

***EX SITU* CONSERVATION OF TWO ORCHID SPECIES VIZ.
CYMBIDIUM ELEGANS LINDLEY. AND *DENDROBIUM*
DENSIFLORUM LINDL. BY TISSUE CULTURE TECHNIQUE**

A DISSERTATION

**SUBMITTED TO INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY, KATHMANDU, NEPAL
IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR
MASTER OF SCIENCE IN BOTANY**

BY

SHREETI PRADHAN

ROLL NO. 28/(060-062)

REG. NO. : 5-2-50-1939-2000

SYMBOL NO. : 659

**CENTRAL DEPARTMENT OF BOTANY
TRIBHUVAN UNIVERSITY, KIRTIPUR**

2006



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

Ref. No.

CERTIFICATE

This is to certify that the dissertation work entitled "***Ex situ conservation of two orchid species viz. Cymbidium elegans Lindley. and Dendrobium densiflorum Lindl. by tissue culture technique***" submitted by Shreeti Pradhan for the partial fulfillment of M. Sc. in Botany, has been carried out under my supervision. The entire work is based on the results of her own work and has not been submitted for any other degree to the best of my knowledge. I recommend this dissertation work to be accepted as a requirement for the partial fulfillment of M. Sc. in Botany, Tribhuvan University, Kirtipur, Nepal.

Dr. Bijaya Pant

Assistant Lecturer

Central Department of Botany

Tribhuvan University

Date: 21st Dec, 2006



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

Ref. No.

APPROVAL LETTER

The dissertation work submitted by **Miss Shreeti Pradhan** entitled "**Ex Situ Conservation of two Orchid Species Viz. Cymbidium elegans Lindley. and Dendrobium densiflorum Lindl. by Tissue Culture Technique**" has been accepted as a partial fulfillment of M.Sc. in Botany.

EXPERT COMMITTEE

Research Supervisor
Dr. Bijaya Pant
Central Department of Botany
Tribhuvan University

Head
Prof. Dr. P.K Jha
Central Department of Botany
Tribhuvan University

Internal Examiner
Prof. Dr. Sanu Devi Joshi
Central Department of Botany
Tribhuvan University

External Examiner
Dr. Belai Meeta Singh
Patan Multiple Campus
Lalitpur, Nepal

Date of Examination: 14th Feb, 2007

Email:- botany @ wlink.com. np, Tel: 977-1-4331322, Fax: 977-1-4331964/ 977-1-4333525

ABSTRACT

Cymbidium elegans Lindley. and *Dendrobium densiflorum* Lindl. are two important orchids especially used for horticultural purposes. Both of these species are listed as rare and critically endangered (CITES, Appendix II) For the conservation and propagation of these species, the present study was conducted to determine the germination of both the species and *in vitro* mass propagation of *D. densiflorum* Lindl. by using MS medium (Murashige and Skoog medium, 1962) and MS medium supplemented with different growth regulators. MS medium supplemented with BAP 1 mg/l was the most effective for *in vitro* seed germination of *C. elegans* Lindley. in which germination started after 9 weeks of inoculation whereas the MS basal medium was most effective for *in vitro* seed germination of *D. densiflorum* Lindl. in which germination was started after 5 weeks of inoculation. Shoot tip and root tip explants obtained from *in vitro* grown seedling of *D. densiflorum* Lindl. was cultured in MS basal medium and MS supplemented with various combinations of BAP and NAA. The maximum number of healthy shoots was observed in MS + BAP 2 mg/l + NAA 0.5 mg/l medium (4.0 shoots/culture). The appropriate medium for root tip development was found to be MS + BAP 1.5 mg/l (7.25 shoots/culture). For rooting, shoot tip explants from *in vitro* multiplied shoots of *D. densiflorum* Lindl. were rooted by using different concentrations of IAA, IBA and NAA. The rooting was observed after 10 weeks of culture of shoot tips. MS + IBA 1.5 mg/l (4.5 roots/culture) was found to be more effective for maximum number and enlargement of roots. The *in vitro* propagated plantlets were subjected for acclimatization.

