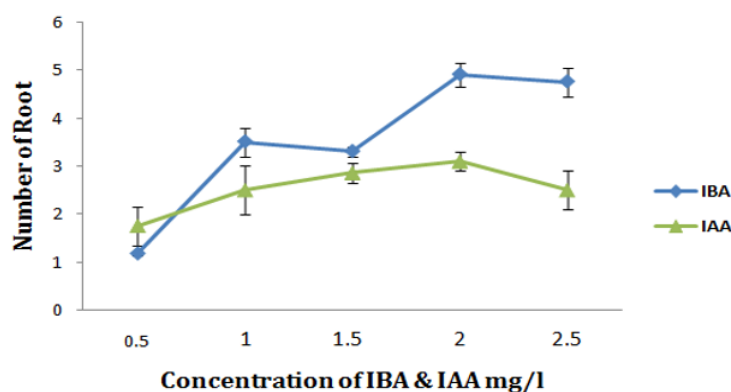


Figure 4. Shoot induction and root formation of *S. album*

Figure 5. Shoot induction and root formation of *S. album*

C. Acclimatization

Shoots that had been successfully rooted were moved to the field to harden. Rooted shoots with four to five completely developed leaves were placed in plastic pots filled with a 3:1:1 sand, soil, and vermicompost. The pots were covered with polyethylene bags and watered twice a week for fifteen to twenty days in order to minimise excessive water loss (Fig. 4). For three to four weeks, plantlets were housed at 25°C ($\pm 2^\circ\text{C}$) in artificial light (16 hours photoperiod) supplied by white fluorescent tubes. After that, the pots were moved outside into the sun. Plantlets that had been successfully developed after three to four months were then moved to field conditions, where they survived 70% of the time. According to Soumen et al. (2010), shoots were able to root on half-strength MS medium that was enhanced with 2% sucrose and 1.5 mg/l IBA. When well-developed plantlets were placed in plastic containers with soil and vermiculite (1:1), 81.1% of them survived. From shoot tip and nodal explants of *Morus nigra* L. several shoots multiplied when cultivated on Murashige and Skoog's media supplemented with BAP or K (0.5–5.0 mg l⁻¹ each). After being moved to rooting media, shoots were given 0.25–1.0 mg l⁻¹ IBA. Plantlets took four weeks to root. Plantlets that had been regenerated and rooted had been successfully inserted into the ground (Yadav et al., 1990).

IV. CONCLUSION

The current investigation established that the best technique for *S. album* micropropagation was MS medium containing 1.5 mg/L of BAP and 2.0 mg/L of IBA. As a result, this procedure can be utilised to conserve and propagate *S. album* tree species that are at risk.

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