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IN VITRO ASYMBIOTIC SEED GERMINATION OF *DENDROBIUM DENSIFLORUM* LINDL

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Abstract

D. densiflorum seeds germinated on all the four nutrient media tried. Maximum seed germination (92.1%) was however, recorded on M medium followed by MS (90.4%) and NN (87.1%) media. The percentage of germination was considerably reduced on KC medium (5.7%). Developmental stages I, II, III, and IV also varied with time on different media tried. Stage IV was obtained on M, MS, and NN media. The germinating entities on KC media failed to develop beyond protocorm stage and subsequently turned brown and died. The development of complete plantlets was observed only on M & MS media.

Introduction

ORCHIDS ARE the most beautiful among the flowering plants and are favourites in horticulture; many genera are cultivated. The North-East Indian region is a rich reservoir for rare, ornamental, and threatened orchids but the natural orchid populations are fast depleting due to ruthless exploitation for commerce and ever increasing deforestation. Consequently, some species are extremely scarce or have already been lost through extinction, whereas several species are endangered of survival. *Dendrobium densiflorum* Lindl. is one such species of rare and threatened orchids in the region (Kataki *et al.*, 1984). With a view to save its natural populations from commercial collection pressures, the posibility of its large scale multiplication was assessed *in vitro* using seeds.

Material and Methods

About eleven-months old capsules of *D. densiflorum* were collected from the plants grown in the glasshouse of the Department of Botany, North-Eastern Hill University,Shillong. These were washed thoroughly with tap water using a mild detergent as a wetting agent, and surface-disinfected with 70 % ethanol for 30 sec prior to flame sterilization. The capsules thus prepared were split-opened using a sterilized surgical blade. The seeds scooped out therefrom were inoculated on the culture medium in 25 x 150 mm glass test tubes each containing 20 ml of medium. Knudson C (KC, 1946), Murashige and Skoog (MS, 1962), Nitsch (NN, 1969), and Mitra *et al.* (M, 1976) media were used as source of nutrition. The medium pH was adjusted at 5.8 prior to autoclaving. The culture tubes were incubated at 25±2°C under 16h photoperiod of 150 μmol m^{-2} s^{-1} light intensity. Ten replicates were used for each treatment and all the experiments were caried out under aseptic conditions. The cultures were examined regularly every wk. The seeds were considered to have germinated upon emergence of their embryos from the testa. The following 5 different developmental stages were distinguished:

Stage I- Non- germinated seeds, embryo slightly swollen but still covered with its seed coat or testa

Stage II- Germinating seeds, embryo greatly swollen forming an ovoid tear-drop shaped protocorms without seed coat or testa

Stage III- Young protocorms showing pointed vegetative apex

Stage IV- Seedlings with leaves

Stage V- Seedlings with roots

Results and Discussion

The seeds of *D. densiflorum*, germinated on all the four nutrient media, were tried. Maximum seed germination (92.1%) was recorded on M medium and it was followed by that in MS (90.4%) and NN (87.1%) media (Table 1). The germination was significantly impaired on KC medium (5.7%). The time taken to attain different developmental stages and the growth behaviour of protocorms varied with the chemical stimulus in the nutrient mix (Fig. 1). The minimum time of two wks was required to attain stage I in M and and KC; 3 wks were needed for the purpose in MS and NN media. Further,

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development to stage II was faster in M and MS medium which took only a wk. The transition period from stage II to stage III was also less (3 wks) on M and MS medium.

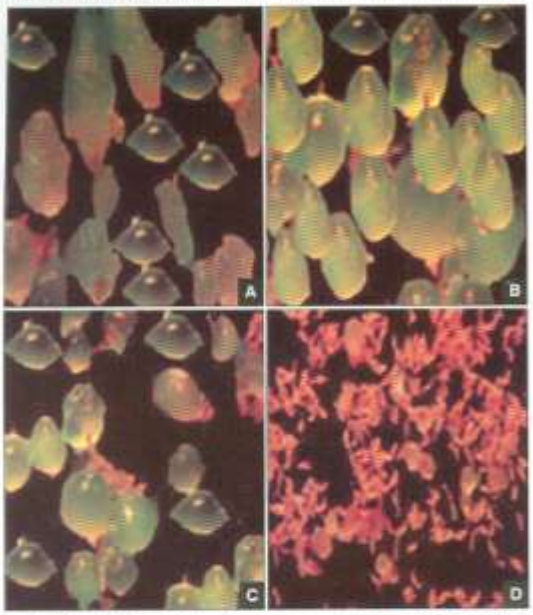


Fig. 1. A-D. Effect of different media on protocorm development in *Dendrobium densiflorum*. A, M medium; B, M S Medium; C, N & N medium; D, KC medium.

