ABSTRACT

Rauvolfia serpentina and *Munronia pinnata* are highly valuable and widely used plants in Ayurveda system of medicine in Sri Lanka. There is no mass scale cultivation of these two plant species due to the poor seed production and low viability. Introduction of micro-propagated medicinal plant stocks to fields will conserve the existing stocks in wild, and will also increase the herbal resources, which are highly used in Ayurveda medicine.

In the present study multiple shoot production response of nodal explants to four combinations of Auxins and Cytokinins in Murashige & Skoog (1962) (MS) basal medium was studied. The meristems of the apical and axillary buds of *R.serpentina* propagated multiple shoots on MS medium containing IAA (0.2 mg/L) and BAP (2.25 mg/L). Three passages of regular subculture on the same medium gave a higher number of shoots, with an average of 10-15/subculture. Transferring the individual shoots to MS medium containing 0.5 mg/L NAA and 0.2 mg/L IBA and 0.1 mg/L BAP hormone combinations induced rooting. 100% rooting was achieved on MS medium containing 0.5 mg/L NAA, followed by callusing at the cut end. Well-rooted plants were obtained in the MS medium without a growth hormone. At the acclimatization stage 80-90% plantlets survived in the trays with a mixture of sterilized sand : soil (1:1 w/w) and after 2 months the plants were transferred to a larger pot containing the same soil mixture for establishment of the root system.

The effects of NAA (2.0 – 5.0 mg/L) in combination with BAP (2.0 –5.0 mg/L) on basic Murashige & Skoog (1962) medium (Hirimburegama *et al.*, 1994) were investigated to assess the induction of callus from leaf explants of *Munronia pinnata*. Cultures were grown at 25 – 27°C separately under a complete dark and 24 h light for 4 weeks. The best response for callus induction and growth was found on medium containing 5 mg/L NAA and 2.0 mg/L of BAP, in complete dark. Callus cultures were transferred to light after 4 weeks in dark. Within 3-4 weeks, tiny green shoots were formed on the callus followed by the formation of green bud like structures. Growth of the tiny shoots of *M.pinnata* increased with the change of medium. Shoots were well developed on the MS medium supplemented with 2.25 mg/L BAP and 0.2 mg/L IAA. The best rooting was obtained in MS liquid medium with 1.0 mg/L IAA and 1.0 mg/L IBA combination within 1-2 months time period. The study reveals the possibility of micropropagating the two plant species through *in-vitro* shoot tip culture of *R.serpentina* and leaf callus culture of *M.pinnata*.

Two types of phytochemical screening methods were carried out for detection of major classes of compounds present in these two plant species. It was revealed that roots of *R.serpentina* (N&TC) contain higher amount of alkaloids, saponins, sterols and cardenolides. Alkaloids occurrence is much higher in comparison to other compounds. An interesting factor find out from the study was the absence of alkaloids in *M.pinnata* plant parts. Whole plant of *M.pinnata* contains higher amount of sterols, saponins and cardiac glycosides, terpenes, tannins as major

group of compounds. Plants established in the field should be tested for the compound/s with medicinal value before commercialize the technology.

The apical and axillary buds obtained through *in-vitro* propagation of *R.serpentina* were successfully encapsulated using 75mM of $CaCl_2.2H_2O$ and 6% Sodium alginate for complexation. Growth of the artificial seeds was observed under both *in-vitro* and *in-vivo* conditions.