

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

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

By

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RECOMMENDATION

This is to certify that the dissertation work entitled "**Micropropagation 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.

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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 (Murashige 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* multiplied 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.

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ABBREVIATIONS

%	=	Percent
~	=	Micron
BA	=	6 – Benzyladenine
BAP	=	6 – Benzylaminopurine
BM	=	Basal Media
CDB	=	Central Department of Botany
<i>et al.</i>	=	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	=	γ - 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