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Optimization of micro propagation protocol of sandal (*Santalum album* L.)

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Abstract
The current study was carried out in a completely randomized design at the Department Plant Biotechnology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Dist. Akola (M.S.) during the academic year 2015–2017 with the aims of producing disease and virus free plantlets, standardizing explant type and media combinations influenceable for tissue culture, and *in vitro* micro propagation of *Santalum album*.

The highest survival percentages were 70.00% and 68.33% for nodal explants after two and three weeks of therapy with MS media supplemented with BAP 1 mg/l + kinetin 1 mg/l, respectively. After two weeks, the nodal explants were inoculated with MS + BAP + Kin @ 1 mg/l for the start of the shoot bud. The highest survival rate, 63.33 percent, was noted. The maximum percent of shoot bud initiation, or 61.66 percent, was achieved after three weeks of inoculation on MS + BAP + Kin @ 1 mg/l each. The highest multiple shot survival rates were seen for multiple shoot induction after three and six weeks of therapy with MS media incorporated with BAP 3 mg/l + Kin 1 mg/l + ADS 2 mg/l, after three weeks (5.06) and six weeks (2.81).

Keywords: Micropropagation, explant, *Santalum album*, survival, nodal explant

Introduction
A priceless tree known as sandalwood (*Santalum album* L.) is connected to Indian culture. The world's second-most expensive wood is this one. One of the best natural carving materials is the tree's heartwood, which is prized for its scent. Pharmaceuticals, cosmetics, aromatherapy, and fragrances all use sandalwood oil.

With an estimated market value of more than $1 billion, sandalwood is one of the most beneficial commercial tree species in the world (Viswanath *et al.*, 2008) [2]. Numerous studies have revealed that sandalwood has a significant genetic diversity for various features.

Santalum album L., a medium-sized evergreen partial root parasitic tree that is highly prized for its aromatic heartwood, is a member of the Santalaceous family. The *Santalum album* possesses the most oil of any member of the genus.

Across tropical and subtropical India, Sri Lanka, and Indonesia, the more well-known Indian sandal, *Santalum album*, grows across a wide range of temperatures and soil types. Western Australia is home to four *Santalum* species: *S. spicatum*, *S. accuminatum*, *S. murrayanum*, and *S. ianceolatum* (Sawyer and Jones, 2000) [4]. The oil content of *Santalum* spp. is highest in *S. album*, while *S. spicatum* and *S. ianceolatum* produce low-quality oil and wood, respectively (McKinnell, 1990) [8]. About 4000 tonnes of sandalwood are produced worldwide. India produces 400 tonnes or such. 1800 tons of Australian variety is produced in Australia; 350 tons are imported, primarily from Timor, Malaysia, Cambodia, Vietnam, Thailand, and Myanmar. The biggest consumers of sandalwood are China and Taiwan, with India and Indonesia as key suppliers.

From Kerala in the south to Uttar Pradesh in the north, *S. album* is naturally spread throughout India in a variety of eco-climatic and edaphic conditions. Kanataka and Tamil Nadu states take up over 90% of the total area. It is primarily found in Kerala's Marayoor, Kasargod, Wayanad, and Thenmalai forest areas (Srimathi *et al.*, 1995 [14]. According to Jeeva *et al.* (1998) [10], sandal is also worn in Andhra Pradesh, Maharashtra, Madhya Pradesh, and Orissa.

To address the rising demand for reforestation efforts, tissue culture or micro propagation technique in forest trees is said to be one of the most effective micro propagation techniques.

It also has the advantage of producing several plantlets in a short amount of time Sandalwood *in vitro* propagation was attempted as early as 1963. On a modified White's medium, the induction of callus from mature endosperm was described; however, the callus did not continue to grow (Rangaswamy and Rao, 1963) [3]. Lakshmi Sita *et al.* (1980) [12] reported on the induction of callus, differentiation of embryoids, and subsequent production of plantlets from endosperm (immature seeds), which can be used to make sandal plants that are more productive commercially. As yet various explants such as embryo (Rangaswamy and Rao, 1963) [3], hypocotyls, shoot tip, nodal segment, leaf disc, endosperm and cell suspension cultures (Dey, 2001) [7] with varying degree of success have been used for rapid multiplication of sandal trees. Only one study on the direct generation of shoot buds from *in vitro* grown leaves for sandal has been published. When it comes to shoots that develop without a callus phase directly on leaves, relatively little information is available for woody species (Preece *et al.*, 1993) [9].

