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An Overview of the Developments in the Propagation of the East Indian Sandalwood, *Santalum album* L.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The East Indian Sandalwood is one of the most highly traded timber, also useful for the extraction of fragrant essential oil possessing medicinal values. Regardless of its vulnerable status, this forest species is now grown in the farmer's field at a faster pace in India; thanks to policy interventions. However, the requirement of quality planting material can be met only if viable methods are made available for large scale propagation. This review summarises the various methods of propagation studied for *S. album*, through seeds and vegetative parts by macropropagation and micropropagation techniques, which can be employed for its mass multiplication. Recent studies are also presented along with research advances and underlying issues in the propagation methods. Although limited numbers of sandalwood plants were produced by various researchers, commercialisation of the methods is still a long way. This may be due to major bottlenecks in host compatibility, *In vitro* rooting and the availability of superior genotypes for clonal propagation.

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1. INTRODUCTION

"Santalum album Linn. belongs to the family Santalaceae and is popularly known as the Dollar earning parasite" (Krishnappa, 1972). It is harvested mainly for its heartwood containing essential oils. The wood is commercially known as "East Indian Sandalwood", whereas its fragrant oil is called the "Queen of Essential oils" (Radomiljac & Mc Comb, 1998). "Historical review shows that its occurrence dates back to at least 2500 - 3000 years, and is one of the oldest plants known to have naturally occurring fragrance as well as medicinal properties. This species is native to the tropical belt comprising the Indian peninsula, eastern Indonesia and northern Australia. In the highlands of southern India, the principal tracts of sandalwood are most parts of Karnataka and adjoining districts of Maharashtra, Tamil Nadu, Kerala and Andhra Pradesh. It grows in geographical areas receiving 850 - 1,350 mm annual rainfall, and having temperature ranging from 25°C to 35°C" (Rao et al., 2007). There are about 20 other sandalwood species belonging to the genus *Santalum* viz., *S. acuminatum, S. austrocaledonicum, S. boninense, S. ellipticum, S. fernandezianum, S. freycinetianum, S. haleakalae, S. insulare, S. involutum, S. lanceolatum, S. leptocladum, S. macgregorii, S. murrayanum, S. obtusifolium S. paniculatum, S. papuanum, S. pyrularium, S. salicifolium, S. spicatum* and *S. yasi*. All the sandalwood species are obligate wood hemi-parasites absorbing certain nutrients like phosphates and nitrates from host trees through root connections termed haustoria (Subasinghe, 2013).

"S. album is primarily grown for its timber and fragrant oil. The timber weighs about 870 kg/m^3 and is strong and durable. It is recognised worldwide as one of the most precious marketable tree species" (Viswanath et al., 2008). India is among the principal exporters of sandalwood and its oil. Indian sandalwood oil is considered to be unique and is preferred for medication, scents, formulations, flavours, cosmetics, toiletries, beauty aids and drugs (Srinivasan et al., 1992). "Sandalwood oil contains mostly sesquiterpenoids, of which α- and β- santalols are the most prominent, and possesses various biological activities. The remaining constituents are hydrocarbons, aldehydes, ketones, phenols, acids, and heterocyclic compounds. The major sesquiterpene viz., α-santalol, is responsible for the pharmacological effects of sandalwood oil, whereas, β-santalol is largely responsible for the highly appreciated creamy, lactonic, sandalwood odour of the oil" (Kim et al., 2005; Hamalton, 2021).

"S. album is currently listed as vulnerable by the International Union of Conservation of Nature and Natural Resources of threatened species. There has been at least 20% loss over the previous three generations, based on actual or potential levels of exploitation. The existing populations are devoid of trees with commercial girth not only due to widespread illicit felling and smuggling, but also grazing, recurrent fires, and the lethal phytoplasmic spike epidemics" (Arunkumar et al., 2019). "Natural regeneration of sandalwood occurs mostly by seeds and also through root suckers, but is slow due to low seed germination percentage, and scavenging of germinated seeds by squirrels and rodents, accompanied with browsing and trampling of young seedlings by cattle and wildlife" (Singh et al., 2013). Moreover, "a major threat to sandalwood trees is the sandal spike disease caused by a mycoplasma-like organism (recently identified as phytoplasma) which has a devastating effect and very often completely eliminates the plantation. Efforts to manage and eradicate the disease have been unsuccessful" (Teixeira da Silva et al., 2016a). "These factors are also responsible for the destruction of *S. album* trees in India. The seedlings are also extremely heterozygous due to out-breeding nature. Alternatively, vegetative propagation is accomplished by grafting, air layering, and with root suckers, but the production of clonal plants is inefficient and time consuming" (Srimathi et al., 1995). Overexploitation, failure of regeneration efforts, and illicit felling have narrowed the gene pool of this heritage species. Consequently, there is a need to develop clonal techniques to produce disease-resistant and high oil-yielding clones at rapid and large scale. The *In vitro* technology, especially somatic embryogenesis, has been employed for studying the regeneration of sandalwood plants for quite some time. Tissue culture techniques can be used to mitigate difficulties of conventional propagation methods by micro-cloning of elite lines.

