

**MORPHOGENETIC STUDIES ON DIFFERENT EXPLANTS OF *RAUVOLFIA
SERPENTINA* (L.) BENTH. EX. KURZ *IN-VITRO* AND ACCLIMATIZATION**

A DISSERTATION

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CERTIFICATE

This is to certify that the thesis entitled **Morphogenetic studies on different explants of *Rauvolfia serpentina* (L) Benth ex Kurz in vitro and Acclimatization**” submitted by **Mr. Basanta Malla** for the partial fulfillment of Master's Degree in Botany is based on the results of experiment carried out by him under my supervision. This thesis has not been previously submitted for any other degree.

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APPROVAL LETTER

The dissertation work submitted by **Mr. Basanta Malla** entitled “**Morphogenetic studies on different explants of *Rauvolfia serpentina* (L) Benth ex Kurz in vitro and Acclimatization**” has been accepted as a partial fulfillment of M.Sc. in Botany.

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ABSTRACT

Morphogenetic studies on different explants of *Rauvolfia serpentina* (L.) Benth. ex Kurz *in-vitro* and *in-vivo* were carried out. Explants were taken from *in-vitro* grown plants on MS media. Various vegetative parts like node, leaf, root and shoot-tip were cultured. The effect of growth hormones on morphogenesis was discussed.

The explants cultured on the MS media with 2,4-D (0.5-3 mg/l) developed into calli from cut part. The node, leaf and root started to develop calli from the cut part in the 7, 8 and 9 days of culture. The growth of calli was found better in the 0.5 mg/l 2, 4-D than in 3 mg/l 2, 4-D and the leaf explants gave more vigorous callus in primary culture than node and root explants. Further sub-culture of the root callus on the fresh media of 2, 4-D only increased the mass which turned brownish in 8 weeks of sub-culture. The 2, 4-D induced callus on sub-culture on BAP and NAA of various concentration differentiated roots only. The transfer of nodal callus on fresh media (MS+BAP+NAA in combination) differentiated shoots and roots.

Nodes were also cultured on MS medium supplemented with nine different combinations and concentrations of BAP and NAA. Maximum number of shoots was found in the MS+2 mg/l BAP (average 16.00 ± 1.10) and best shoot elongation was found in the MS+1.5 mg/l BAP (average 7.42 ± 0.62 cm) in the 8 weeks of culture. Similarly, the leaf explants were cultured on MS medium supplemented with nine different concentrations of BAP and NAA. Response was observed only in combination and 0.5 mg/l NAA singly. MS+ BAP (2 mg/l) + NAA (0.5 mg/l) gave plantlets from cut end while in other hormone concentrations green callus with red and white patches was produced which differentiated only roots.

Shoot tips cultured in MS+Kn (0-3 mg/l) was not effective to produce multiple shoots as compared to BAP. Shoot tips at basal part produced callus which differentiated shoots (1, 3 mg/l Kn)

6-week old *in-vitro* multiplied shoot without rooting was taken for *in-vivo* rooting. *In-vivo* rooting was done with or without treating the shoots with IAA (3 mg/l for 1 hour). 100% shoots were rooted in IAA treated shoots in the 3 weeks of plantation while 90% of shoots were rooted in the 4 weeks of plantation in shoots that were not treated with IAA.

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ABBREVIATIONS

±	:	Plus or minus
2, 4-D	:	2, 4 - Dichlorophenoxy acetic acid
BA	:	6-Benzyl Aminopurine
BAP	:	6-Benzyl Aminopurine
BM	:	Basal Medium (Murashige and Skoog's Medium 1962)
C.D.B.	:	Central Department of Botany
et al.	:	et alibi
GA ₃	:	Gibberellic Acid
IAA	:	Indole-3- Acetic Acid
IBA	:	Indole-3-Butyric Acid
Kn	:	Kinetin
Mg/l	:	Milligram Per liter
MS	:	Murashige and Skoog's Medium 1962
NAA	:	-Naphthalene-acetic Acid
ppm	:	Parts per million
Spp.	:	species
T.U.	:	Tribhuvan University
TD-Z	:	Thidiazuran