2. Materials and Methods

2.1 Preparation of Nodal explants for inoculation

The nodal explants taken from *Santalum album* plants that were 8 years old and mature were properly cleansed under running water for 20 minutes before being treated for 25 minutes with a Bavistin @ 1% solution diluted with a few drops of Twin 20. The explants were then surface sterilized once more using the antibiotic Streptomycin 1% for 20 minutes and again, wash four times with sterile distilled water to remove all the traces of antibiotics. The cleaned explants will then be washed four times with sterile distilled water to eliminate any remaining $HgCl_2$ residues before being treated with 0.1% (w/v) $HgCl_2$ for 5 min under aseptic conditions. After surface sterilization, put each piece separately onto blotting sheets that have been autoclaved before inoculating them onto MS (Murashige and Skoog, 1962) [13] basal media.

3. Results and Discussion

Percentage of nodal explants that survive On MS media, four explants per bottle were inoculated with BAP at concentrations of 1 and 2 mg/l, Kinetin at concentrations of 1 and 2 mg/l, and BAP combined with Kinetin at a concentration of 1 mg/l each. As a control, MS medium without a growth regulator was used.

Table 3.1: Percent survival of nodal explants after two and three weeks on different media combinations

Treatment	Treatment details	% of survival of nodal explants after two weeks	% of survival of nodal explants after three weeks
T_1	MS Control	59.30	55.00
T_2	MS + BAP 1mg/L	65.00	60.00
T_3	MS + BAP 2mg/L	63.33	58.33
T_4	MS + KIN 1mg/L	68.33	65.00
T_5	MS + KIN 2mg/L	61.66	56.66
T_6	MS + BAP 1mg/L + KIN 1mg/L	70.00	68.33
	CV%	7.25	8.91
	CD 1%	2.35	2.69

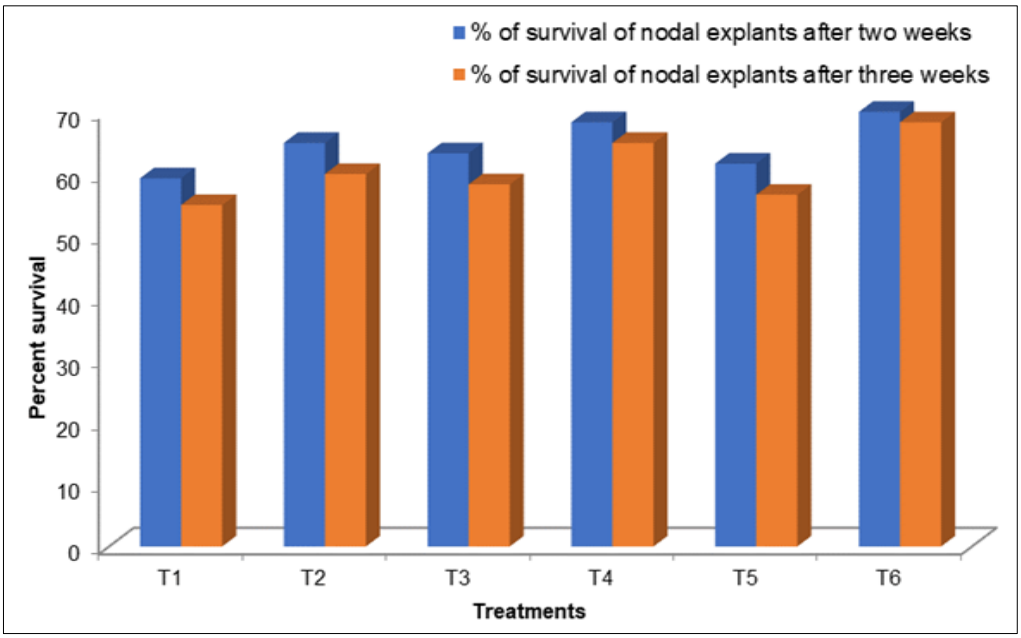
Table 3.1 showed that the percent survival of nodal explants after two weeks on various media combination ranges from 59.30 to 70% whereas the percent survival on different media combination after three weeks ranges from 55 to 68.33%.