CONTENTS

	Page
1. INTRODUCTION	1-6
1.1 Background	1
1.2 Objectives	6
1.3 Justification	6
2. LITERATURE REVIEW	7-20
3. MATERIALS AND METHODS	21-29
3.1 Materials	21
3.2 Methodology	21
3.2.1 Methods for seed germination	21
3.2.1.1 Preparation of stock solution	21
3.2.1.2 Hormones used for the experiments	24
3.2.1.3 Preparation of hormones	24
3.2.1.4 Sterilization of Glassware's and Metal instruments	24
3.2.1.5 Preparation of Media	25
3.2.1.6 Sterilization of plant material	26
3.3 Inoculation of seeds of <i>D. densiflorum</i> and <i>C. elegans</i>	26
3.4 Histomorphological study of <i>D. densiflorum</i>	27
3.5 Inoculation of explant of <i>D. densiflorum</i>	27
3.6 Shoot Multiplication	27
3.7 Rooting of Shoots	27
3.8 Methods of Acclimatization	28
3.9 Statistical Analysis	29
4. RESULT	30-60
4.1 <i>In vitro</i> culture of seeds <i>Cymbidium elegans</i> Lindley. and <i>Dendrobium densiflorum</i> Lindl.	30
4.1.1 <i>In vitro</i> germination of seeds	32

4.2	Histomorphological Study of <i>Dendrobium densiflorum</i> Lindl.	37
4.3	Micropropagation of <i>Dendrobium densiflorum</i> Lindl.	38
4.3.1	Culture of Shoot tip of <i>D. densiflorum</i> Lindl.	40
4.3.1.1	Development of Shoot tip explant of <i>D. densiflorum</i> Lindl.	41
4.3.1.2	Shoot Multiplication	45
4.3.2	Culture of Root tips of <i>Dendrobium densiflorum</i> Lindl.	48
4.3.2.1	Development of Rhoot tip explant of <i>D. densiflorum</i> Lindl.	51
4.4	Rooting of Shoots of <i>Dendrobium densiflorum</i> Lindl.	55
4.5	Acclimatization	59
5.	DISCUSSION	61-66
5.1	<i>In vitro</i> seed germination <i>Cymbidium elegans</i> Lindley. and <i>Dendrobium densiflorum</i> Lindl.	61
5.2	Shoot Tip Culture of <i>Dendrobium densiflorum</i> Lindl.	63
5.3	Root Tip Culture of <i>Dendrobium densiflorum</i> Lindl.	64
5.4	Rooting of Shoots of <i>Dendrobium densiflorum</i> Lindl.	65
6.	CONCLUSION	67
7.	RECOMMENDATION	68
8.	REFERENCES	69-80
APPENDIX		

LIST OF TABLES

	Page
Table 1 : Effect of growth regulators supplemented in MS media on seed germination and seedling growth of <i>Cymbidium elegans</i> Lindley.	31
Table 2 : Effect of growth regulators supplemented in MS media on seed germination and seedling growth of <i>Dendrobium densiflorum</i> Lindl.	31
Table 3 : Effect of BAP and NAA on shoot tip culture of <i>Dendrobium densiflorum</i> Lindl.	40
Table 4: Effect of BAP and NAA on root tip culture of <i>Dendrobium densiflorum</i> Lindl.	49

LIST OF DIAGRAM

	Page No.
Diagram 1 : Average number of shoot after 12 weeks of culture of shoot tip of <i>Dendrobium densiflorum</i> Lindl.	46
Diagram 2 : Average shoot growth after 12 weeks of culture of shoot tip of <i>D. densiflorum</i> Lindl.	46
Diagram 3 : Average number of leaves after 12 weeks of culture of shoot tip of <i>D. densiflorum</i> Lindl.	47
Diagram 4 : Average number of root after 12 weeks of culture of shoot tip of <i>D. densiflorum</i> Lindl.	47
Diagram 5 : Average number of shoot after 24 weeks of culture of root tip explant of <i>D. densiflorum</i> Lindl.	50
Diagram 6 : Average number of root after 24 weeks of culture of root tip explant of <i>D. densiflorum</i> Lindl.	50
Diagram 7 : Average number of root after 10 weeks of rooting of shoot tip of <i>D. densiflorum</i> Lindl	56
Diagram 8 : Average length of root after 10 weeks of rooting of shoot tip of <i>D. densiflorum</i> Lindl.	56

ACRONYMS AND ABBREVIATIONS

ANOVA	-	Analysis of Variance
B ₅	-	Gamborg's Medium
BA	-	Benzyl Adenine
BAP	-	6-Benzyl Amino Purine
BM	-	Basal Medium
CDB	-	Central Department of Botany
CITES	-	Convention on International Trade In Endangered Species of Wild Fauna and Flora
EDTA	-	Ethylene Diamino Tetra Acetate
IAA	-	Indole -3-Acetic Acid
IBA	-	Indole -3-Butyric Acid
ICIMOD	-	International Center for Integrated Mountain Development
Kn C	-	Knudson Medium
KN	-	Kinetin
MS	-	Murashige and Skoog (1962)
NAA	-	α -Naphthalene Acetic Acid
PLBs	-	Protocorm like bodies
S.D.	-	Standard Deviation