EX-SITU CONSERVATION OF PROTECTED MEDICINAL PLANTS: *VALERIANA JATAMANSI* JONES AND *RAUVOLFIA SERPENTINA* L. BENTH. EX KURZ. BY TISSUE CULTURE TECHNIQUE

A Dissertation
Submitted to Central Department of Botany
For the partial fulfillment for the requirement of M. Sc. in Botany

Submitted by
Yadu Ram Sharma Nepal
Roll No.: 41
Batch: 059/60
Exam No.: 599
T.U. Regd. No.: 9-2-268-1997

Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu, Nepal
2006

CERTIFICATE

This is to certify that the dissertation entitled "***EX-SITU* CONSERVATION OF PROTECTED MEDICINAL PLANTS: *VALERIANA JATAMANSI* JONES AND *RAUVOLFIA SERPENTINA* L. BENTH. EX KURZ. BY TISSUE CULTURE TECHNIQUE**" submitted by **Mr. Yadu Ram Sharma Nepal** for the partial fulfillment of Master Degree in Botany. The result of the investigation was carried out by him under my supervision in the Tissue Culture Laboratory of Central Department of Botany. The result of the present work has not been submitted for any degree to the best of my knowledge. I recommend this dissertation to be accepted for the partial fulfillment of Master Degree in Botany from Tribhuvan University, Nepal.

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Dr. Bijaya Pant
(Supervisor)
Central Department of Botany
Tribhuvan University
Kathmandu, Nepal

December 3, 2006

APPROVAL LETTER

This is to certify that the dissertation entitled ""***EX-SITU*** **CONSERVATION OF PROTECTED MEDICINAL PLANTS:** ***VALERIANA JATAMANSI*** **JONES AND** ***RAUVOLFIA SERPENTINA*** **L. BENTH. EX KURZ. BY TISSUE CULTURE TECHNIQUE**" submitted by **Mr. Yadu Ram Sharma Nepal** has been accepted as a partial fulfillment of Master Degree in Botany.

EXPERT COMMITTEE

……..
Supervisor
Dr. Bijaya Pant
Central Department of Botany
Tribhuvan University, Kirtipur
Kathmandu, Nepal

……..
Head
Prof. Dr. Pramod Kumar Jha
Central Department of Botany
Tribhuvan University, Kirtipur
Kathmandu, Nepal

……..
Internal Examiner
Prof. Dr. Sanu Devi Joshi
Central Department of Botany
Tribhuvan University, Kirtipur
Kathmandu, Nepal

……..
External Examiner
Dr. Bilaimita Singh
Patan Multiple Campus
Lalitpur, Nepal

Date of Examination: December 12, 2006

ACKNOWLEDGEMENT

I express my gratitude and in-depth appreciation to my supervisor Dr. Bajaya Pant for her constant guidance and encouragement, which have provided me the foundation in the field of basic research in plant biotechnology.

I also express my gratitude to Prof. Dr. Pramod Kumar Jha, Head of Central Department of Botany for providing me the necessary laboratory facilities during this investigation. My profound gratitude and appreciation to Prof. Dr. Sanu Devi Joshi, Prof. Dr. B. N. Prasad, Prof. Dr. V. N. Gupta and Mr. Sankhar Pahadi for their theoretical and practical guidance in the field of tissue culture and biotechnological research. I thank my friends: Ramesh Basnet, Dhan Chand, Roshan Sharma Poudyal, Robin Uprety, Apsana Giri, Kalpana Sharma, Kuber Poudel (photographic work), Saraswati Aryal, Lil Jung Gurung, Basanta Sharma and others for their co-operation and valuable help during my investigation period. I am highly indebted to Madan K. D. Shrestha for the photography; and to his unwavering encouragement throughout my work. My appreciation to the laboratory assistants of CDB for their co-operation. I acknowledge the support of Mr. Prem Khanal and Mr. Gopi Khanal, Jhapa, for the supply of the seedlings of *Rauvolfia serpentina* at the right time of my need. I also acknowledge the entire computer service of Mr. Anil Kumar Bajracharya, Natole, Lalitpur. I acknowledge and thank the friendly staff of University Computer Service, Kirtipur, for their valuable help in the final phase of the work i.e. page setting and binding.