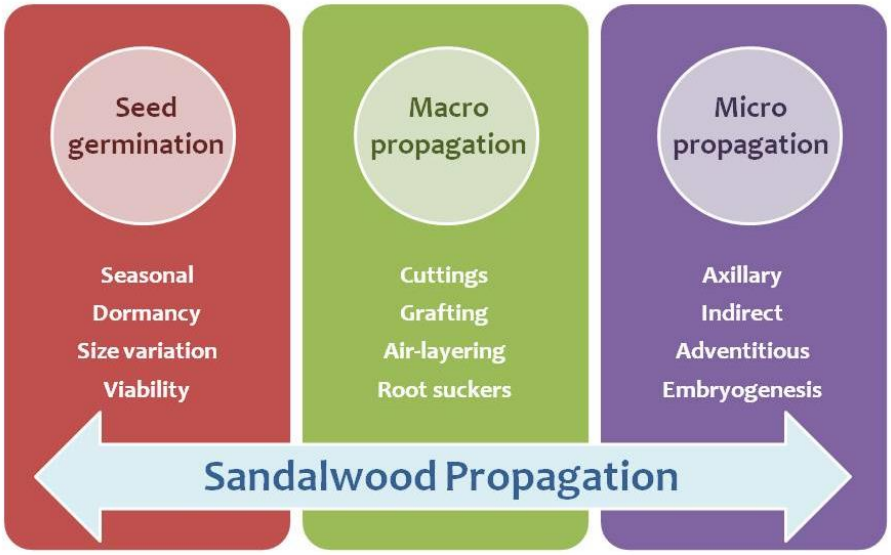

Fig. 1. Techniques for propagation of *Santalum album*

"The destruction caused by the pandemic spike disease, hemi-parasitic nature and deliberate-cultivating character of the plant dictated investigation towards the extension of modern unconventional tools of *in vitro* propagation through callusing in the early 1960s. Substantial work has been done so far in sandalwood by several researchers all over the world by employing an array of explants such as embryo, hypocotyls, shoot tip, nodal segment, leaf discs, endosperm, zygotic embryo, cotyledons, protoplast and cell suspension cultures, for inducing morphogenesis followed by plantlet regeneration with varied degree of success" (Bapat et al., 1978; Lakshmi Sita et al., 1979; Rugkhla, 1997; Dey, 2001; Rai & Mc Comb, 2002; Bele et al., 2012; Singh et al., 2013, 2015; Zhang et al., 2016; Tripathi et al., 2017). "Later on, attempts of somatic embryo development and maturation in bioreactors were successfully documented. The uniform and synchronous suspension cultures are also suitable for producing phytochemicals at large scale" (Bapat et al., 1990; Lakshmi Sita & Raghava Ram, 1995; Rugkhla & Jones, 1998; Sanghamitra & Chandni, 2010; Ilah et al., 2016). Tissue culture studies have been reported using juvenile as well as mature explants (Rao et al., 1978 & 1984; Lakshmi Sita & Raghava Ram, 1995). Artificial seeding using somatic embryo encapsulation and its recovery has also been successful (Bapat & Rao, 1988). Although few plants were regenerated, some of these methods still need to be optimised. Hence, this review explores the various propagation techniques (Fig. 1) to produce clonal plant material of sandalwood for planting as well as for conservation.

2. PROPAGATION OF SANDALWOOD

2.1 Propagation through Seeds

Sandalwood is mainly propagated by seeds, which are orthodox in nature (Fig. 2). For artificial regeneration, methods such as broadcasting of seeds, dibbling seeds in pits with different host, wounding mother plant roots by trenching and planting of nursery grown seedlings were adopted. During 1971, hoeing around the mother trees just before rains was reported to give promising results by Rao & Rangaswamy. Scientists have been exploring seed propagation techniques using various chemicals for promoting seed germination, as well as, growth for mass propagation of sandalwood (Table 1). Sandalwood seeds germinate faster on completely removing the seed coat, or when seeds are soaked for 12-16 hours in 0.05% gibberellic acid (GA_3) (Nagaveni & Srimathi, 1980, 1981). The season of seed collection also plays an important role in *S. album* seedling establishment. Nagaveni & Srimathi (1985) experimented on the viability and germination percent of floating and sinking seeds. "As the time of soaking increased, germination capacity in sunken seeds decreased due to deterioration of the seed, suggesting that soaking for longer duration makes the seeds non-viable. In 1995, they also reported that sandalwood seeds collected during September-October and sown in

April-May give maximum germination in minimum time period. Though seed germination and early seedling growth in *S. album* are fully independent of the host, seedling establishment seems to be dependent on accomplishing host contact" (Sahai & Shivanna, 1984; Rai & Mc Comb, 2002). Ananthapadmanabha et al. (1984, 1986) proved that the host plants are necessary for healthy and good growth of sandalwood plants.

Nagaveni & Ananthapadmanabha (1986) studied germination percentage and survival based on size and weight of seeds. Smaller seeds germinated more quickly (starting after 15 days and reaching a maximum at 70 days), than the medium and large seeds which started germination after 30 days and reached a maximum at 90 days. Seedling growth and survival increased with seed size; survival of seedlings from small seeds was only 55-60%, compared to 70-75% from medium seeds, and 90-95% from large seeds. Bagchi & Kulkarni (1985) observed germination and survival percentage from selected trees of *S. album* and noted genotypic differences. Sandalwood seeds show variation in size, germination percentage, rate and time duration which may affect the adoptive variability of the species (Sindhuveerendra et al., 1991). Germination rate was highly variable for seeds categorised based on weight from 9 different girth class trees, suggesting that the germination was entirely dependent on the genetic factors, which may reflect on their adoptive variability. Gamage et al. (2010) have reported that germination efficacy of stored *S. album* seeds decreases over time, reaching 0% after 28 weeks, suggesting that seeds should be sown once shed rather than using stored seeds, and germination trials should be started early. Das & Tah (2013) noted that the duration of germination is much prolonged after the dormancy period, starting at 25 days and reaching hardly 50% in 90 days with 0.05% GA_3 soaking for 16 hours. Highest germination rate after pretreatment with 500ppm GA_3 was also reported by Karmakar et al. (2017).

Fig. 2. Propagation of *S. album* through seed germination on sand

Table 1. Studies on propagation of *S. album* using seeds

Research study	References
Seed viability and germination	Nagaveni & Srimathi 1980, 1981, 1985
Seed germination and survival percentage	Sahai & Shivanna 1984; Bagchi & Kulkarni 1985; Sindhuveerendra et al.1991; Xiao Jin et al. 2010
Seed dormancy	Jayawardena et al. 2015
Response to gibberellins	Xiao Jin et al. 2010; Das & Tah 2013; Karmakar et al. 2017
Seed germination and Seedling growth	Sahai & Shivanna 1984; Nagaveni & Ananthapadmanabha 1986; Srimathi & Nagaveni 1995

2.2 Macropropagation

Vegetative propagation is preferred for forestry species especially when natural regeneration through seeds is a limiting factor (Teja et al., 2023). Macropropagation through root suckers, stem cuttings, grafting and air layering has been reported in *S. album* (Table 2). Though these methods can be employed for conservation, this species is not easily amenable to vegetative propagation. It is recalcitrant to *in vivo* and *in vitro* propagation, and only limited success has been achieved (Sanjaya et al., 2003).

2.2.1 Propagation through cuttings

Vegetative propagation of industrial species is an alternative option for maximising the end uses within very short period. For large scale plantation, vegetative reproduction of the species could be a possibility in forest tree improvement, especially when the original characteristics of the parent tree need to be maintained in the offspring. Development of roots from stem / branch cutting may be the fastest, easiest and inexpensive way for propagation, but only few studies were conducted in *S. album* for rooting of cuttings. (Alam, 2001; Azad et al., 2016). Rao & Srimathi (1976) achieved vegetative propagation of mature sandal through root suckers by inducing shoot primordia in radiating roots and then rooting them with indole-3-acetic acid (IAA), while on the original roots as well as after excising them.

Uniyal et al. (1985) reported successful establishment of sandalwood cuttings with shoots and roots transplanted to pots. Vijayakumar et al. (1995) attempted vegetative propagation of 2-12 month seedlings to study the role of juvenility in rooting of stem cuttings and found maximum rooting (96%) at the third month. As the seedling aged, the rooting ability of the cutting decreased; in 11-12 month seedlings, there was no rooting. Stem cuttings treated with different hormones gave limited to less than 3% rooting under mist conditions (Srimathi et al., 1995). Later, the ability of the shoot cuttings arising as root-suckers to root and sprout in *S. album* was investigated by Balasundaran (1997). Batabyal et al. (2014) have noticed the responses of some phytohormones at different higher concentrations for vegetative propagation of *S. album* with stem cuttings, without mentioning the rooting, sprouting and survival success. Whereas, Azad et al. (2016) attempted to study the effects of indole-butyric-acid (IBA) on rooting and sprouting of branch cuttings of *S. album*, biomass production of adventitious shoots and roots and survival of cuttings with no significant differences among the treatments.

