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The work described in this thesis was carried out by me under the supervision of Dr.(Mrs.) Nazeera Salim and a report on this has not been submitted in whole or in part to any University for another Degree/ Diploma.

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I certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation

31/10/03
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MICROPROPAGATION OF *OPHIORRHIZA MUNGOS L.* FOR THE PRODUCTION OF CAMPTOTHECIN

By

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ABSTRACT

Ophiorrhiza mungos L. (Dathketiya) is a medicinal plant used in traditional medicine in Sri Lanka and India. This plant produces potent antileukemic and antitumor compounds, camptothecin (CPT) and 10 methoxy camptothecin.

Since the propagation of this plant by seeds is slow and viability of seeds is very low, feasibility of propagation *in vitro* has been studied. Studies were conducted to determine the best explant type for callus culture, to select the suitable nutrient medium and plant growth regulators for callus & root cultures, regeneration of plants from callus and axillary buds. The camptothecin contents in callus, root, *in vitro* and *in vivo* grown plant organs were determined.

Young and mature leaves, stems and petioles from greenhouse grown plants and young leaves and roots from *in vitro* grown plants were used as explants for callus formation. Different concentrations of auxins, 2.4-dichloro phenoxy acetic acid (2.4-D), Indole butyric acid (IBA) and Naphthalene acetic acid (NAA) in combination with different concentrations of cytokinins, Benzyl aminopurine (BAP) or kinetin in Murashige and Skoog (MS) or Gamborg B_5 (GB_5) medium were tested. Almost all the growth regulator combinations tested showed 100% callus formation. Young leaf explants obtained from *in vitro* grown plants in MS medium with 1.0 mg/l 2.4-D + 0.1 mg/l kinetin gave the best callus formation within 10 days. Same explants obtained from greenhouse grown plants also showed better callus formation but it took more time to

initiate callus than the leaf explants from *in vitro* grown plants. Callus initiated on GB_5 medium was hard and dried out within several days.

For the regeneration of shoots from callus, MS medium with different concentration of auxins (2.4-D and NAA) in combination with different concentrations of BAP, kinetin alone and Woody plant medium (WPM) with different combinations of 2.4-D and BAP were used. Among the tested growth regulator combinations, MS medium with 0.1 mg/l 2.4-D with 1.0 mg/l BAP gave mean of 19.3 shoots within 10 weeks and 0.3 mg/l kinetin gave mean of 15.4 shoots within 8 weeks which were higher than other treatments tested. MS medium with 1.0 mg/l IBA + 5.0 mg/l BAP gave the highest number of shoots per node.

MS half strength medium containing 0.5 mg/l NAA with 1.5% sucrose gave 100% rooting with long and normal appearance roots while treatment with kinetin resulted short, thick roots. The concentration of 0.1 mg/l gibberellic acid was suitable for shoot elongation of *O. mungos*.

Eighty days old root cultures grown in WP medium with 2% sucrose and NAA (0.5, 1.0 and 1.5 mg/l) gave the highest root yield (0.53-0.58g, dry weight) and no significant difference in dry weights were observed with different concentrations of NAA. GB_5 medium with 4% sucrose also showed very similar results (0.47-0.53 g dry weight).

Flowers of field grown plants contained the highest percentage of camptothecin (0.08%) compared to roots, leaves and stems. Similar amount of camptothecin was

produced in 80 day old root cultures grown in WP medium with 2% sucrose. Although NAA increased the root yield, it inhibited the production of camptothecin.

Forty day old root cultures in MS, GB_5 and WP medium without NAA gave 0.06% of camptothecin. Callus cultures too produced the similar amount of camptothecin (0.06%), but it took longer time than above. Among the tested callus, 9 month old callus grown on MS medium with 1.0 mg/l 2.4-D + 0.1 mg/l kinetin gave the highest amount of camptothecin.