The maximum survival rate, or 70%, was observed when nodal explants were incorporated with MS + BAP + Kin @ 1mg/l each, with MS + BAP 1mg/l displaying a 68 percent survival rate after two weeks. 65% of those receiving MS plus BAP at 1 mg/l survived. On MS + BAP at 2 mg/l, 63.33 percent of the patients survived, compared to 61.66 percent on MS + Kin at the same concentration, and 55 percent in the control group.

The maximum percentage of survival for nodal explants inoculated on MS + BAP + Kin @ 1mg/l each was reported, i.e., 68.33 percent, with MS + Kin 1mg/l demonstrating 65 percent survival after three weeks. On MS + BAP at 1 mg/l, 60% survival was seen, however on MS + BAP at 2 mg/l, only 58% survival was seen. The control group showed the lowest survival rate, 55 percent.

The results from Table 3.1 and Fig. 2 revealed that MS + BAP and Kin @ 1 mg/l each, or 70 and 68.33 percent, respectively, had the highest nodal explant survival rates after two and three weeks. As a result, the same was applied to additional research.

According to Supatmi *et al.* (2016), after two weeks of observation, MS medium supplemented with IAA (0.5 and 1 mg/l) or IAA and 0.2 mg/l Kinetin (P0.05) provided the optimum response for the survival of explants when matched to other media combinations. Surprisingly, sandalwood explants also had a high survival rate when grown in MS media at half concentration and supplemented with 1 mg/l GA3. Explants cultivated in regular MS medium concentration with 1 mg/l GA3 supplement exhibited the lowest overall survival rate among those obtained in other media.



T_1 - MS control T_4 - kinetin 1 mg/l
T_2 - BAP 1 mg/l T_5 - kinetin 2 mg/l
T_3 -BAP 2 mg/l + T_6 - BAP + kinetin 1 mg/l