2.2.2 Grafting

The scion required for grafting in sandalwood is fresh shoots easily available throughout the year. *In vivo* micrografting can be done by incision into decapitated 45-day-old greenhouse grown seedling using a surgical blade. A drop of 1% diethyldithiocarbamate was placed on the wound and 2-3 cm scion collected from a candidate plus tree was inserted into the incision and elastic strip or paper bandage was applied to cover the grafted zone. Thus, micrografting was advantageous to produce clonal plants from selected trees (Sanjaya et al., 2006b). Earlier, cleft grafting method was adopted for clonal multiplication using 8 to 12 months old seedlings which gave more than 60% success. Improved grafting technique developed using one month old seedlings, gave more number of grafts in short duration. The survival rate of the grafted plants in the field was more than 70% compared to the plants produced through tissue culture (Srimathi et al., 1995). Li & Zhong (1997) studied the best season and practical method for grafting as well as selection of shoots for scion (shoot apex). Their study revealed that the best season for grafting in Guangzhou district, China is from June to October with side graft and scion from 1-5 years old tree. Side grafting of *S. album* has given up to 80% success. Prastyo et al. (2022) reported differences in the grafting success and survival percentage when diverse sandalwood variants were used as rootstock and scion, following the top cleft grafting method. Ratnaningrum et al. (2022) observed that root-suckers emerging from horizontal roots survived more grafts (57%) compared to those of rootstock from 8 months old seedlings.

2.2.3 Cloning using root suckers

Root-sucker formation induced by trenching around the sandalwood tree has been successful. This method of root regeneration resulted in building up dense sandal patches around the mother trees only (Vijayakumar et al., 1981; Mathew, 1995). In 1995, Srimathi reported more than 25% success through root suckers, but the scope of their application in the field is limited. Shanthi et al. (2020) propagated *S. album* by coppicing, pruning of the selected trees by root suckers and treating it with 1000ppm IBA for 5 min. Successful rooting of sandal was observed with host plants.

2.3 Micropropagation

Micropropagation is one of the most important applications of plant biotechnology used for rapid and large-scale production of true-to-type plants which are difficult to propagate, for plantation program and germplasm conservation. *In vitro* cloning of superior genotypes/clones for rapid and mass production of genetically uniform planting material is in practice. The mass production of clonal planting material of high oil yielders of *S. album* can be accomplished through micropropagation (Rathore et al., 2022). Different methods of *in vitro* plant regeneration have been developed for sandalwood viz., (i) regeneration through axillary shoot proliferation, (ii) direct organogenesis without callus phase (adventitious mode of regeneration), (iii) somatic embryogenesis and (iv) micrografting.

2.3.1 Axillary shoot proliferation

The axillary bud is an embryonic shoot, which lies at the junction of the stem and petiole of a plant which can develop into a stem or flower. Axillary shoot proliferation has become more and more popular in commercial micropropagation as it is the most reliable method of propagation, and produces direct shoots from the lateral meristems avoiding the risk of genetic variation and thus maintaining clonal stability. Although the rate of multiplication is generally less in axillary mode of regeneration than adventitious and somatic modes of regeneration, there is less likelihood of associated callus development and the formation of adventitious shoots, so that subculture carries very little risk of induced genetic irregularity. For this reason, axillary shoot culture has been increasingly recommended by research workers as the micropropagation method that is least likely to induce somaclonal variation. (McManus & Veit, 2002; Bairu et al., 2010)

The availability of good quality dormant axillary buds at the right stage is one of the most important factors to be considered for high frequency shoot initiation. The ideal period for the collection of *S. album* explant is January to March. There are very few reports that deal with *in vitro* propagation from the mature tree either through axillary shoot proliferation (Fig. 3) or through somatic embryogenesis (Sanjaya et al., 2006a & b; Goyal, 2007; Mamatha, 2007; Rathore et al., 2008a, b & c). The first report on axillary mode of regeneration in *S. album* with numerous shoot buds from two node segments obtained from 30 year old trees was by Rao et al. (1984). Later, Sanjaya et al. (1998) obtained multiple shoots from 50-60 year old trees from single nodal segments, but failed to induce rooting in the regenerated shoots. Parthiban et al. (1998) induced excellent axillary shoot multiplication in less than four weeks after inoculation on Murashige & Skoog (MS) medium supplemented with a 2.0 mg/l Kinetin (Kn) and 1.0 mg/l 6-benzyl-amino-purine (BAP) with 80% of the cultures showing shoot induction. These shoots were further separated, subcultured for multiple shoots, and also for root induction for which different host species were also introduced into the medium. He also reported that MS medium with IBA 2.0 mg/l induced sporadic single roots after 15 weeks period.

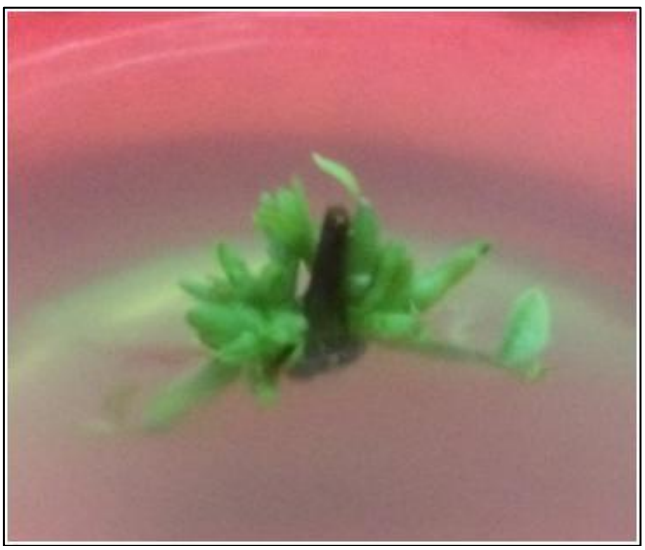

Fig. 3. Sprouting of axillary buds from nodes of *S. album*

Table 2. Macropropagation studies performed for sandalwood

Research study	Parts used	References
Vegetative propagation	Air layering	Rao & Devar 1982
	Root cuttings	Uniyal et al. 1985
	Stem cuttings	Rao & Srimathi 1976; Batabyal et al. 2014
	Stem cuttings 2-12 month seedlings	Vijayakumar et al. 1995
	Grafting, air layering, Stem cuttings	Srimathi et al. 1995
	Branch cutting	Azad et al. 2016
	Root suckers	Vijayakumar et al. 1981; Mathew 1995; Srimathi et al. 1995; Shanthi et al. 2020
In vivo / *in vitro* grafting	45-day-old greenhouse grown seedling	Sanjay et al. 2006a
	45-day-old seedling	Sanjaya et al. 2003 & 2006b

For the first time, complete plantlet from nodal shoot segment of mature and high oil yielding trees of *S. album* was reported by Sanjaya et al. (2006a). Multiple shoots were obtained (5 shoots/explant) in MS medium supplemented with naphthalene acetic acid (NAA) 0.1 mg/l and BAP 2.5 mg/l medium, followed by shoot multiplication on MS medium with NAA (0.53 µM) and BAP (4.44 µM) and additives like ascorbic acid, citric acid, cysteine, glutamine and coconut milk. Micro-shoots pulse treated with IBA for 48 hours, followed by transfer to hormone free medium favoured 41.67% rooting.

