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RESPONSE OF SANDAL WOOD (*Santalum album* L.) LEAF EXPLANTS UNDER *IN VITRO* CONDITIONS

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ABSTRACT

Sandalwood is a commercially important plant species belonging to the family Santalaceae and the genus *Santalum.* Sandalwood oil extracted from the heartwood has been used for medicinal, cultural and other purposes. The objective of this study was to find out the *in vitro* regenerative responses of sandalwood leaf explants using different combinations of plant growth regulators such as BAP and 2, 4 D. Half immature leaf explants were excised vertically with midrib from newly formed shoots of two years old seedlings. They were then subjected to sterilize by using 70% ethanol for 30 sec and immersed in 25% Clorox^{TM} (Sodium hypochlorite, 5.25% active ingredient) with two drops of tween 20 for 20 min. Sterilized explants cultured on MS medium supplemented with different concentrations of (0.5–1.0 mg/l) BAP and 2,4 D and kept in culture environment. The half immature leaf explants showed remarkablely higher (73.3%) callus formation within four weeks of culture on MS medium containing 1.0 mg/l 2, 4 D and after that there was no considerable proliferation in callus. Subcultured callus on MS medium supplemented with 1.0 mg/l BAP showed better callus proliferation. One fourth of half leaf explant produced 73.6 ± 1.73 mg of callus mass and the colour was changed from greenish yellow to light yellow at four weeks after subculture. Leaf explants were cultured on 1.0 mg/l BAP exhibited yellowish white calli. Leaf explants near the margin produced more callus compared with explants from the rest of the leaf lamina. Leaf lamina proximal to the leaf base region responded well rather than distal area. The cells on both the sides of the midrib were observed to be small in size and the cells towards middle of the leaf and towards the margin were found to be larger. It can be concluded that MS medium containing 1.0 mg/l BAP was more suitable to produce embryogenic callus from immature leaf explants of sandalwood.

KEYWORDS: 2, 4 D, BAP, sandalwood, callus formation

INTRODUCTION

Santalum album L. (sandal wood) belongs to the Santalaceae family and highly valued for its heartwood, which contains sandal oil. It is an essential tree species cultivated in a wide range of areas and adapts in different agro-climatic and soil conditions for plant regeneration (Rao et al., 2007: Batabyal and Tah 2012). There is a possibility to establish sandal wood plantations in Sri Lanka over the other vital forest plantation species because of high value and demand (Subasinghe, 2013). Therefore, large numbers of plant materials are required for the establishment of sandal wood plantations. There are some limitations in natural seed germination. As a result, an alternative propagation is needed. Leaves of sandal wood are good

source of explants and indirect plant regeneration via leaves is possible under *in vitro* conditions. The objective of this study was to determine the *in vitro* response of sandalwood leaf explants cultured in different concentrations of plant growth regulators such as BAP and 2, 4 D.

MATERIALS AND METHODS

This study was carried out in 2015 at the Tissue Culture Laboratory of the Department of Crop science, Faculty of Agriculture, Eastern University of Sri Lanka. Newly formed shoots were collected from two years old mother plants and immature leaf explants were excised. Then they were kept under running tap water for one hr and rinsed followed by distilled water. Thereafter, the explants were dipped in 70% ethanol for 30 sec and immersed in 25% Clorox^{TM} (Sodium hypochlorite, 5.25% active ingredient) with two drops of tween 20 for 20 min. After that, explants were rinsed three times with sterilized distilled water. Half sterilized explants were vertically excised with midrib and inoculated on the different MS (Murashige and Skoog) media supplemented with various concentrations (0.5 mg/l, 1.0 mg/l) of BAP and 2, 4 D. All cultures were incubated at 25±0.5 ^{o}C under white fluorescent light in photoperiod of 16 hrs light and 8 hrs dark. The light intensity of 2000 – 2500 lux and 70% humidity were maintained. The data obtained were subjected to Analysis of variance (ANOVA) using SAS 9.1.3 Portable version. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% significant level.

RESULTS AND DISCUSSION

Leaf lamina proximal to the leaf base region was responded well rather than distal area (Plate 1). The cells on both the sides of the midrib were observed to be small in size and the cells towards middle of the leaf were found to be larger (Plate 2). By this investigation, it showed that the callus cells near leaf base margin were in a status of active cell division. It was further noted that these cells contained small-large nuclei (Plate 3). This statement supported by (Devi *et al.*, 2015) who reported that mitotic activity is in the cells near the midrib.

Plate 1: Morphology of leaf explants cultured on MS medium with 1.0 mg/l BAP at 4th week of culture (arrow- midrib).