Table1. Effect of different basal media on seed germination and growth of protocorms of *D. densiflorum*

Medium	Germination (%)*
M	92.1 ± 3.2
MS	90.4 ± 4.4
NN	87.1± 3.2
KC	5.7 ± 0.28

Mean ± SE * Data recorded after 2 months, n = 10

This was attained in 3 wks time; this period was however delayed to 4 wks on NN medium. However, in KC medium, stage II and III was not attained at all. Here, the germinating entitity failed to grow beyond protocorm stage; these turned brown after 5 wks of culture and subsequently died. Out of all the four media tried, stage IV was obtained only on M, MS, and NN media. Here also, the transitional period was faster on M and MS media, which took 3 wks. The development of complete plantlets with shoots and roots from the protocorms was observed on M and MS media only (Table 2; Graph,1; Figs. 2-3).

A perusal of literature revealed that the orchid seeds respond differently to different nutrient media for germination (Pathak, *et al*., 2001; Vij *et al*., 1995). They require a balanced supply of both organic and inorganic nutrients (Arditti, 1982; VanWaes and Debergh, 1986). Complete plantlets of *Dendrobium densiflorum* were obtained on M and MS media. M medium also proved more effective for inducing early and better germination of seeds. The seeds require a nutrient rich medium which is present in M medium that contains riboflavin, biotin, folic acid, sodium hydrogen phosphate, calcium nitrate, and ammonium sulphate essential for better germination and protocorm development. The efficiency of M medium during seed germination has already been indicated by many workers (Pathak *et al*., 2001; Seeni and Latha, 2000; Vij *et al*., 1995). Seed germination as well as protocorm growth and development were better on MS medium. Higher concentration of nitrogen (60.05mM) *i.e.* ammonium nitrate and potassium nitrate, present in MS medium was required for the optimal germination of seeds. A better efficacy of MS medium in maintaining healthy growth of the seedlings may also be attributed to their Fe-EDTA contents, a growth promotory nature of Fe-EDTA is already available on record (Lee *et al*., 1983). The nutrient requirement of orchid seeds in terms of quality may vary at different

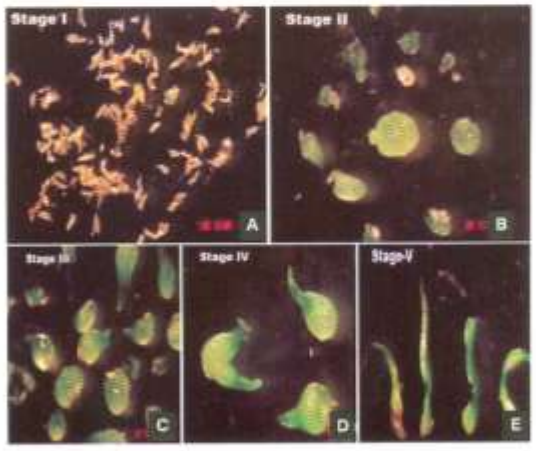


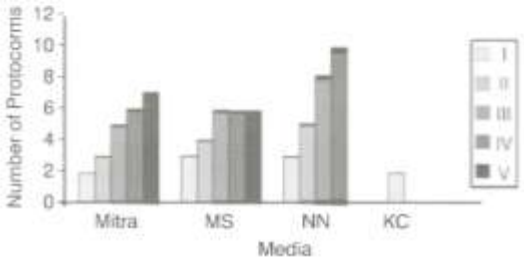
Fig. 2 A-E. Different developmental stages of *Dendrobium densiflorum* in Mitra *et al*. medium: A, Non-germinating seeds after 2 wks; B, Germinating seeds after 3 wks; C, Young protocorms with pointed vegetative apex after 6 wks; D, Protocorms with leaves after 9 wks; E, Development of shoot and root after 13 wks.