Detailed studies by Goyal (2007) revealed that nutrient media and genotypes had a significant effect on frequency of multiple shoot induction and growth. Amongst the five genotypes studied, shoot induction varied from 2.13 - 4.11 shoots/explant with shoot multiplication fold ranging from 2.62 to 4.21 also. A combination of IAA and IBA was effective in inducing rooting (70.39%) followed by 12 weeks hardening in nursery for high rate of survival. Rathore et al. (2008a & b) reported 4-fold shoot multiplication in selected clones/plus trees of sandalwood on MS medium with additives + NAA 0.1 mg/l + BAP 1.0 mg/l. Incorporation of thidiazuron (TDZ) in the medium did not improve the multiplication rate, but induced callus. Genotypes influenced shoot multiplication rate and shoots could be multiplied for two years without loss of multiplication rate and vigour. Alternatively, Krishnakumar & Parthiban (2018) used shoot tips as explants for multiple shoot induction on MS medium with 5.0 mg/l Kn + 2.0 mg/l BAP, and these shoots were further rooted with 3 mg/l IBA. Whereas, Bhargava et al. (2018) reported highest shoot multiplication of *S. album* with maximum shoot length (2.9 cm) achieved on MS medium containing 0.5 mg/l BAP and 5.0 mg/l IBA after 30 days of culture. Later, Manokari et al. (2021) proved that meta-Topolin (mT) improved the quality of shootlets, rate of shoot proliferation, and biochemical contents of the leaves. The concentrations of the various plant growth hormones resulting in successful axillary shoot proliferation in sandalwood are summarised in Table 3.

Table 3. List of plant growth hormones used for axillary shoot proliferation

Growth regulators with MS basal media	References
0.5 & 1.0 mg/l BAP	Rao et al. 1984
5.0 mg/l BAP	Sanjaya et al. 1998
2.0 mg/l Kn + 1.0 mg/l BAP	Parthiban et al 1998
0.1 mg/l NAA + 2.5 mg/l BA; 20 mg/l IBA	Sanjaya et al. 2006a
0.1 mg/l IAA + 1 - 2.5 mg/l BAP; IAA + IBA	Goyal 2007
0.1 mg/l IAA + 1.0 mg/l BAP	Rathore et al. 2008a & b
5.0 mg/l Kn + 2.0 mg/l BAP; 3.0 mg/l IBA	Krishnakumar & Parthiban 2018
5.0 mg/l IBA + 0.5 mg/l BAP	Bhargava et al. 2018
0.5 mg/l mT + 0.25 mg/l NAA	Manokari et al. 2021

2.3.2 Adventitious Shoot Regeneration

The induction of shoot buds from a place other than its usual site, depicted by no involvement of primary meristem, is known as adventitious shoot regeneration. This mode of regeneration manifests shoot induction without any callus phase involved. Direct adventitious shoot regeneration is efficient and can be a reliable technique of sandalwood tissue culture (Fig. 4). Production of numerous clonal plants via adventitious mode can be used for the large scale propagation and also for genetic transformation investigations. Plant tissues such as roots, internodes, leaf sections, shoot segments, petioles and flower/fruit parts can be used for inducing adventitious shoots. (Kulkarni et al., 2000; Hartmann et al., 2002). In sandalwood also, adventitious shoot regeneration has been reported from various explants including leaves and internodes (Table 4).

During initial studies, stem nodal segments of *S. album* were the optimal explants to induce adventitious shoots on MS medium supplemented with BAP and NAA (Rao & Bapat, 1978; Bapat & Rao, 1984). Later, Rao & Bapat (1980, 1993) used hypocotyl segments, excised from *in vitro* grown 4-week-old seedlings. Bud formation and proliferation occurred to a high degree on basal medium supplemented with Kn (1 mg/l) and adenine (10 mg/l) with 15-20 buds on a single explant. Mujib (2005) reported direct shoot induction from leaf explant of seedlings of *S. album* on both MS and woody plant (WP) media. Whereas, Rathore et al. (2008c) reported direct adventitious shoot induction from mature sandalwood tree explants after pulse treatment using TDZ (0.05 - 0.1 mg/l) in MS liquid medium for a week, followed by transfer to hormone free MS medium with additives. However, Mamatha (2007) cultured explants directly on MS medium with TDZ and observed that none of the TDZ (0.5 and 1.0 mg/l) containing medium induced shoots, but produced compact and nodular callus.

Janarthanam & Sumathi (2011) obtained high frequency of shoots from the internodes of *S. album* on MS medium supplemented with 1 mg/l 2-iP and 10% coconut milk. Later, Solle & Semiarti (2016) reported emergence of adventitious shoot from hypocotyls of *in vitro* germinated seeds of *S. album*. Shoot induction (13 ± 6.11) was achieved in leaves using MS + 2 mg/l BAP with 100% explants producing shoots in 10 days after inoculation. Simultaneously, Zhang et al. (2016) reported effect of BAP and NAA on shoot proliferation from stem segments of F1 hybrids. *Santalum yasi* x *S. album*, *S. album*, and *S. yasi* initiated shoots from adventitious buds on MS medium supplemented with 0.089 - 0.89 µM BAP and 0.11 - 0.27 µM NAA after 2 weeks of culture. On MS medium supplemented with 0.89 - 8.88 µM BAP alone, or combined with 0.27 - 2.69 µM NAA or 1.43 - 5.71 µM IAA, multiple adventitious shoots formed in the two species as well as in the hybrid. In 2019, Bele et al. studied direct organogenesis on MS medium supplemented with a moderate concentration of TDZ (1.0 mg/1) in combination with a comparatively lower concentration of NAA (0.5 mg/1) in *S. album*, wherein shoots developed from somatic embryoids formed on explant's surface.