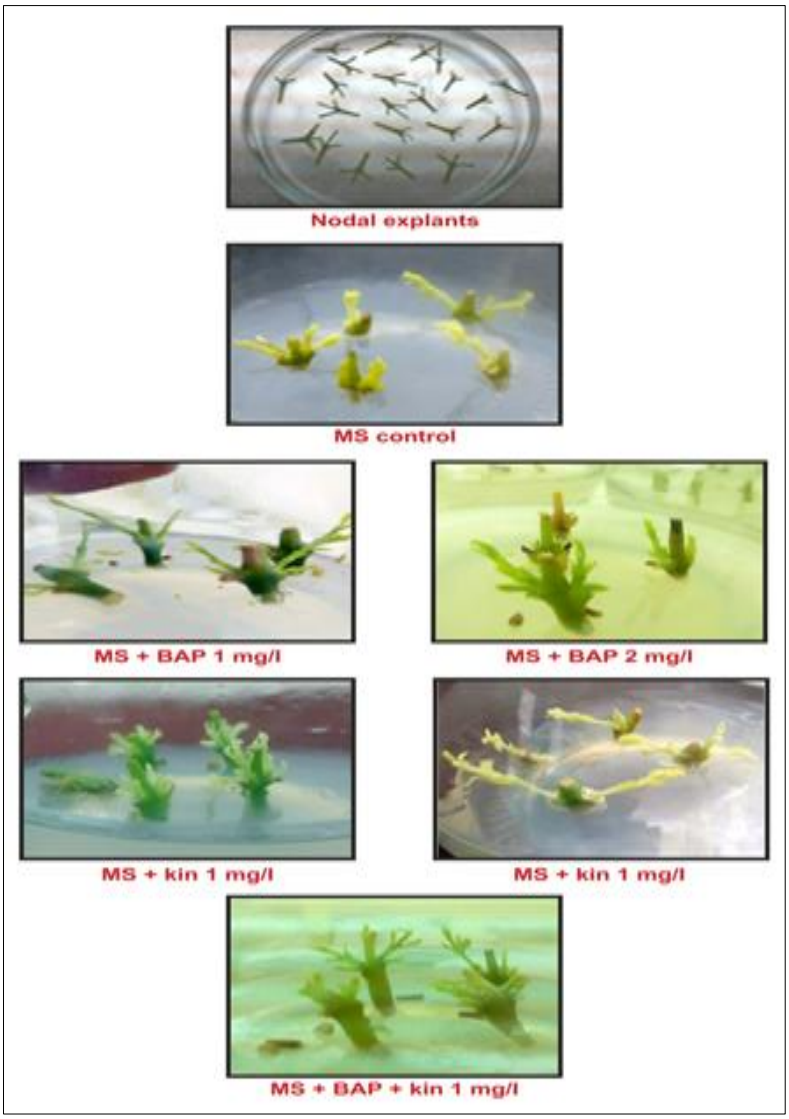


Plate 1: Survival of nodal explants on various media combination

Conclusions

According to the results of the experiment, nodal explants responded differently to several media combinations; nevertheless, MS + BAP + Kinetin @ 1 mg/l demonstrated to be the optimum media combination for explants survival.

References

1. Bapat VA, Rao PS. Somatic embryogenesis and plantlet formation in tissue culture of sandalwood *(Santalum album* L.). Annals of Botany. 1988;44:629-630.
2. Viswanath S, Dhanya B, Rathore TS. Financial Viability of sandal (*Santalum album* L.) cultivation practices. In: Proceedings of national seminar on conservation, improvement, cultivation and management of sandal-wood. Institute of Wood Science and Technology, Bangalore India; c2008. p. 158-164.
3. Rangaswamy NS, Rao PS. Experimental studies on (*Santalum album* L.). Establishment of tissue culture of endosperm. Phytomorphology. 1963;14:450-454.
4. Sawyer B, Jones P. Western Australian sandalwood resource statement, Department of Conservation and Land Management, Western Australia; c2000.
5. Lakshmi Sita G, Raghava Ram NV. Tissue Culture - A technique for rapid multiplication of sandal trees. In: Recent advances in research and management of sandal (*Santalum album* L.) in India; c1980.
6. Rao PS, Bapat VA. Vegetative propagation of sandalwood plants through tissue culture. Canadian Journal of botany. 1978;56:1153-1156
7. Dey S. Mass cloning of *Santalum album* L. through somatic embryogenesis. Wanatca Yearbook. 2001;25:23-26.
8. McKinnell FH. Status of management and silviculture research on sandalwood in Western Australia and Indonesia. In: Proc. of the Symposium on Sandalwood in the Pacific (Hamilton L and Conrad CE, Eds.); c1990. p. 19-25.
9. Preece JE, Imel MR, Shevade A. Regeneration of Rhododendron PJM group plants from leaf explants. Journal of the American Rhododendron Society. 1993;47:6871.
10. Jeeva V, Saravanan S, Devaraj P, Lakshmidevi R. Malady and remedy of sandal cultivation in farmlands and private lands an overview. In: Sandal and Its Products (Radomiljac AM, Ananthapadmanabha HS, Welboum, RM and Satyanarayana Rao K, Eds.); c1998. p. 16-19.
11. Sharma KK, Thorpe TA. Asexual embryogenesis in vascular plants in nature. In: Thorpe TA (Ed) in vitro Embryogenesis in Plants. Kluwer Academic Publishers; c1995. p. 17-72.
12. Lakshmi Sita G, Raghava Ram NV. Tissue Culture - A technique for rapid multiplication of sandal trees. In: Recent advances in research and management of sandal (*Santalum album* L.) in India; c1980.
13. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiology Plantarum. 1962;15:473-497.
14. Srimathi RA, Kulkami HD, Venkatesan KR. Recent advances in research and management of sandal (*Santalum album* L.) in India. Associated Publishing Co., New Delhi; c1995. p. 416.