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Somatic embryogenesis in *Santalum album* L.

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Abstract
Profuse somatic embryoids were obtained from leaf segments cultured on MS media supplemented with IAA (0-1.5 mg/l) and 2, 4-D (0-1.5 mg/l) after 80 days in no light condition. The leaf segments were collected from *in vitro* shoot multiplication cultures. Embryoids were cultured on MS media supplemented with BA (0.5-2.0 mg/l) and 2, 4-D (0-1.0 mg/l) for development and maturation. Different shaped embryos were developed after four weeks. Mature embryos developed into shoots and roots when placed on hormone free half strength MS media, after 6 weeks.

Keywords: 6-benzyleaminopurine (BAP), 2, 4-dichlorophenoxyacetic acid (2, 4-D), indole acetic acid (IAA), embryoids, somatic embryos (SES), embryogenesis

1. Introduction
Santalum album is popular semi parasitic tree belonging to family Santalaceae. Originally it is distributed in dry deciduous forests and western Peninsula. It is now grown in Andhra Pradesh, Gujarat, Kerala, Madhya Pradesh, Maharashtra, North India, Rajasthan, Tamil Nadu apart from Mysore/Karnantak - a natural habitat. *Santalum album* has status of an endangered plant SPS. Due to felling of the tree, grazing by animals, haphazard cutting of the tree for heartwood and poor seed germination.

Fig 1: Host

This root parasite grows on all kind of plants i.e. herbs, shrubs, grasses and trees like Neem, *Pongamia*, Lemon, *Casuarina* and some leguminous plants. Tree develops haustoria at the tip of the roots (Bulging of the root tip) which enter the roots of the host plant. The host plant supply water and nutrients to the *Santalum album*. In the early phase of growth of the *Santalum album*, the *haustoria* are very active. The tree obtain some of the nutrients and water from host. It is able to produce complex compounds on its own (Wanntorp and De Craene, 2009).

Medicinal uses
Sandalwood is the best antiseptic used since centuries due to its antimicrobial properties. Chandanasava – ayurvedic preparation using *Santalum album* along with other ayurvedic hebs is a well-known ayurvedic medicine to gain strength and stamina and improve heart health naturally. Also used in blood pressure, UTI, kidney stones, skin disorders etc. Apart from these it is used in small pox, chronic bronchitis, mental troubles, thirst, burning sensation, headaches. Widely used in making perfumes, soaps, incense sticks etc.

Popular Ayurvedic medicines using *Santalum album* as main ingradient is Chandanasava, Chandraprabhavati, Chandanbalaxadi tailum, Chanadanavati, Chandanadi tail. Used in other preparations like ashokaristam, Anutailam, Dhanvantaram lkasayam, Kalyanak Ghritam etc.

Other uses

Due to its antimicrobial properties long lasting preparations were made from the plant would like Garlands, pen-stand, pens, wrist-watches, combs, wall clocks, walking sticks, hand fans etc. Now a days aromatic ornaments are also made from the wood and decorative items as well from the seeds of the sandalwood.

Royal people were using Sandalwood for their furniture's like chair, cupboard, dressing table, table tops and different articles like flower-vase, jewellery boxes, small temples, toys, pens, pen stand, key chain, key holders etc. Also used to make the coffin boxes. The Sandal wood is widely used in temples, jambalayas for puja and religious rituals. It enhances the spirituality and beauty of the mankind.

Chemical ingredients

The oil contains Santalene, phenols, lactones and borneols. It also contains Santalic acid, Santalal α and β, Santalol, α and β. The higher the Santalol content of the oil, greater the value of oil. Different parts of the plant has different values. The seeds are used as beads of rosaries. The heartwood is aromatic, medicinal and economically important part of the tree. Sapwood/softwood is used for making some articles like garlands (The peels of the softwood entered in a string and arranged in a way that look like a garland) toys, pens etc. and powder for medication. The Sandalwood bitter in taste but is the most precious woods of all.

Materials and Methods

Explant collection

Explants were **c**ollected from *in vitro* regenerated shoots from the Plant tissue culture lab., Pl. Biotechnology, ICAR-DMAPR, Boriavi, Anand, Gujarat. The shoot tip alongwith the 1-2 leaves were selected as explants. As the explants were collected from *in vitro* condition, these were cut into the required size. As they were aseptically grown and being sterile, were directly cultured on somatic embryo induction media.

