ABSTRACT

Red sandalwood (*Pterocarpus santalinus*) is not cultivated locally and Sri Lanka entirely depends on Indian exports. Therefore, an extensive cultivation system is needed to satisfy the local demand as imports from India is no longer possible.

For the propagation of red sandalwood, conventional clonal propagation methods have not been very successful and natural germination by seeds also highly restricted due to low seed set, high recalcitrant nature and low survival rate. Hence, an alternative rapid propagation system is needed and for that a series of experiments were conducted using red sandalwood seeds to study *in-vitro* growth performances with ultimate focus on developing an effective protocol for micro-propagating the plant in large scale.

To select a suitable surface sterilization procedure for *in-vitro* establishment of seeds, 0.1 % mercuric chloride was used with five different exposure times (5, 10, 15, 20, and 25 min). Pods with a range of external diameter (< 3, 3-4, 4-5, and > 5 cm) were used to examine the effect of pod size on *in-vitro* germination. Pods harvested at light brown stage and stored at ambient temperature (28 ±2 °C) for 1, 2, 3, and 4 weeks were used to examine the effect of storage time on *in-vitro* germination. To study the effect of different culture media on *in-vitro* seed germination Murashige & Skoog (MS) (1962), Anderson (1980), Vitis (1987), and Woody Plant medium (WPM) (1980) with (1 g/l) or without activated charcoal were used. To study the *in-vitro* growth performance of the seedlings, MS, Anderson, and WPM incorporated with 1 g/l of activated charcoal were used.

Surface sterilization of seeds using 0.1 % $HgCl_2$ with 2-3 drops of Tween 20 for fifteen minutes was found to be effective. Pods less than 3 cm of diameter did not contain viable seeds. Seeds obtained from the pods of more than 4 cm showed significantly higher germination ability (90 %). Seeds cultured within one week of harvest showed the highest germination rate (96 %) and the shortest germination time (6 days), while pods stored for 4 weeks showed the minimum germination rate (61 %) and prolonged germination time (10 days).

Germination percentage was significantly higher (92%) in Anderson medium without charcoal, and lower germination of 62, 62 and 61 % were recorded on seeds cultured on WPM with charcoal, MS and Vitis medium without charcoal respectively. Seeds cultured on Anderson, Vitis and WPM took almost similar time (6-8 days) for germination. Seeds cultured on Vitis medium with charcoal showed the longest hypocotyl length of 13.8 mm.

The highest plant height of 9.9 cm and the number of nodes per seedling (7 nods/shoot) were promoted by WPM while the lowest plant height of 7.3 cm and the number of nodes (6 nodes/shoot) was resulted by MS medium. Best Leaf formation was achieved with WPM, and the average leaf diameter was 10.8 mm. A well developed root system was observed in all media tested. However, significant difference in growth performance among the media, was not observed.

To study *in-vitro* shoot proliferation ability of nodal cuttings, 6-Benzylaminopurine (BAP) was used in different concentrations with 0.2-mg/l α-Naptheleneacitic acid (NAA). As basal media, MS and WPM were tested with 1 g/l activated charcoal or without charcoal. To induce *in-vitro* rooting from nodal cuttings, two-steps culture procedure was

practiced. In the first step, 1 mg/l Indoleacitic acid (IAA) and 1 mg/l Indolebuteric acid (IBA) were used with MS basal medium either with or without activated charcoal. MS medium supplemented with, different concentrations and combinations of IAA (0.2, 0.5, 0.7 mg/l) and IBA (0.5, 1.0, 1.5 mg/l) were used for the sub culture.

No multiple shoot proliferation was observed on WPM with or without charcoal. However, single shoot elongation from the axillary buds of the nodal cuttings was observed. The highest shoot length of 16.0 mm was recorded in WPM supplemented with 6 mg/l BAP and 0.2 mg/l NAA. Among the tested media and hormone combination, 4 mg/l BAP and 0.2 mg/l NAA in MS basal medium was found to be effective for shoot proliferation (3.7 shoots/ nodal cutting).

In-vitro rooting was observed when two-steps culture procedure was practiced. The first stage of 1 mg/l IAA and 1 mg/l IBA followed by sub culture in 0.5 mg/l IAA and 0.5 mg/l IBA in MS basal medium was the successful procedure. The highest rooting percentage recorded was 50 %, whereas 99 % of nodal cuttings produced roots through callus formation, not direct adventitious rooting.