ABSTRACT

Propagation of Red sandalwood either through seeds or vegetative means is not given satisfactory results. Tissue culture has proved to be a promising technique for conservation and large scale multiplication of several woody species. Therefore the overall objective of this research was to develop a feasible *in vitro* micro propagation protocol for red sandalwood through tissue culture, which would be a reliable mass propagation method.

Shoot tips and semi hardwood cuttings were surface sterilized using different concentrations of clorox with different exposure time durations followed by 70% ethanol with 2 minutes exposure time. Surface sterilized shoots were transferred to Murashige and Skoog (MS) and Mc Cowns Woody Plant (WPM)) media with or without 0.1 % activated charcoal. Browning, survivals, number of new leaves produced and rate of forming new shoot buds were evaluated. Ex vitro derived shoot tips and semi hard wood cuttings, shoot tips excised from twenty- day- old *in vitro* germinated seedlings, cotyledonary nodal segments, mesocotyls and twenty- day- old *in vitro* derived seedlings were evaluated to identify best proliferation medium and best Benzyl Amino Purine (BAP) and Napthalene Acetic Acid (NAA) concentrations by evaluating number of shoots, leaves, branches and shoot height.

Proliferated shoots were excised for *in vitro* as well as ex vitro rooting. Three Indol Butric Acid (IBA) concentrations with 12 to 24 hours shaking periods were applied as the pulse treatment and subsequently transferred to 0.1 and 1 mg/l IBA containing liquid and solid half strength MS media. Number of roots produced, root length and browning of shoots were evaluated. *In vitro* rooted plantlets were transferred to pots containing sand: coir dust, cow dung and top soil in different ratios and placed under laboratory conditions and then transferred to plant house and maintained under high humidity. Finally plantlets were transferred to poly bags containing cow dung: top soil: sand (1:1:2). Survival rates, time taken

to appear new shoot branches, number of new leaves formed and height increment of the plants were evaluated. Experiments were designed according to Complete Randomized Design and all parametric data were analyzed using SAS statistical software. Mean separations were carried out using Least Significant Difference test (LSD). Non-parametric data were analyzed using chi square test. Each treatment was replicated twenty times.

Results revealed that 15% Clorox with 10 minutes exposure time followed by 70% ethanol for two minutes exposure time was the best treatment combination for surface sterilization of shoot tips by getting 80% aseptic cultures, with 80% survival. WPM medium incorporated with 0.1% activated charcoal is the best establishment medium where shoot tips showed 95% survival, without browning and producing highest number of new leaves and shoot buds. B5 medium with 2 mg/l BAP and 0.4 mg/l NAA gave maximum number of shoots and shoot branches from cotyledonary nodal segments (4.95, 4.20), mesocotyl segments (4.00, 3.75), *in vitro* derived shoot tips excised from 20- day- old seedlings (3.00, 2.5), shoot tips (2.95, 3.15) and semi hardwood cuttings (0.00, 3.45) detached from one- year- old plantlets maintained under plant house conditions and *in vitro* germinated seedlings (3.25, 3.5) respectively during four weeks time period. Explant, mesocotyls gave the highest mean shoot height (4.55 cm) and ex vitro derived shoot tips produced the highest mean number of leaves per shoot (4.00) in B5 medium with 2 mg/l BAP and 0.4 mg/l NAA, after four weeks. Adventitious roots were formed directly on 2 cm length stem cuttings when they were exposed to pulse treatment for twelve hours. After pulse treatment, stem cuttings were transferred to half strength solid MS medium containing 0.1 mg/l IBA to form roots.

In vitro rooted plantlets were successfully acclimatized in potting media containing sand: coir dust 1: 1 ratio. It is recommended to place plantlets in a humid chamber during the first six weeks time for better survival. Before repotting or field establishment, plants have to be maintained under plant house conditions for another four weeks or more.