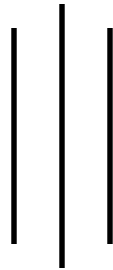


IN VITRO SEED GERMINATION OF CYMBIDIUM  
ALOIFOLIUM (L.) Sw. AND MICROPROPAGATION OF  
COELOGYNE FUSCESCENS Lindl. BY TISSUE CULTURE  
TECHNIQUE.



A Dissertation Submitted for the Partial Fulfillment of  
the Requirements for M.Sc. in Botany



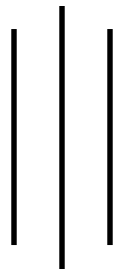
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2007



**TRIBHUVAN UNIVERSITY  
INSTITUTE OF SCIENCE AND TECHNOLOGY  
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Kirtipur, Kathmadu  
Nepal

**APPROVAL LETTER**

This dissertation work entitled “*In vitro* seed germination of *Cymbidium aloifolium* (L.) Sw. and micropropagation of *Coelogyne fuscescens* Lindl. by tissue culture technique.” has been accepted for partial fulfillment of master's Degree in Botany.

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.....  
Dharma Raj Koirala (2007)

## ABSTRACT

The present study was carried out to find out the effect of growth hormones on seed germination of two Orchid species viz. *Coelogyne fuscescens* Lindl. and *Cymbidium aloifolium* (L.) Sw. and *in vitro* propagation of *C. fuscescens* Lindl.

The seeds of both orchid species cultured in different concentrations of BAP (0.5 mg/l - 2mg/l) and NAA (0.5 mg/l), singly and in combination showed different responses on germination and seedling development.

MS medium supplemented with BAP (1 mg/l) and NAA (0.5 mg/l) was found to be most favourable for the germination of *C. fuscescens* Lindl. Similarly MS medium supplemented with BAP (2 mg/l) plus NAA (0.5 mg/l) was found to be the most effective for the germination of *C. aloifolium* (L.) Sw.

For shoot multiplication, BAP (0.5 mg/l –2 mg/l ) and NAA (0.5 mg/l) were used singly as well as in combination. Highest number of healthy shoots (6 shoots/culture) was obtained in the combined concentration of BAP (1.5 mg/l) plus NAA (0.5 mg/l). To investigate the organogenesis from root tips, media with similar hormonal concentration were used. BAP (1 mg/l) was found to be the most favourable for the shoot multiplication. Two and one half shoots/culture were observed in this condition. For rooting of *in vitro* multiplied shoots, 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 (1 mg/l ) with 3.5 roots per culture, was found to be most favourable for rooting. To find out the combined effect of two rooting hormones namely IAA and NAA on rooting of shoot tips MS media supplemented with 0.5 mg/l to 1.5 mg/l of IAA and 0.5 mg/l to 1 mg/l of NAA in combination were used. Maximum rooting with healthy roots (2.75 roots/culture) were observed in MS plus IAA (0.5 mg/l) plus NAA (0.5 mg/l). The *in vitro* developed shoots were acclimatized in clay pot containing coco-peat and club moss.

This protocol may be helpful in conserving highly threatened orchid species via tissue culture.

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## APPENDIX

## ABBREVIATIONS

- BAP - 6 – Benzylaminopurine
- BM - Basal Media
- CDB - Central Department of Botany.
- EDTA - Ethylene Diamino Tetra Acetate.
- G<sub>5</sub> - Gamborg<sub>5</sub>
- IAA - Indole-3-Acetic Acid.
- IBA - Indole-3-Butyric Acid.
- mg/l - milligram per litre.
- MS - Murashige and Skoog.
- NAA - Napthalene Acetic Acid
- ppm - Parts Per Million
- Plbs - Protocorms like bodies.
- SPSS - Statistical Package for Social Sciences.
- T.U. - Tribhuvan University.