MICROPROPAGATION OF *CYMBIDIUM ELEGANS* LINDL. (ORCHIDACEAE).

A Dissertation submitted for the partial
fulfillment of the Requirements for
M.Sc. in Botany.

By
Januka Pathak
T.U. Regd. No. 5-2-50-1034-2001
Exam Roll No. 1116
Batch: 2061/63

Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu, Nepal
2008

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY
KIRTIPUR
KATHMANDU, NEPAL

Kirtipur, Kathmandu
Nepal

RECOMMENDATION

This is to certify that the dissertation work entitled "**Microprogation of *Cymbidium elegans* Lindl. (Orchidaceae)**" submitted by **Ms. Januka Pathak** for the partial fulfillment of the M.Sc. Degree in Botany under my supervision. The result of this work has not been submitted for any other degree.

I therefore, recommend this dissertation to be accepted for the partial fulfillment for Master of Science in Botany with specialization in Biotechnology Degree in Tribhuvan University, Kirtipur, Nepal.

Date: 14th January, 2008

.....

Dr. Bijaya Pant
Supervisor
Assistant Professor
Central Department of Botany
Tribhuvan University, Kirtipur, Kathmandu

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY
KIRTIPUR
KATHMANDU, NEPAL

Kirtipur, Kathmandu
Nepal

APPROVAL LETTER

The dissertation work submitted by Ms. Januka Pathak entitled "**Microprogation of *Cymbidium elegans* Lindl. (Orchidaceae)**" has been accepted as a partial fulfillment of Master of Science in Botany.

EXPERT COMMITTEE

.....	.....
Research Supervisor	Head
Dr. Bijaya Pant	Prof. Dr. Krishna Kumar Shrestha
Central Department of Botany	Central Department of Botany
T.U., Kathmandu	T.U., Kathmandu
.....	.....
Internal Examiner	External Examiner
Prof. Dr. Sanu Devi Joshi	Dr. Mukunda Ranjit
Central Department of Botany	Academician NAST

T.U., Kathmandu Executive Chairman, GREAT Nepal

Date of Examination: 29th Feb, 2008

ACKNOWLEDGEMENTS

I express my sincere thanks and gratitude to my supervisor Dr. Bijaya Pant for her constant guidance and encouragements during the research period.

I wish to express my sincere gratitude to Prof. Dr. Krishna Kumar Shrestha, Head of Central Department of Botany, and Prof. Dr. Pramod Kumar Jha, Former Head of the Central Department of Botany for providing the necessary library and laboratory facilities throughout this research work.

I am grateful to Prof. Dr. Sanu Devi Joshi and Prof. Dr. Vimal Narayan Prasad Gupta for their valuable suggestions.

I express my courteous gratitude to Mrs. Banu Ojha, the Director of Cornell Nepal Study Programme (CNSP), Kirtipur for providing me partial financial support during the research work. I am thankful to all teaching and non teaching staff of Central Department of Botany, T.U. Kirtipur.

I am thankful to my senior Miss Shreeti Pradhan for the research material from CDB. I am also thankful to my senior Mr. Nabin Bhattarai and Mr. Dharma Raj Koirala for statistical analysis. I am thankful to my friends Basanta Malla, Deepak Mahat, Ravi Kumar Poudel, Sarala Sitaula, Sarmila Gurung, Tara Adhikari, Umesh Shrestha, Vivek Poudel, Trilok Nath Thakur and all others who help me during the research work.

I would like to express my sincere thanks to my parents and all relatives for their moral and financial support and encouragement during the research work.

Lastly, I am thankful to the Proprietor, Vikram Maharjan & Sanish Maharjan for typing this thesis work in this Skill Promotion Centre.

Januka Pathak

ABSTRACT

Cymbidium elegans Lindl. is a special orchid because of its long lasting beautiful colour range characteristics and especially used for horticultural purposes. It is listed as rare and critically Endangered Species (CITES, Appendix II).

The present study was carried out to find out the *in vitro* mass propagation of *C. elegans* Lindl. by using MS (Murashinge and Skoog medium 1962) and MS medium supplemented with growth regulators. *In vitro* development and differentiation of single protocorm like bodies (PLBs) was studied either on hormone free medium or on MS medium supplemented with growth hormones. MS medium supplemented with BAP (1 mg/l) and NAA (0.5 mg/l) was most ideal condition for multiplication of plantlets from single protocorm segment. Callus induction was vigorous from PLBs segment on MS medium supplemented with BAP (1.5 mg/l). The shoot tip and root tip explants obtained from *in vitro* grown seedling of *C. elegans* Lindl. was cultured in MS basal media and MS medium supplemented with various combinations of BAP and NAA. The maximum number of healthy shoots was reported in MS medium supplemented with BAP (1 mg/l) and NAA (0.5 mg/l) i.e. 6.75 shoots /culture within 16 weeks of culture of shoot tip explant.

In the investigation of the organogenesis from root tips, it was found that MS medium supplemented with BAP (1 mg/l) was appropriate medium for the regeneration from root tip culture, i.e. 3 shoots/culture within 24 weeks of culture of root tip explant. For rooting of *in vitro* multipled shoots, MS medium supplemented with IBA, IAA and NAA in the range of 0.5 mg/l to 2 mg/l in concentration were used. MS medium supplemented with IBA (0.5 mg/l) was most ideal condition for the development of maximum number of healthy roots i.e 3.25 roots/culture within 12 weeks of culture of rooting of shoot explant. *In vitro* propagated plantlets were subjected to acclimatization in clay pot containing cocopeat and moss.

CONTENTS

LIST OF TABLES

Page No.

ABBREVIATIONS

%	=	Percent
«	=	Micron
BA	=	6 – Benzyladenine
BAP	=	6 – Benzylaminopurine
BM	=	Basal Media
CDB	=	Central Department of Botany
et al.	=	et alebi
EDTA	=	Ethylene Diamine Tetra Acetate
Fig.	=	Figure
G_5	=	Gamborg $_5$
GN	=	Government of Nepal
IAA	=	Indole – 3 – Acetic Acid
IBA	=	Indole – 3 – Butyric Acid
KnC	=	Knudson Medium
KN	=	Kinetin
MS	=	Murashige and Skoog (1962)
NAA	=	~ - Napthalene Acetic Acid
PPM	=	Parts Per Million
PLBs	=	Protocorm Like Bodies
S.D.	=	Standard Deviation
SPSS	=	Statistical Package for Social Science
T.U.	=	Tribhuvan University