

**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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..... 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.

A handwritten signature in blue ink, appearing to read 'Senarath', with a horizontal line underneath.

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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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TABLE OF CONTENTS

Table of contents.....	i-vi
<i>List of figures</i>	iv
<i>List of plates</i>	v
<i>List of tables</i>	vi
Acknowledgments.....	vii
Dedication	ix
Abstract.....	x
CHAPTER ONE: INTRODUCTION.....	01-11
1.1 Family Orchidaceae	01
1.2 Plant tissue culture	04
1.3 Habit of <i>Zeuxine regia</i> (Lindl.) Trimen (Iruraja).....	05
1.3.1 Taxonomical classification of <i>Zeuxine regia</i> (Lindl.) Trimen	05
1.4 Habit of <i>Zeuxine flava</i> (Wall.) Trimen (Sandaraja).....	07
1.4.1 Taxonomical classification of <i>Zeuxine regia</i> (Wall.) Trimen.....	07
1.5 Importance of mass propagation of <i>Z.regia</i> and <i>Z. flava</i>	07
1.6 Study sites	08
1.6.1 Kanneliya Man and Biosphere Reserve	08
1.6.2 Peak Wilderness Sanctuary.....	09
1.7 Objectives of the study.....	11
CHAPTER TWO: REVIEW OF LITERATURE	12-28
2.0 General.....	12
2.1 <i>In vitro</i> culture.....	12
2.2 Types of culture	13
2.3 Vegetative propagation of Orchids	14
2.4 Outline of tissue culture of Orchidaceae.....	16
2.5 Sources of explant.....	20
2.6 Organogenesis.....	22

2.6.1 Shoot initiation.....	22
2.6.2 Root initiation	23
2.7 Somatic embryogenesis	24
2.7.1 Somatic embryogenesis in Orchids.....	25
2.8 Medicinal importance of selected species.....	27
CHAPTER THREE: MATERIALS AND METHODS	29-42
3.0 General.....	29
3.1 Comparison of habitats of <i>Zeuxine regia</i> and <i>Zeuxine flava</i>	32
3.2 Effect of different growth regulators and basal media for induction of callus from leaf disc explants.....	36
3.3 <i>In vitro</i> shoot induction of <i>Z. flava</i> and <i>Z. regia</i> from shoot tips and nodal segment explants	38
3.4 <i>In vitro</i> shoot multiplication of <i>Z. flava</i> and <i>Z. regia</i>	38
3.5 Effect of different growth regulators on root induction of <i>in vitro</i> produced shoots.....	40
3.6 Effect of different potting mixtures on acclimatization	40
3.7 Comparison of <i>in vitro</i> propagated plants with vegetatively propagated plants under greenhouse condition.....	42
3.8 Reintroduction of <i>in vitro</i> produced plants into natural habitat.....	42
CHAPTER FOUR: RESULTS AND DISCUSSION	44-80
4.1 Comparison of habitats of <i>Zeuxine regia</i> , and <i>Zeuxine flava</i>	44
4.2 Effect of different growth regulators and basal media for induction of callus from leaf disc explants	50
4.3 <i>In vitro</i> shoot induction of <i>Z. regia</i> and <i>Z. flava</i>	52
4.4 <i>In vitro</i> shoot multiplication of <i>Z. flava</i> and <i>Z. regia</i>	57
4.5 Effect of different growth regulators on root induction of <i>in vitro</i> produced shoots.....	62
4.6 Effect of different potting mixtures on acclimatization	65
4.7 Comparison of <i>in vitro</i> propagated plants with vegetatively propagated plants under greenhouse condition.....	72

4.8 Re introduction of <i>in vitro</i> produced plants into natural habitat	78
CHAPTER FIVE: CONCLUSION	80-83
Micropropagation protocol for <i>Z.flava</i>	82
Micropropagation protocol for <i>Z.regia</i>	83
CHAPTER SIX: RECOMMENDATIONS	84
<i>References</i>	85
Appendices	