2.3.3 Indirect Shoot Regeneration

It is possible to quickly capture the outcome of breeding or genetic engineering programmes following techniques for indirect organogenesis and thereby improve the quality and uniformity of the nursery stock. *In vitro* indirect organogenesis is determined by the application of plant growth regulators and also on the response of the tissues to the hormonal changes during culture. The presence of auxins, cytokinins and other hormones are necessary for indirect organogenesis to occur from different tissues of sandalwood (Table 5). This procedure mainly involves callus induction, followed by shoot stimulation and development (Fig. 5), and the required levels of exogenous hormones may be different in each step. Although small numbers of plants were regenerated, this method is still a long way from being optimised (Singh et al., 2015). In 1998, Parthiban et al. reported callus cultures using inflorescence and hypocotyl segments after 3-4 weeks from inoculation. The callus cultures were induced in MS medium containing 1.5 mg/l and 2.0 mg/l 2,4-D and periodically subcultured for shoot bud induction (organogenesis) and somatic embryo development (embryogenesis). Shoot bud organogenesis was achieved in callus cultures using 3.0 mg/l each of BAP and Kn as growth supplements, which were further elongated and separated for root induction.

Upadhayay & Samantray (2010) induced multiple shoots from nodal shoot segments from 3 to 6 years old tree and calli via indirect organogenesis in *S. album*, which is also in agreement with the results of Sarangi et al. (2000) and Radhakrishanan et al. (2002). Singh (2011) reported regeneration via organogenesis

from callus raised from leaf explants. High frequency callus was induced on WP medium with TDZ, whereas highest shoot buds (24.6) per callus was obtained on WP medium along with BAP and NAA. 91.6% rooting of the *in vitro* shoots could be achieved with successful acclimatization in greenhouse. Bele et al. (2012) and Singh et al. (2013) reported *in vitro* regeneration through callus phase from leaf explants of 4 weeks old *in vitro* raised seedlings and field grown plants on MS and WP medium with 2,4-dichlorophenoxy acetic acid (2,4-D) and TDZ, respectively. Singh et al. (2015) later reported optimal callus from nodal segments on WP medium containing 0.6 mg/1 TDZ and 1.5 mg/1 2,4-D. Shoot bud initiation was achieved from the surface of callus when transferred to shoot induction medium supplemented with BAP and NAA. Highest number of shoot buds (16.0) per callus was observed in medium containing 2.5 mg/1 BAP and 0.4 mg/1 NAA in 8 weeks period.

Crovadore et al. (2012) used young hypocotyl segments obtained from aseptically germinated seeds of *S. album* (5 weeks old) to induce callus formation, and pointed out that MS and Gamborg basal medium (B5 medium) containing 2,4-D (0.5 µM) and Kn (10 µM) are the most appropriate media for the initiation and proliferation of *S. album* calli, from which shoots can be successfully regenerated. Barpanda et al. (2017) attempted adventitious regeneration from leaf disc through callus phase but failed to induce shoots. Bele et al. (2019) showed that in *S. album* higher concentration of BAP (1.0 - 2.0 mg/l) in combination with a lower concentration of NAA (0.5 mg/l) promoted frequency of indirect somatic embryogenesis. Maximum plantlets regenerated via direct and/or indirect somatic embryogenesis on regeneration medium supplemented with 2.0 mg/l TDZ and 1.0 mg/l GA_3, while plantlets were obtained in higher frequencies via indirect organogenesis with regeneration medium containing comparatively lower concentration of TDZ (1.0 mg/l) and 0.5 mg/l GA_3 with 0.5 mg/l NAA.

2.3.4 Somatic Embryogenesis

Embryogenesis is an important step in the life cycle of a plant, and somatic embryogenesis is a developmental process in which a bipolar structure develops from a somatic (non-zygotic) cell that has the ability to give rise to an embryo under appropriate conditions, without any vascular connection with the original tissue. Somatic embryogenesis occurs through several series of stages viz. globular, heart shape, torpedo and bipolar, which are also characteristic of zygotic embryogenesis (Williams & Maheshwaran, 1986). *In vitro* cultures have several advantages over the field grown plants as a source of explants viz., better rejuvenated explants, minimum accumulation of phenolics and inhibitors, better uniformity of plants and no carryover effect of sterilant. There are large numbers of reports in which *in vitro* shoot cultures of mature trees have been used as explants for somatic embryogenesis. Leaf as a source of explant has been used for the induction of embryogenic callus (Fig. 6). But some limitations like poor development of embryos, limited choice of explants for inducing somatic embryogenesis, and somaclonal variation which is very specific in somatic embryogenesis technique have hindered commercialisation of the technique in case of forest tree species (Jain et al., 2003).

Table 4. Studies on adventitious shoot regeneration from various explants

Explant	Basal media	Growth regulators	References
Nodal	MS	BA + NAA; 2.0 mg/l BAP + 15% CW; 2.5 mg/l BA + 0.4 mg/l NAA	Rao & Bapat 1978, 1993; Zhang et al. 2016; Solle & Semiarti 2016
Hypocotyl	MS WPM	1.0 mg/l Kn + 10 mg/l adenine 0.1 mg/l TDZ	Rao & Bapat 1992 Mujib 2005
Leaf	MS MS	0.05 - 0.1 mg/l TDZ 1.0 mg/l TDZ + 0.5 mg/l NAA	Mamatha 2007; Rathore et al. 2008c Bele et al. 2019
Leaf, internode and hypocotyl	MS	0.1 mg/l NAA, 2.5 mg/l BAP and additives; 0.1 mg/l IAA, 1.0 mg/l BAP	Janarthanam & Sumathi 2011; Janarthanam et al. 2012; Dubey et al. 2014

