IN-VITRO MASS PROPAGATION OF *WITHANIA SOMNIFERA* (L.) DUNAL

A DISSERTATION
SUBMITTED TO CENTRAL DEPARTMENT OF BOTANY
FOR THE PARTIAL FULFILLMENT FOR THE REQUIREMENTS
OF M. Sc. DEGREE IN BOTANY

BY
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INSTITUTE OF SCIENCE AND TECHNOLOGY
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Nepal

CERTIFICATE

It is to certify that the research work entitled "***IN-VITRO* MASS PROPAGATION OF *WITHANIA SOMNIFERA* (L.) DUNAL"** submitted by **Mr. Durga Dutt Shukla** for the partial fulfillment of Master's 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 partial fulfillment of Master of Science in Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

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Date: December 5, 2007

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APPROVAL LETTER

The dissertation work submitted by **Mr. Durga Dutt Shukla** entitled "***IN-VITRO* MASS PROPAGATION OF *WITHANIA SOMNIFERA* (L.) DUNAL"** has been accepted as a partial fulfillment of Master's Degree in Botany.

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ABSTRACT

Withania somnifera (L.) Dunal (Ashwagandha), of the family Solanaceae is an important medicinal plant and a major source of alkaloid and steroid (withanolids), which is regularly used in pharmaceutical industries. The present study describes the procedure for micropropagation of *W. somnifera*. The morphogenetic responses of various vegetative parts (shoot-tip, node, root and leaf) were studied in MS medium supplemented with different concentrations and combinations of phytohormones.

Seeds were pretreated with GA_3 (50 and 100 mgl^{-1}) for 24 hours and 80% germination was achieved. All the explants were taken from *in-vitro* germinated plant. Among the different explants tested, multiple shoot formation was achieved from shoot-tip and nodal explants in MS + 0.25, 0.5, and 1.0 mgl^{-1} kinetin. Nodal explants were selected for mass propagation protocol because: it formed maximum number of shoots. Shoots were started to differentiate after one week of culture and after 8 weeks an average of 16.25 shoots/explant were formed on MS + 1 mgl^{-1} kinetin. Increase in concentration of kinetin was most effective for callus formation. For further multiplication of shoot, adventitious shoots with callus at the base were sub cultured on MS + 0.5 mgl^{-1} kinetin and multiple shoots (14.25 to 14.5 shoots/culture) were protruded out.

Auxins, (IAA, IBA and NAA) were supplemented in MS medium to induce rooting. Among these auxins, IAA at 0.5 mgl^{-1} was found to be the most effective concentration for rooting of *in-vitro* propagated shoots (50 roots/shoot). Rooted plantlets were transferred on the pot containing coco-pit, sand, sand-soil mixture (50% each) and coco-pit + garden soil mixture (50% each). Only few weeks of survival is recorded in last two combination of hardening medium.

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ABBREVIATIONS

BAP	:	6-Benzyleaminopurine
BM or MS	:	Basal medium (Murashig and Skoog medium 1962)
CDB	:	Central Department of Botany
Conc.	:	Concentration
GA_3	:	Gibberellic acid
IAA	:	Indol-3-Acetic acid
IBA	:	Indol-3-Butyric acid
Kin.	:	Kinetin (6-Furfurylamino Purine)
MAPs	:	Medicinal and Aromatic Plants
Mgl^{-1}	:	Milligram per Liter
NAA	:	Napthalene Acetic acid
No.	:	Number
NTFPs	:	Non Timber Forest products
ppm	:	Parts per million (mgl^{-1})
SD	:	Standard Deviation
Spp	:	Species
TU	:	Tribhuvan University
UV light	:	Ultra Violet Light