Nutrient medium

The experiment on induction of Somatic Embryos was carried out by using Murashighe and Skoogs tissue culture media and hormones. Only auxins were supplemented as SE inducing hormones. These hormones were 1) IAA and 2) 2, 4-D. Different concentrations of both the auxins were supplemented to MS media i.e. from 0 to 1.5 mg/l IAA and 0 to 1.5 mg/l 2, 4-D. Shoot tips from *in vitro* shoot multiplication cultures of *Santalum album* were taken as explants. As they were *in vitro* regenerated plantlets, did not undergo sterilisation process. They were directly taken out of the culture vessel, cut into required size and cultured on the somatic embryo induction media.

Culture conditions

The *in vitro* cultures were given no light until the embryos were induced. Temperature was maintained 24±1 ^{0}C. Regularly monitored all the required parameters**.**

Somatic embryoids (1) were obtained on MS media supplemented with 1.0 mg/l IAA and 1.0 mg/l 2, 4-D after 12-14 weeks of culture. Very small, creamy [1] to yellowish green [3] somatic embryoids were obtained after 8 weeks of culture. These embryoids were cultured on freshly prepared MS media supplemented with high concentration of cytokinin and low concentration of axing.

Initiation of Somatic embryos and maturation

The somatic embryos were produced from the initial cultures ranged from 0.5mm to 1mm size, globular to v-shaped shaped and some leafy in structure [6]. The small sized somatic embryoids [1, 2, 3, 4] were transferred to maturation media which is MS media supplemented with BAP 1.0-2.0 mg/l + 2,4-D 0-1.0 mg/l. Among all the media tried, MS media supplemented with BAP 2.0 mg/l + 2, 4-D 1.0 mg/l found the medium for embryo development and maturation. In the development and maturation phase, various shapes of the embryos were obtained i.e. dumb-bell, v shape, globular to torpedo and cotyledonary [6]. Colour variations also observed in SEs. White, Ivory to dull cream, yellowish with tinge of light green [5]. The embryos became brownish after 4 or more weeks on the same culture media.

The embryos were cultured on fresh media after every 4 weeks. After subsequent cultures, embryos developed into plantlets [8, 9]. These plantlets were separated from intactly grown embryos and these plantlets were cultured on freshly prepared MS basal media devoid of any hormones. After the plantlets were developed in required size with sufficient shoots and roots, they were transferred to polythene bags containing sterilised soil and FYM in 1:1 [10].

Regeneration of Plantlets

The somatic embryos thus matured and grown into two peculiar polars or growing tips i.e. shooting tip and rooting tip [5, 6, 7]. Small plantlets produced when the matured embryos were cultured on hormone free MS media. The germination of somatic embryos were about 50%. Rest of the SEs were further transferred to freshly prepared basal media where they were germinated and developed shoots and roots. Leaves became broader and lengthier, stem elongated and root length also increased after 2-3 months. Initially SEs were single rooted [6, 7], after 6 weeks the branching of roots or secondary roots were observed [8, 9].

These plantlets were taken out of the culture medium after 6-7 months when roots and shoots were fully developed. The fully grown plantlets with 4 to 8 leaved shoots and 4-5 cm. Long roots, were selected for hardening.

Hardening

The plantlets thus produced were taken out from the culture medium and washed thouroughly with water for 4-5 times to remove agar from the roots. These cleaned plantlets were transferred to the polythene bags containing peatmoss and soil in 1:1 ratio [10]. They were kept in laboratory for hardening at lab condition.

Field transfer

The plantlets which were hardened in laboratory conditions [10], were taken to net house for pre hardening for transfer to the field [11]. These plantlets were taken to the field after 4 weeks. It required proper care from the direct sunlight.

The *in vitro* produced Sandal wood saplings were hardened with *Citrus limon* -Lemon plant. The host and sandal wood saplings flourish together with very good growth of the Sandal wood plants initially. After the plants established well in the field condition, the host plants are not required.

Fig 2: Numbers in bracket denotes stages of the embryos and its development

Conclusion

Santalum album has an endangered plant status, it was necessary to develop a reliable protocol. Hence the objective of the study was to produce healthy and elite plant material through somatic embryogenesis.

The plantlets produced through somatic embryogenesis were found growing faster than the naturally growing plants, best quality, disease free and easily rooting in the soil, flourish well in the field condition.

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