Studies on *in vitro* regenerative performance of different explants of sandal wood (*Santalum album* L.)

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2015

ABSTRACT

Sandalwood plant (*Santalum album* L.) is a commercially and culturally important plant species, known for its fragrant heartwood and oil. Due to the high value of heartwood and oil, this species is illegally harvested in large amount which can lead to possible extinction. The present study was aimed to examine the *in vitro* regenerative performance of different explants of *Santalum album*. Therefore, various types of explants namely, shoot tips, stem segments, immature leaf, immature leaf segments, mature leaf, petiole, and single nodal segments were excised from the healthy stem cuttings of two years old seedlings and also mature seeds from ten years old healthy mother plant. The excised explants were dipped in 70% ethanol for 30 sec and immersed in 25% Clorox™ (Sodium hypochlorite, 5.25% active ingredient) with 1 - 2 drops of tween 20 for 20 min then rinsed three to four times thoroughly in sterilized distilled water until free from Clorox residues. Sterilized segments of plant parts were separately cultured on MS medium containing 0.5 mg/l BAP aseptically. The result revealed *in vitro* response percentage of the cultured explants clearly showed significant difference ($P<0.01$) among the explants. It ranged from 13.3% to 56.6%. Immature leaf segments were showed higher (56.6%) *in vitro* response and better survival rate (65%). Single nodal segment and shoot tip explants showed moderate *in vitro* response but the survival rate was significantly high (80%) in shoot tips at four weeks of culture. Petiole and mature leaf explants failed to show *in vitro* response and lower survival rate. Seed explants cultured on MS media supplemented with 0.5 - 1.0 mg/l GA_3 were swollen after four weeks of culture. These swollen seed explants were transferred to MS medium containing 1.0 mg/l BAP at eight weeks of culture and there was no germination response.

Further study was done to optimize the growth regulators and their concentrations for efficient direct or indirect organogenesis from shoot tip, single nodal segments and immature leaf explants of sandal wood. Therefore, the explants were cultured on MS medium supplemented with two different concentrations of BAP (0.5 mg/l, 1.0 mg/l) and 2,4 D (0.5 mg/l, 1.0 mg/l). Shoot tip explants were showed significantly higher (63.3%) *in vitro* response on 1.0 mg/l BAP. Shoot elongation and growth were occurred at the second week of culture then the succulent shoot growth and the callus formation were observed within four weeks of culture. 2,4 D supplemented medium was showed significantly lower (16.6%) *in vitro* responses. The single nodal explants were exhibited axillary shoot formation significantly high on 1.0 mg/l BAP within four weeks of culture thereafter no further proliferation. Immature half leaf explants was exhibited significantly higher (73.3%) *in vitro* response on 1.0 mg/l 2,4 D than other media. Better nodule formations were observed after ten days of culture. Then, the nodules were developed into compact greenish yellow callus within four weeks beyond this period no proliferations occurred and prone to browning. The extent of nodular formation was slightly lower on 0.5 mg/l 2,4 D medium. Then the explants were subcultured on MS medium supplemented with 1.0 mg/l BAP. After four weeks of subculture showed better proliferation on callus was noted and the colour was changed from greenish yellow to creamy white. The callus formed in 1.0 mg/l 2,4 D medium failed to show any morphogenesis response within eighth week of culture.

Furthermore another study was done to induce the embryogenic callus from the shoot tips and half leaf explants (vertically cut with midrib). Therefore, the explants were cultured on MS medium containing 1.0 mg/l BAP and 1.0 mg/l ascorbic acid with or

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