Fig. 4. Adventitious shoot regeneration from *S. album* leaf

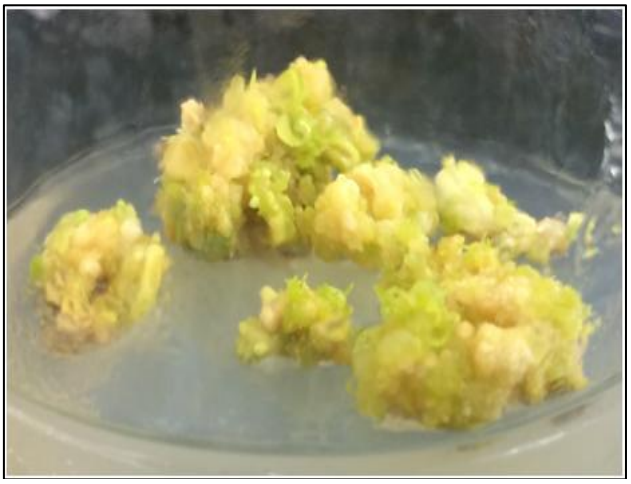

Fig. 5. Shoot regeneration from callus of *S. album*

Fig. 6. Somatic embryos regenerated from embryogenic callus of *S. album*

Table 5. Studies on indirect shoot regeneration in different tissues

Explant	Basal media	Growth regulators	References
Inflorescence, Hypocotyl	MS	1.5 & 2.0 mg/l 2,4-D; 3 mg/l BAP + Kn	Parthiban et al. 1998; Crovadore et al. 2012
	B5	0.5 µM 2,4-D + 10 µM Kn	
Hypocotyl	MS WPM	2.5 mg/l BAP and 1.5 mg/l 2,4-D	Barpanda et al. 2017
Leaf	MS	2,4-D	Bapat & Rao 1992
		2 mg/l 2,4-D + 0.5 mg/l TDZ	Bele et al. 2012
	WPM	1.5 mg/l 2,4-D + 0.5 mg/l	Singh et al. 2011, 2013, 2015
	MS	TDZ; 0.4 mg/l TDZ; 2.5 mg/l BAP + 0.4 mg/l NAA	
Leaf and internodes	WPM	0.4 mg/l TDZ; 2.5 mg/l BAP + 0.4 mg/l NAA; 5 mg/l BAP + 3 mg/l Kn	Singh et al. 2013
Nodal	MS	4 mg/l BAP	Sarangi et al. 2000;
		4 mg/l 2,4-D	Radhakrishanan et al. 2002; Upadhayay & Samantray 2010
	WPM	0.6 mg/l TDZ + 1.5mg/l 2,4-D; 2.5 mg/l BAP + 0.4 mg/l NAA	Singh et al. 2015
	MS	1 - 2 mg/l BAP, 0.5 mg/l NAA; 2 mg/l TDZ + 0.5 mg/l GA_3 + 0.5 mg/l NAA	Bele et al. 2019

There are several reports on *in vitro* regeneration of sandalwood through somatic embryogenesis (Table 6), but most of them are from seedling material. These reports deal with different factors involved in the somatic embryogenesis of *S. album* from seedling explant or 20 year old plants. Successful protocols also exist for the induction of somatic embryos from young shoots, nodal shoot segments, zygotic embryos, endosperm, and leaves. As early as 1963, Rangaswamy & Rao reported callus initiation and multiplication from endosperm tissues of *S. album* on White's medium. Later, Rao & Rangaswamy (1971) observed callus induction from embryos on White's medium with yeast extract and development of plantlets from differentiated embryos. Lakshmi Sita et al. (1979) have reported somatic embryos induction on the MS medium supplemented with 2, 4-D (1.0 mg/l) with Kn (0.2 mg/l) within 4-5 weeks from the nodal and internodal segments in *S. album*. They observed small embryoids from embryogenic callus with white colour, and non- embryogenic nature in brown colour callus. In 1980, regeneration of plantlets through somatic embryogenesis from seedling explant has been reported by Lakshmi Sita et al., whereas Rao & Bapat reported callus from hypocotyl on MS medium containing 2,4-D.

Rao & Ram (1983) reported the use of 5 mm long stem internodes from 20 year-old plus tree of *S. album* for callus induction on MS basal medium containing 2, 4-D (4.52 µM) and sucrose (87.6 mM). They also reported that incorporation of IAA (2.85 µM) and BAP (2.22 µM) in the MS medium developed somatic embryos in all stages from globular to torpedo in *S. album*. Bapat & Rao (1984) used the hypocotyl explants from the *in vitro* germinated seedling and obtained 20% cultures with extensive induction of embryogenic callus within 4 weeks period. Later, Rao & Ozias-Akins (1985) claimed to derive somatic embryos from callus induced from protoplasts derived from shoot segments. Bapat et al. (1985) and Rao & Bapat (1992) also reported protoplast isolation from mesophyll, stem and hypocotyl followed by callus and suspension cultures, resulting in differentiation to somatic embryos. The induction of callus from mature explants and shoot bud differentiation in somatic embryogenesis through callus-mediated cultures was reported by Bapat & Rao (1992a & b). Embryogenic callus can be induced from hypocotyl and stem explants of *S. album* on 2,4-D and Kn medium. Although different combinations of auxin-cytokinin induced embryos in the callus, high regeneration of somatic embryos was observed on MS medium containing IAA and BAP (Rao & Bapat, 1993). Complete plantlet production through callus-mediated somatic embryogenesis was reported by Lakshmi Sita & Raghava Ram (1995).

Rugkhla & Jones (1998) have reported that *S. album* and *S. spicatum* can spontaneously produce direct somatic embryos from cells of several types of explants from seedling explant and field grown plant in MS medium with TDZ (1 or 2 µM), or indirectly in medium containing 2,4-D + TDZ. Multiplication of embryoids was carried out on MS with IAA + Kn and germination on medium containing GA_3. Concurrently, Shiri & Rao (1998) used hormone free medium with 2% mannitol for the induction of somatic embryos in *S. album*. Rai & Mc Comb (2002) reported direct somatic embryo development from zygotic embryos of *S. album* plated on MS medium containing TDZ or BAP, which were isolated and transferred to hormone free MS medium, and converted into secondary embryos by repetitive cycle. Isolated somatic embryos cultured on half-strength MS medium with GA_3 (1.4 µM) resulted in germination and development of plants. Ilah et al. (2002) found that WP medium, which contains lesser amounts of inorganic compounds performed better than MS medium for development and maturation of somatic embryos in *S. album*.

Direct somatic embryogenesis was obtained in *S. album* from zygotic embryo and cotyledon on MS medium with NAA, BAP and coconut milk (Sanjaya et al., 2000). Mamatha (2007) checked the effect of agar-agar (0.4 – 0.7% w/v) and sucrose (0.0 – 6.0% w/v) concentrations and pH range (4.0 – 7.0) for standardising the optimum condition for somatic embryogenesis, in MS or MS modified with additives, and then reported synchronised somatic embryo induction from fragile callus on WP medium containing additives and IAA (1.0 mg/l), followed by maturation of the somatic embryos on WP medium with abscisic acid (ABA) and mannitol. Rathore et al. (2008a) reported embryogenic callus induction in *S. album* from the leaf segments obtained from multiple shoot culture of mature trees on MS medium with additives and 1.0 mg/l 2,4-D. Initial growth of callus was slow, but produced fragile and whitish embryogenic callus from the second subculturing onwards, which were maintained by subculturing on fresh medium within 4 weeks period, and obtained complete plant through somatic embryogenesis.

Revathy & Arumugam (2011) reported direct somatic embryogenesis from leaf of *in vitro* raised seedlings, with about 60% of the cultures exhibiting embryo induction. Herawan et al. (2014) observed callus induction in MS medium and leaf explants gave the best response on development of embryogenic callus. High number of direct somatic embryo proliferation from the leaves was observed in MS medium containing 3 mg/l 2,4-D. Peeris & Senarath (2015) have carried out studies on somatic embryogenesis from single nodal segments, mature & immature seeds and leaf discs. Nodal segments gave 95.64% of callus on MS medium under dark conditions, followed by somatic embryo induction when supplemented with 0.5 mg/l BAP, 1.0 mg/l IAA and 0.5 mg/l Kn. Zhang et al. (2016) induced embryogenic callus when *S. album* x *S. yasi* and *S. album* nodal segments were cultured on MS medium supplemented with 4.52 - 9.05 µM 2,4-D. Friable embryogenic callus of *S. yasi* x *S. album* was subcultured for somatic embryo induction, maturation and germination in MS containing BAP (0.89 µM), NAA (0 - 0.27 µM) or GA_3 (1.44 - 5.77 µM).