List of figures

Figure 1: Laying of quadrates along transects35

Figure 2: Dominant families associated with *Z. regia*45

Figure 3: Dominant families in areas without *Z. regia*46

Figure 4: Dominant families associated with *Z. flava*48

Figure 5: Dominant families in areas where *Z. flava* plants were not found48

Figure 6: Variation in growth of tissue cultured plants and natural plants
grown in the greenhouse over a period of one year75

List of plates

Plate 1: (a) Habit of <i>Z. regia</i> (Lindl.) Trimen (Iruraja); (b) Habitat of <i>Z. regia</i> in Kanneliya MAB reserve	06
Plate 2: (a) Habit of <i>Zeuxine flava</i> (Wall.) Trimen (Sandaraja); (b) Habitat of <i>Zeuxine flava</i> in Kanneliya MAB reserve	06
Plate 3: Mother plants collected from Kanneliya MAB reserve, Peak Wilderness Sanctuary and Haldummulla Nursery maintaining inside humid chambers	30
Plate 4: Swollen edges of leaf discs of <i>Z. flava</i> in 1/10 WPM.....	51
Plate 5: Bud elongation of <i>Z. flava</i> in full-strength WPM medium supplemented with 2.0 mg/L BAP and 1.0 mg/L 2,4-D	53
Plate 6: (a) Elongation of axillary buds (b) Elongation of apical buds (after 5 days of incubation) of <i>Z. flava</i> in 1/10 WPM supplemented with 2.0 mg/L BAP and 1.0 mg/L 2,4-D	56
Plate 7: (a) Elongation of axillary bud: (b) elongation of apical bud of <i>Z. regia</i> in 1/10 WPM supplemented with 2.0 mg/L BAP and 1.0 mg/L 2,4-D after 12 weeks of incubation	56
Plate 8: Multiple shoot induction of <i>Z. flava</i> in MS medium supplemented with 0.1 mg/L NAA, 1.0 mg/L BAP and 1.0 mg/L Kin after 8 weeks of incubation	60
Plate 9: Formation of root nodules in <i>Z. flava</i> in multiple shoot induction medium.	64
Plate 10: Formation of root nodules in shoot induction medium without multiple shoots	64
Plate 11: Growth of <i>in vitro</i> produced plants in coconut husks medium.....	68
Plate 12: Growth of <i>in vitro</i> produced plants in leaf litter: top soil (1:1) medium	68
Plate 13: (a) Natural plant with flowers (b) <i>In vitro</i> produced plants with flowers...	75

List of tables

Table 1: Different basal media and growth regulator combinations used for callus induction from leaf disc explants of <i>Z. flava</i> and <i>Z. regia</i>	37
Table 2: Different combinations of BAP and Kin used with MS basal medium supplemented with 0.1 mg/L NAA	39
Table 3: Calculated IVI values for species with <i>Z. regia</i>	46
Table 4: IVI values for species in areas where <i>Z. regia</i> plants were not found	47
Table 5: IVI values for species found with <i>Z. flava</i>	49
Table 6: IVI values for species found in areas without <i>Z. flava</i>	49
Table 7: Time taken for bud elongation, mean shoot length from axillary buds and apical buds after six weeks	53
Table 8: Time taken for multiple shoot induction in different combinations of BAP and Kin for <i>Z. regia</i> and <i>Z. flava</i> in MS basal medium supplemented with 0.1 mg/L NAA	57
Table 9: Mean number of shoots and shoot length in different weeks of incubation in MS medium supplemented with 0.1 mg/L NAA, 1.0 mg/L BAP and 1.0 mg/L Kin	59
Table 10: Mean number of root nodules, time taken for root nodule and hairy root formation in different growth regulator combinations in MS medium with 0.1 mg/L NAA	63
Table 11: Comparison of growth parameters for <i>in vitro</i> produced plants grown in two potting mixtures after six months	66
Table 12: Comparison of growth parameters of <i>in vitro</i> produced plants and natural plants after six months of growth in the greenhouse	73
Table 13: Comparison of growth parameters of <i>in vitro</i> produced plants and natural plants after six months of growth under natural conditions	79

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Dedication

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

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

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 die 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 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.