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STUDIES ON NATURAL HABITAT AND CLONAL PROPAGATION OF *Zeuxine regia* (IRURAJA) AND *Zeuxine flava* (SANDARAJA)

By

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy in Botany on Plant Tissue Culture

Declaration

The work described in this thesis was carried out by me under the supervision of Prof. W.T.P.S.K. Senarath and a report on this work has not been submitted in whole or in part to any university for any other degree or diploma

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Samangi Hewage (BSc)

11.11.2011

Date

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 Degree of Master of Philosophy.

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I hereby certify that all corrections, additions and amendments have been done accordance with the comments and suggestions of the examiners.

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Dedication

To my parents and my sister for their unconditional love, care and support to achieve goals in my life

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S. HEWAGE

ABSTRACT

Zeuxine flava and *Zeuxine regia* are two endangered medicinal plants belong to family Orchidaceae. The objectives of this study were to identify the natural distribution of these species in wet zone of Sri Lanka and associate tree species in their natural habitats, to develop a feasible mass propagation protocol and greenhouse establishment of the tissue cultured plants. Finally introduce the mass propagated plants back to their natural habitats.

During the ecological study in Kanneliya MAB reserve and Peak Wilderness Sanctuary it was observed that the common most families associated with *Z. regia* were Ebanaceae, Anacardiaceae and Dipterocarpaceae. There were only four tree species found in association with Z. *flava*. All these were represented by one stem and due to the higher diameter (dbh) *Homalanthus populifolius* become dominant.

No calli initiation was observed in any of the species, when the leaf discs were used as explants. Different basal media Murashige and Skoog (MS), Woody Plant Medium (WPM), and Knudson's C (KC) at different concentrations were supplemented with 2.0 mg/L Benzyle Amino Purine (BAP) and 1.00 mg/L 2,4-dichlorophenoxyaceticacid (2,4-D) were used to initiate shoots from apical and axillary buds of *Z. flava* and *Z. regia*

In the shoot initiation medium (SIM) (1/10 WPM supplemented with 2.0 mg/L BAP and 1.0 mg/L 2,4-D) bud initiation was observed in *Z. flava* and *Z. regia* within 5 days, but no multiple shoots were induced even after three subcultures. Elongated buds of *Z. flava* and *Z. regia* in SIM were transferred to MS basal medium supplemented with 0.1 mg/L Naphthalene Acetic Acid (NAA), 1.0 mg/L BAP, 1.0 mg/L Kinetin (Kin), induced multiple shoots of *Z. flava*, and mean number of shoots per shoot was 4.1 ± 0.87. *Z. flava* cultures were further maintained up to 14 weeks for bud elongation. Multiple shoots were induced in *Z. regia* when MS medium supplemented with 0.1 mg/L NAA, 1.0 mg/L BAP and 0.5 mg/L Kin within 12 weeks time and mean number of 2.8 ± 0.42 shoots per shoot was observed. However, multiple shoots were not elongated further and root nodules were not produced in *Z. regia.* Therefore, further experiments were not carried out for *Z. regia.*

Z. flava shoots induced root nodules after ten weeks of incubation in SIM. Time taken to produce roots in multiple shoot induction medium was 5.7 ± 0.67 weeks. Further incubation (4.1 ± 0.74 weeks) in the same medium produced hairy roots around the root nodules.

Mixtures of top soil and leaf litter (1:1) and coconut husks were used in this study for acclimatization. 100% survival was observed in both potting mixtures when plants were gradually introduced to the greenhouse after covering with a polypropylene bag and maintained in culture room temperature for seven days before being transferred to greenhouse.

Mean number of leaves in *in vitro* produced plants was higher (5.90 ± 1.45) than those of vegetatively propagated plants (4.50 ± 0.87) after maintaining the same height *in vitro* produced plants and vegetative propagated plants for six months in greenhouse. Mean plant heights were also higher in *in vitro* produced plants (6.63 ± 2.5) cm than vegetatively propagated plants. *In vitro* plants produced two to three multiple shoots per node after 6 months where no multiple shoot production in vegetatively propagated plants, indicating tissue cultured plants are more vigorous than natural plants.

In vitro produced plants grown under greenhouse conditions initiated floral spikes during early March, which is an unusual habit. Although under natural conditions, once the flower spike is fallen and plant dries off, *in vitro* produced plants do not died after falling off the flower spike, instead they initiated axillary buds and produced multiple shoots.

Mean number of leaves in *in vitro* produced plants after six months of growth in natural habitat were higher (7.9 ± 0.74) than natural plants. Mean height of the plant also higher in *in vitro* produced plants (14.73 ± 0.46 cm).

In *in vitro* produced *Z. flava* plants, leaf texture and colour of the leaves were prominent than plants collected from the wild. Tissue cultured plants produced multiple shoots and flowers under greenhouse conditions. These factors indicate that mass production of *Z. flava* through tissue culture is possible for ornamental purposes and conservation of the species.