In vitro Morphogenesis of *Asparagus racemosus* Willd. and *Rauvolfia serpentina* (L.) Benth. ex Kurz.

THESIS SUBMITTED TO THE INSTITUTE OF SCIENCE AND TECHNOLOGY TRIBHUVAN UNIVERSITY, KATHMANDU, NEPAL FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY

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Certificate

This is to certify that the thesis entitled '*In vitro* Morphogenesis of *Asparagus racemosus* Willd. and *Rauvolfia serpentina* Benth. Ex. Kurz.' submitted to Institute of Science and Technology, Tribhuvan University for the degree of Doctor of Philosophy in Botany, is the outcome of original research work carried out by Mr. Krishna Kumar Pant, Lecturer, Department of Environmental Science, Rampur Campus, Chitwan Nepal, under my supervision. Almost all the research work, especially the laboratory experiments on *in vitro* morphogenesis was performed at the Central Department of Botany, Tribhuvan University, Nepal. This work has not been submitted for a degree of any other University.

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DECLARATION

I hereby declare that the work presented in the thesis entitled '*In vitro* Morphogenesis of *Asparagus racemosus* Willd. and *Rauvolfia serpentina* Benth. Ex. Kurz.' has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

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DEDICATED TO MY LATE FATHER MR. YAGYA PRASAD PANT

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Krishna Kumar Pant

ABSTRACT

Asparagus racemosus willd. and *Rauvolfia serpentina* Benth. Ex. Kurz. Belonging to the families Lilliaceae and Apocynaceae respectively are among the most important natural resources of Nepal. Both these plants have high medicinal value and are decreasing from the natural forests due to unsustainable collection and destruction of their habitats for various reasons. *R. serpentina* is listed in the "Appendix II" of the CITES and is under the category "E" of IUCN. Similarly, *A. racemosus* is facing towards its extinction due to its increasing demand in the local as well as international markets. Understanding these facts, the GoN has prioratised these species for research in the areas of multiplication and cultivation. Considering these facts, the present research was undertaken to support the national mission of conservation through *ex-situ* way. Plant tissue culture method was selected because it is one of the promising methods of rapid plant propagation in short time and less space without damaging the mother stock. From the present work, most effective explant for the rapid multiplication of these plant species using tissue culture technique has been identified to be the nodes. Calli induced from the nodes are suitable for organogenesis as well as somatic embryogenesis. Induction of buds from the shoots in *A. racemosus* is another potential area where more works are necessary. In the overall experiments, NAA was found to be the most effective hormone among the auxins in all respects and BAP among the cytokinins tested.

From the seed germination experiments of *Asparagus racemosus* on MS basal medium a single seed produced up to 13 shoots. In the tissue culture experiments, the nodes produced more callus when cultured on the medium containing NAA alone in higher concentrations or when combined with BAP. The tough friable calli induced from the nodes on high auxin containing media were capable of inducing somatic embryoids. Generally, high auxin either singally or in combination with higher concentration of cytokinin produced large number of somatic embryoids from the calli. Shoot formation from the calli was supported by lower cytokinin whereas root induction by higher cytokinin levels in the media. In case of multiple shoot induction either a combination of low NAA (0.1 mg/l) and high Kn (1.0 or 2.0 mg/l) concentration or various combinations of IBA and BAP in the MS medium were promising. Similarly, for the root induction, NAA at low concentration (0.1 mg/l) was found to be the best although its other higher concentrations also induced roots at significant levels. Agar

manipulation experiment for the induction of storage roots produced insignificant result. A preliminary study on bud induction from the shoots has shown very interesting results. This can be an alternative method of rapid multiplication of plants. Vitrification of shoots is one of the major problems in tissue culture and generally the vitrified shoots are discarded. We here, have used the vitrified shoots as explants and produced normal shoots using very low amount of cytokinin or auxin in the sub-culture medium. Sand rooting using NAA 100 mg/l pulse treatment, acclimatizing in a shade house on the coco-peat and transferring the acclimatized plantlets on the gaden soil in an open environment were the final steps. Another important and threatened medicinal plant *Rauvolfia serpentina* also was taken for the present research. Here, callus induction from the shoot and leaf explants were supported by the incorporation of 2,4-D or NAA above 1.0 mg/l and 0.5 mg/l singally in the MS medium respectively. Incombination of auxin and cytokinin, NAA 1.0 mg/l along with all concentrations of BAP yielded highly significant amount of callus especially from the shoots. The calli obtained from the leaf were rarely embryogenic as well as caulogenic. The shoot induced friable green calli were able to go for organogenesis as well as somatic embryogenesis. The multiple shoot induction was generally supported by a lower auxin and higher cytokinin concentrations. The maximum induction was observed on the MS medium incorporated with IBA 0.1 + BAP 2.0 mg/l with 7.83±1.01 shoots per explant. For the induction of roots from the shoots, NAA singly at almost all concentrations were found to be significant, however NAA o.5 mg/l induced the maximum average roots upto 12.50 per explants after 12 weeks of culture. IAA and IBA did induce some roots when alone but in combination with cytokinins their performances were very poor. Finally, the shoots rooted both *in vitro* and *in vivo* after pulse treatment with 100 mg/l auxin survived in the open environment after acclimatization in the shade house.

CONTENTS

List of Tables

List of Figures

ABBREVIATIONS AND ACRONYMS

˚C	Degree Celsius
µl	micro litre
µm	micro meter
2,4-D	2,4-Dichlorophenoxyacetic Acid
ANOVA	Analysis of Variance
BA	Benzyl Adenine
cm	centimetre
CTAB	Cetyl TrimethylAmmonium Bromide
cZ	cis Zeatin
DMRT	Duncan's Multiple range test
DNA	DeoxyriboNucleic Acid
Fig	Figure
FAO	Food and Agriculture Organisation
GA	Gibberellic Acid
M	molar
mM	milli molar
mm	milli meter
mRNA	messenger RiboNucleic Acid
MS	Murashige and Skoog
NAA	α-Naphthalene Acetic Acid
NPK	Nitrogen, Phosphorus, Kalium (Potassium)
PCR	Polymerase Chain Reaction
RNA	RiboNucleic Acid
tZ	trans Zeatin
UV	Ultraviolet