The shoot tip explants also exhibited the potential for embryonic callus and somatic embryoid formation, especially on MS basal medium supplemented with 1.0 mg/l BAP and antioxidant (Vanajah & Seran, 2016). Akhtar & Shahzad (2019) studied the ontogenic differences between the structures of directly differentiating shoot buds and somatic embryos from seedling derived hypocotyls. Tripathi et al. (2021) optimised different factors for somatic embryo induction in sandalwood through cell suspension cultures using 2,4-D, and found that calli derived from mature embryonic axis were better than calli derived from mature cotyledon for raising cell suspension cultures. Recently, Manokari et al. (2022) reported the histochemical basis of somatic embryogenesis in *S. album* by inducing direct somatic embryos from the internode explant, and analysed the developmental anatomy and histochemical features during embryogenesis. The induction of direct somatic embryos has an advantage of genetic stability and is also a more desirable system to facilitate genetic transformation (Rose et al., 2010; Ochoa-Alejo, 2016).

2.3.5 Micrografting

Under aseptic condition *in vitro* micrografting can be achieved by splitting 0.5 - 1 cm on the top of the decapitated root stock of the 45 day old seedling using a sharp surgical blade. 1 - 2 cm long *in vitro* derived shoot apex scion is inserted into the stock incision and cultured on liquid MS/2 medium with 3% sucrose. The radical of the above plant is pushed through the hole in the filter paper bridge as a support (Sanjaya et al., 2003 & 2006b).

Table 6. Somatic embryogenesis studies using a variety of explants

Explant	Basal media	Growth regulators	References
Seeds	WPM	1.0 mg/l 2,4-D	Rani et al. 2018
Seedling	MS	1.0 mg/l 2,4-D + 0.5 mg/l Kn	Lakshmi Sita et al. 1979, 1980
	MS	4.52 mM 2,4-D	Misra & Dey 2013a & b
	WPM	2.85 mM IAA, 3.99 mM BAP	
Hypocotyl	MS	1.0 mg/l BAP, 1.0 mg/l 2,4-D;	Bapat & Rao 1979, 1984; Rao & Bapat 1980; Rao &
	B5	4.54 mM 2,4-D; 0.5 µM 2,4-D + 10 µM Kn	Ram 1983; Das et al. 2001
Hypocotyl segments and root junction	MS	2.5 µM BAP & 7.5 µM BAP	Akhtar & Shahzad 2019
Hypocotyl, Stem and internodes	MS	2,4-D; IAA + BAP	Rao & Bapat 1993, 1995
Hypocotyl and nodes	WPM	2.26 µM 2,4-D & 2.68 µM CPA	Ilah et al. 2002
	MS	2.70 µM NAA + 2.22 µM BAP	
Zygotic embryo	MS	14 µM/l GA_3	Mo et al. 2010
Zygotic embryo and cotyledon	Whites media	2.0 mg/l 2,4-D + 5.0 mg/l Kn + 0.25% yeast extract	Rao & Rangaswamy 1971; Sanjaya et al. 2000;
	MS	NAA + BAP + coconut milk; TDZ/BAP; 2,4-D	Revathy & Armugam 2011; Tripathi et al. 2021
Endosperm	Whites media	2.0 mg/l 2,4-D + 5.0 mg/l Kn + 0.25% yeast extract; 2,4-D and BAP	Rangaswamy & Rao 1963; Rao & Rangaswamy 1971; Lakshmi Sita et al 1980 & 1986; Rao & Bapat
	MS	2,4-D and Kn; 2,4-D / 2% Mannitol	1992; Rao et al. 1996; Anil & Rao 2000; Radhakrishanan et al. 2002
Protoplast	MS	1.0 mg/l IAA + 1.0 mg/l BAP; TDZ/BAP with 1.0 mg/l 2,4-D	Rao & Ozias-Akins 1985; Ozias-Akins et al. 1985; Bapat et al. 1985, 1992b
Mature stem segments	MS	1.0 mg/l 2,4-D	Bapat & Rao 1992 a & b; Shekhawat et al. 2008, 2010
Leaf and internodes	MS	2.0 mg/l 2,4-D;	Mamatha 2007; Rathore et al. 2008a; Mamatha &
		1.0 mg/l 2,4-D	Rathore 2014
		1.0 mg/l 2,4-D + 0.5 mg/l TDZ;	Janarthan & Sumathi 2011
		2.0 mg/l TDZ + 1.0 mg/l GA_3	
		3 mg/l 2,4-D;	Herawan et al. 2014; Barpanda et al. 2017
		2.5 mg/l BAP + 1.5 mg/l 2,4-D	
		2.5 mg/l 2,4-D & 3 mg/l Kn	Peeris & Senarath 2015
	WPM	0.4 mg/l TDZ;	Singh et al. 2015
		2,4-D;	
		1.0 mg/l IAA, ABA + mannitol	

Explant	Basal media	Growth regulators	References
	MS WP liquid medium	2.0 mg/l 2,4-D with additives. 3.75% PEG, 1.0 mg/l ABA; 1.0 mg/l IAA and 1.5 mg/l GA_3	Somashekar et al. 2014
Shoot tip	MS	1.0 mg/l BAP	Rao et al. 1996; Vanajah & Seran 2016
Nodal	MS	2.0 mg/l NAA + 0.5 mg/l 2,4-D + 0.5 mg/l BAP + 15% CM	Lakshmi Sita et al. 1979
		2,4-D and IAA + Kn + GA_3; 0.25 or 0.5 mg/l TDZ, 2,4-D	Rugkhla & Jones 1998; Rai & Mc Comb 2002
		2,4-D; 0.89M BAP + NAA + GA_3	Zhang et al. 2016
		0.2 - 1.5 mg/l TDZ	Cheng et al. 2019
Internodes	MS	4.52 µM 2,4-D; 2.85 µM IAA, 2.22 µM BAP	Rao & Ram 1983
		2.5 mg/l BAP, 0.5 mg/l NAA, 50 ml/l deproteinised coconut water and 25 mg/l each of myo-inositol, adenine sulphate and L-arginine.	Manokari et al. 2022
	MS	1 mg/l TDZ + 0.5 mg/l NAA; 1 - 2 mg/l BAP+ 0.5 mg/l NAA	Tripathi et al. 2022a
Shoot tips, Leaves, mature embryos	MS	1.1 µM/l TDZ; 1.4 µM/l GA_3 + 4% Sucrose	Mo et al. 2008
Mature cotyledons, mature embryos and hypocotyls	MS	0.5 - 2 mg/l 2,4-D + 0.5 mg/l BAP; 0.5 mg/l TDZ + 1 - 2 mg/l NAA; 2 mg/l TDZ + 1 mg/l GA_3; 1 mg/l TDZ + 1 mg/l GA_3 + 0.5 mg/l NAA	Tripathi et al. 2022b