My special appreciation to my brother Ranga Lal Nepal and friends Chhabi and Kuber Poudel for their financial support and moral encouragement during my academic pursuit. I appreciate my sister Menuka for the timely tea and nutritious meals that have encouraged me for early hour work.

Kathmandu, Nepal
November 10, 2006

Yadu Ram Sharma Nepal
Examination Roll No. 99

ABSTRACT

Subculture of *in-vitro* nodes of *Valeriana jatamansii* Jones was carried out for six consecutive batches or passages on MS medium supplemented with 1 ppm BAP and 0.5 ppm NAA. Optimum number of shoots (average 11 shoots) was obtained in the fifth batch. The shoots were acclimatized and hardened in three different media: cocopit, sand and the mixture of sand and soil (1:1 v/v). The highest percentage of survival *in-vivo* (91.6%) was achieved in cocopit followed by sand (83.3%). The shoots and their roots appeared healthy and longer in cocopit. However, the *in-vitro* plants hardened in sand adapted to the soil relatively faster and with over 95% survival rate. The density of roots was relatively higher in the plants hardened in sand. The size of the rhizome seemed bigger in those plants which were hardened in sand and then transferred them into the soil. The *in-vitro* raised plants hardened in cocopit were slow and ineffective for rapid adaptation in the soil.

Nodes from the *in-vitro* developed shoots of *Valeriana jatamansii* Jones were subcultured in MS medium supplemented with four different concentrations of NAA: 0.5 ppm, 1 ppm, 1.5 ppm and 2 ppm NAA for the production of root mass. profuse of root mass of root was observed in MS + 0.5 ppm NAA. Next, the most effective strength of MS for the optimum proliferation of hairy roots was verified by inoculating the *in-vitro* developed nodal explants in four different strengths of MS medium: $\frac{1}{4}$ MS, $\frac{1}{2}$ MS, $\frac{3}{4}$ MS and full strength of MS, supplemented each with 0.5 ppm NAA. Maximum proliferation of hairy roots was observed in $\frac{1}{2}$ MS + 0.5 ppm NAA.

Next, shoot tips and nodes from the *in-vivo* plants of *Rauvolfia serpentina* L. Benth. ex Kurz were inoculated on MS media with fifteen different combinations and concentrations of BAP and NAA. Shoot tip explants responded with healthy and distinct multiple shoots in MS + 2 ppm BAP + 0.5 ppm NAA. Multiple shoots were diffirentiated from the callus. The nodal explants differentiated into, healthy and relatively sturdy shoots in MS + 0.5 ppm BAP + 0.5 ppm NAA. There was no callus formation. This protocol may be appropriate to produce genetically homogenous

progeny of the plant. Shoot tip explants differentiated optimum callus, without any shoot and leaf, in MS + 0.5 ppm BAP + 1 ppm NAA. Nodal explants produced soft, dark-brown callus with upper green surface in MS + 1.5 ppm BAP + 1 ppm NAA. Single shoot appeared from the callus.

CONTENTS

ABBREVIATION

2, 4-D	:	2, 4 – Dichlorophenoxy Acetic Acid
B A	:	6 – Benzyl adenine
B S	:	Bikram Sambat (Nepali Calendar Year0
BAP	:	6 - benzylaminopurine
CDB	:	Central Department of Botany, T. U.
IAA	:	Indole 3 – Acetic Acid
IBA	:	Indole 3 – Butyric Acid
Kn	:	Kinetin (6 – furfurylamino purine)
MS	:	Murashige and Skoog medium (1962)
NAA	:	Napthalene Acetic Acid
NTFP	:	Non-Timber Forest Product
PPM	:	Parts Per Million (mg/l)