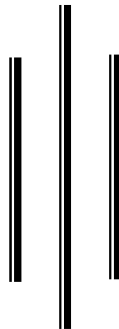


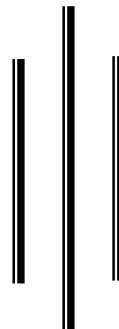
***EX-SITU* CONSERVATION OF PROTECTED
MEDICINAL PLANTS: *VALERIANA JATAMANSI*
JONES AND *RAUVOLFIA SERPENTINA* L. BENTH.
EX KURZ. BY TISSUE CULTURE TECHNIQUE**



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



**Submitted by
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**Central Department of Botany
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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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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.

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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 differentiated 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.

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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)