3. ADVANCEMENTS OF *In vitro* STUDIES IN *S. album*

Endogenous plant growth regulators also play a significant role in haustorial development and function, and their application in the *in vitro* environment can increase the number of haustoria, which can serve to overcome host incompatibility issues in sandalwood (Rocha & Santhoshkumar, 2022). Khannam & Hamalton (2021) have reported the use of DNA markers viz., RAPD, SSR & ISSR for genetic fidelity testing of *in vitro* raised clonal sandalwood plants. Although protocols for the induction of somatic embryos are available, the rate of conversion to healthy plants is low, making the application of somatic embryogenesis in the *Santalum* genus difficult for biotechnological applications such as large-scale plant multiplication, cryopreservation or genetic transformation (Teixeira da Silva et al., 2016b). Pandey et al. (2022) have characterised the gene expression patterns during direct and indirect organogenesis, which can be used as transcriptional markers for early prediction of the organogenesis stage in sandalwood.

3.1 Synthetic Seed Production and Germination

Very few studies have been carried out on synthetic seed production in *S. album*. The first report on synthetic seed formation was by Bapat & Rao (1988) using embryogenic cell suspensions. Later Rao & Bapat (1992) reported that encapsulation of somatic embryos can be achieved by using calcium alginate. Beads were kept for 40 minutes on a shaker (40 rpm) under light, and the encapsulated embryos were germinated in petriplates containing MS basal medium and finally into plantlets. Bapat & Rao (1992b) reported regeneration of plantlets from encapsulated and non-encapsulated desiccated somatic embryos for 10, 20 and 30 days, and revival of growth was exhibited in both types of embryos when rehydrated on White's medium.

3.2 Scale up and Secondary Metabolite Production

To induce large-scale stable somatic embryogenesis in sandalwood, Das et al. (1999a) established a bioreactor-based production system using liquid medium for scale-up study. Later, they used air-lift bioreactors to obtain 3000 seedlings from somatic embryos, although 59.3% of them were abnormal. However, metabolite production was not evaluated in their bioreactor. In 1998 & 1999b, Das et al. investigated the type and concentration of carbon source, inorganic nitrogen and ABA during the maturation and conversion stages required for embryo production. Their team also studied the influence of media pH and found that 4% sucrose was best for somatic embryogenesis, and constant pH at 6.0 promoted maximum embryo production with minimum abnormalities in *S. album*. Anil & Rao (2000) have also reported the use of endosperm of sandalwood for induction of embryogenic callus, to carry out studies on calcium mediated signalling during somatic embryogenesis and ascertained the role of exogenous calcium as second messenger.

Valluri et al. (1991) reported production of phenolics by two heterotropic suspension cultures cultivated in 2.5 litre bioreactor. Crovadore et al. (2012) conducted studies on selection and production of calli for inducing sequiterpenes. Various elicitors in different concentration were used for testing of sesquiterpene in callus and GC-MS analysis revealed that a range of terpenic molecules including some desired terpene in sandalwood odour profile were illustrated. Misra & Dey (2013a & b) were able to induce somatic embryos in an air-lift-type bioreactor and produce β-santalol, epi-β-santalol, and α-santalol within 28 days. They have reported that suspension cultures of sandalwood grown in bioreactor and shake flask cultures are also an alternative and renewable resource of shikimic acid. Similarly, accumulation of squalene in the suspension cultures of *S. album* was studied by Rani et al. (2018) in shake flasks and air-lift bioreactor. They reported that in flask, 3.2 mg/g dry weight was accumulated in 6 weeks whereas in bioreactor accumulation of squalene was better with 5.5 mg/g dry weight in 4 weeks. Cheng et al. (2019) developed a reproducible method for the induction and proliferation of sandalwood callus using 2, 4-D, Kn and/or TDZ, which accumulated santalenes and bisabolene that are precursors of santalol when co-cultured with *C. gloeosporioides*.

3.3 Genetic Transformation Studies

For the first time, Shiri & Rao (1998) reported genetic transformation and regeneration of sandalwood plants. Their study deals with introducing and expressing foreign genes in sandalwood using *Agrobacterium tumefaciens* strains carrying β-glucuronidase uidA (GUS) and

neomycin phosphotransferase II (NPT II) genes on binary vector. 20% of the inoculated embryos induced callus and developed embryos which were confirmed by analysing in the presence of kanamycin, GUS and NPT II assays. After two months, transgenic embryos were identified and developed into rooted plants. Later Shekhawat et al. (2008) reported an efficient method for genetic transformation using cell suspension cultures. Expression of β-glucuronidase was assessed by RT-PCR and GUS assays, and transformation and stable insertion of T-DNA into the host genome was confirmed by Southern blotting. This is the first report of a stable and high level of foreign protein expression in cell suspension cultures of *S. album*, followed by transformation using hepatitis B small surface antigen by Shekhawat et al. (2010). Transformed cell suspension colonies were selected using kanamycin in the medium and subsequently by PCR analysis. The use of various additives in the medium was also studied for increasing the expression of the antigen. The genes involved in sesquiterpene biosynthetic pathway in *S. album* have also been studied extensively by cloning and expression in other organisms including yeast and tobacco (Zha et al. 2020; Chen et al. 2023).

4. CONCLUSION AND FUTURE PERSPECTIVES

Due to the out breeding nature of sandalwood, seed base progenies are highly variable and will not be true to type to the mother plant. In order to exploit full worth of plus trees/elite genotypes which consist high oil content or heartwood, cloning technique by macro- and micro-propagation is essential. With ageing, it is difficult to clone mature tree through macropropagation, and micropropagation has potential to overcome this problem. It is the only method for rapid and mass production of clonal planting material, especially for sandalwood. *In vitro* cloning through axillary shoot proliferation, somatic embryogenesis and adventitious shoot regeneration has tremendous scope for production of quality planting material for farmers and other stakeholders in future. The bottleneck which limits the widespread application of micropropagation techniques in sandalwood is the establishment of hardened plants with suitable host and further survival for planting. Hence the hardening process of tissue culture plantlets should be standardised and improved for paving the way for commercialization. The decline in number of trees in the natural populations formerly subject to outcrossing, has resulted in inbreeding and ensuing deleterious effects on the gene pool. Mass propagation of genetically variable material and introduction in plantations can help in reversing these ill effects. Stringent techniques for mass production of sandalwood planting material are also needed to check future insufficiencies. Also tissue culture raised plantlets should be intensified for elite trees, and lab to land programme should be strengthened so that superior planting material is made available for future afforestation programmes. Since spike infected explants are also amenable to tissue culture, production of disease free planting material and research on screening of micropropagated plants (or phytosanitation) is also necessary.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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