Immature half leaf (vertically cut with midrib) explants were cultured on 1.0 mg/l BAP exhibited yellowish white calli. The morphology of callus was observed at regular intervals. Explants excised near leaf margin produced better *in vitro* response compared with explants from the rest of the leaf lamina. Similar results have also been addressed by Mujib (2005) in sandalwood leaf disc culture. *In vitro* response was prominent in the midrib and margin region of the leaf explants.

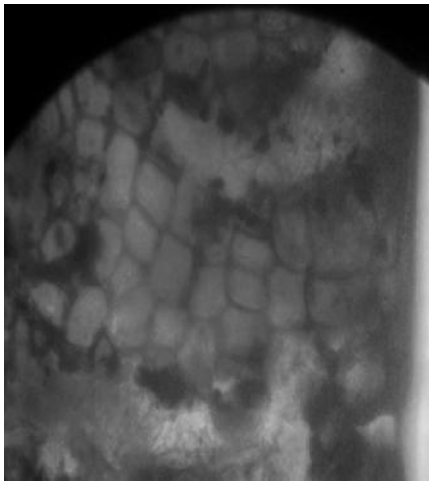

Plate 2: Leaf tissue cultured on MS medium with 1.0 mg/l BAP at 2nd week of culture (x400) (arrow- cell structure near midrib).

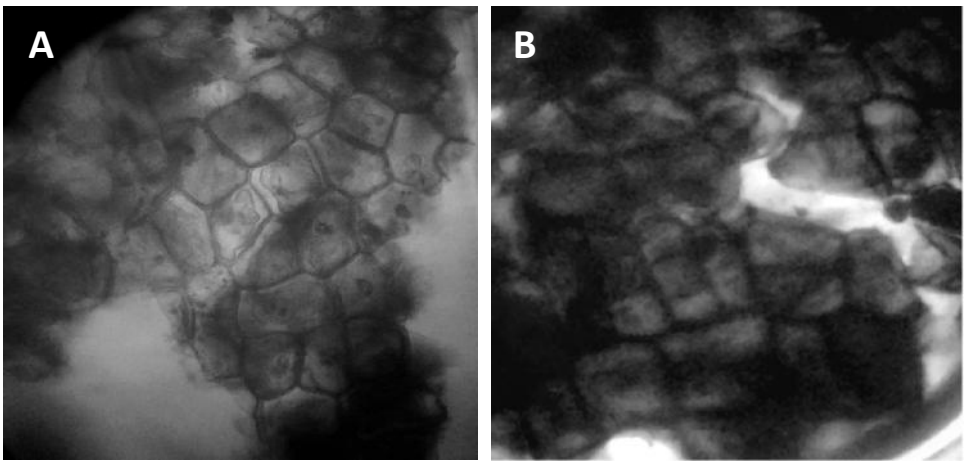

Plate 3: Histology of cultured leaf explant on MS medium with 1.0 mg/l BAP (x 400) at 3rd (A) and 6th week (B).

The present results revealed the half immature leaf explants (vertically cut with midrib) produced significantly high percentage of callus within four weeks of culture on MS medium with 1.0 mg/l 2, 4 D (Table 1, Plate 4) and after that there was no considerable callus proliferation and these callus turned to brown colour. Therefore, the explants were subcultured on MS medium supplemented with 1.0 mg/l BAP. After four weeks of subculture, better callus proliferation was noted and the callus colour was changed from greenish/whitish yellow to light yellow. Morphology and growth of the calli were influenced by the concentration of growth regulators (Singh *et al.,* 2013).

Table 1: *In vitro* response of leaf explants cultured on different media at 4th week.

Culture media	*In vitro* response	Response %
MS + 0.5 mg/l BAP	Greenish nodules	6.6 ± 1.73c
MS + 1.0 mg/l BAP	Greenish nodules	23.3 ± 1.73b
MS + 0.5 mg/l 2,4 D	Greenish yellow compact callus	26.6 ± 2.08b
MS + 1.0 mg/l 2,4 D	Greenish yellow compact callus	73.3 ± 1.15a
F value		P<0.05

Data based on the availability of survived explants. The percentage values are means ± standard error of three replicates. Means followed by the same letter are not significantly different from each other at 5% significant level according to the Duncan's Multiple Range Test.

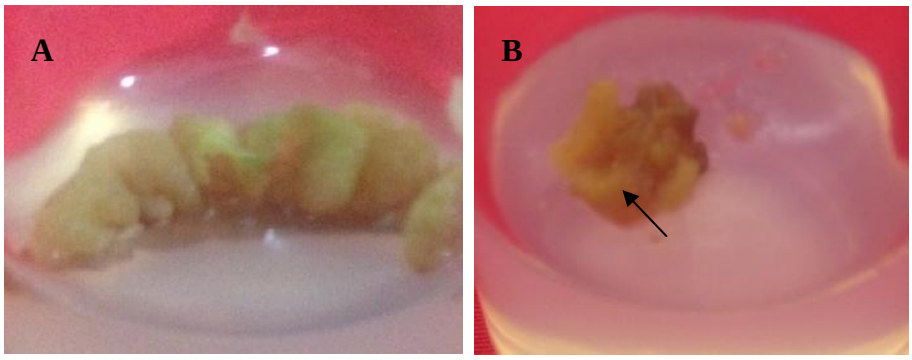

Plate 4: Callus formation from cultured leaf explants on MS medium with 1.0 mg/l 2, 4 D (A) at 4th week and leaf callus subcultured on MS medium with 1.0 mg/l BAP (B).

Fresh and dry weights of callus were taken by using electric digital balance at 8th week of culture. For this investigation, 1/4th of callus formed from initial half leaf explant was taken. The fresh weight of entire leaf before culture was 27.0 ± 0.5 mg which was cultured in MS medium with 1.0 mg/l BAP or 1.0 mg/l 2, 4 D for callus formation.

Table 2: Fresh and dry weights of callus at 8th week of culture.

*Culture media	Fresh weight (mg)	Dry weight (mg)
MS + 1.0 mg/l BAP	48.3 ± 1.53	2.3 ± 0.40
MS + 1.0 mg/l 2,4 D	73.6 ± 1.73	3.4 ± 0.72
't' test	P< 0.05	P> 0.05

*Initially culture media.

The callus weight values are means± standard error of three replicates.

The result showed that the callus formation in MS medium with 1.0 mg/l 2, 4 D was significantly differed from that in MS medium with 1.0 mg/l BAP by considering the fresh callus. This study showed that 1/4th of half leaf explant produced 73.6±1.73 mg of callus mass at 8th week of culture. However, the callus formed initially in MS medium with 1.0 mg/l 2, 4 D failed to show

any morphogenesis response within 8th week of culture. Literature survey stated that the MS medium with 2, 4 D did not produce any reproducible callus (Joulain *et al.*, 2012).

CONCLUSIONS

The results revealed that Leaf explants near leaf margin produced better *in vitro* response compared with explants from the rest of the leaf lamina. The half immature leaf explants exhibited significantly higher response of callus formation within four weeks of culture on 1.0 mg/l 2,4 D medium. The callus subcultured on MS medium supplemented with 1.0 mg/l BAP observed yellowish white appearance with better callus proliferation.

CONFLICT OF INTEREST

None

REFERENCES

Batabyal, S., Tah, J., 2012. Effect of agro-climatic and edaphic factors on *Santalum album* L. population in Burdwan and Bankura forest divisions of West Bengal. World Journal of Science and Technology 2(7), 56-62.

Devi, K. N., Madhavi, C., Sathyamurthy, B., 2015. Studies on the *In vitro* Regenerative Response of *Santalum Album*. International Journal of Innovative Research and Development. *4*(3)

Joulain, D., Nengone, S. N., DeGuahma, D. dit Lyo, *L*., 2012. New insights into the qualitative and quantitative analytical chemistry of sandalwood essential oils New Caledonia, France. In: Proceedings of International Sandalwood Symposium 21-24 October, 2012, Honolulu, Hawaii.

Mujib, A., 2005. *In vitro* regeneration of sandal (*Santalum album* L.) from leaves. Turkish Journal of Botany. 29(1), 63-67.

Rao, R. V., Hemavathi, T. R., Sujatha, M., Shashikala, S., 2007. Status on wood structure, heartwood formation, gae, gaps research needs of *Santalum album* L. In: Proceedings of National Seminar held in Institute of Wood Science and Technology (ICFRE) 12-13 December, Bangalore, India, pp165-171.

Singh, C. K., Raj, S. R., Patil, V. R., Jaiswal, P. S., Subhash, N., 2013. Plant regeneration from leaf explants of mature sandalwood (*Santalum album* L.) trees under *in vitro* conditions. *In Vitro* Cellular and Developmental Biology-Plant. 49(2), 216-222.

Subasinghe, S. M. C. U. P., 2013. Sandalwood research: A global perspective. Journal of Tropical Forestry and Environment. 3(1), 1-8.