stages of development (Arditti and Ernst, 1984). The importance of ammonium or nitrate ions (individually or in combination) during the *in vitro* germination of orchid seeds as a source of nitrogen is well established (Curtis and Spoerl, 1948; Withner, 1959). The seeds require biotin and folic acid for better germination which

Table 2. Effect of different basal media on the developmental stages of protocorms of *D. densiflorum*

Nutrient Medium	Developmental stages (wks)					Remarks
	I	II	III	IV	V	
M	2	3	5	6	7	Protocorms differentiated into healthy shoots and roots.
MS	3	4	6	6	6	Protocorms differentiated into complete plantlets developed into shoots and roots.
NN	3	5	8	10	-	Protocorms were differentiated up to shoot stage.
KC	2	-	-	-	-	Only swelling of seeds; they did not germinate, turned yellow and died.

are essentially present in NN medium. Protocorms of *D. densiflorum*, however, differentiated only upto shoot stage on NN medium. Roots were not developed in



Graph 1. Effect of different basal media on the protocorm formation in *D. densiflorum*.

this medium which conforms to the findings of Nath *et al.* (1991), who reported that the growth of *Vanda*

Fig. 3 A-E. Different developmental stages of *Dendrobium densiflorum* in MS medium: A, Non-germinating seeds after 2 wks; B, Germinating seeds after 3 wks; C, Young protocorms with pointed vegetative apex after 6 wks; D, Protocorms with leaves after 9 wks; E, Development of shoot and root after 13 wks.

coerulea seedlings remained arrested prior to rooting in NN medium. In our study, the protocorms turned yellowish brown and perished in KC medium in accord with similar earlier findings (Chaturvedi *et al.*, 1987; Nath *et al.*, 1991). The orchid seeds have been reported to show both promotary as well as inhibitory response for germination on KC medium (Oliva and Arditti, 1984; Pyati and Murthy, 1995). Although there are some reports of orchid seed germination in KC medium (Bopiah and Jorapur, 1986; Pyati and Murthy, 1995; Sharma and Tandon, 1987), several Indian orchids including species of *Cymbidium* and *Dendrobium* are reported to have germinated poorly on KC medium. Chaturvedi *et al.* (1987) and Yam and Weatherhead (1989) reported that the nutritional requirement of germinating orchids vary due to their physiological state. Healthy growth of orchid protocorms in medium containing balanced supply of organic and inorganic nutrients has been reported by some workers (Arditti and Ernst, 1984). Initiation of seed germination, protocorm development and subsequent growth and development of seedlings seems to vary with the species and the medium employed.

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References

Arditti, J. 1982. *Orchid Biology: Reviews and Perspectives* II. Cornell. University. Press, Ithaca, New York, US.

Arditti, J. and R. Ernst. 1984. Physiology of germinating orchid seeds. *In: Orchid Biology, review and perspectives* III. (ed. J. Arditti) pp. 177-222. Cornell University Press, Ithaca, New York, US.

Bopaiah, A. K. and S. M. Jorapur 1986. Studies on growth and development of *Cymbidium aloifolium* Sw. seedling *In vitro*. In: *Biology, Conservation, and Culture of Orchids*. (ed. S. P Vij) pp. 423-27. Affiliated East West Press. New Delhi, India.

Chaturvedi, H. C., A. K. Sharma, R. N. Prasad, and M. C. Sharma. 1987. Plant tissue culture of some ornamentals. *In: Proc. National Seminar on Plant Tissue Culture*. pp. 76-90. ICAR, New Delhi, India.

Curtis, J. T. and E. Spoerl. 1948. Studies on the nitrogen nutrition of orchid embryos. II. Comparative utilization of nitrate and ammonium nitrogen. *Am. Orchid Soc. Bull.*, **17**: 111-14.

Kataki, S. K., S. K. Jain, and A. R. Sastry. 1984. *Threatened and Endemic Orchids of Sikkim and North East India*. Botanical Survey of India. Howrah, India.

Knudson, C. 1946. A new nutrient solution for the germination of orchid seeds. *Am. Orch. Soc. Bull.*, **15**: 214-17.

Lee, H. S., K. Y. Peak, and J. K. Lee. 1983. Studies on the non symbiotic germination of seeds of *Laelia briegeri*. II. Effect of anionic and cationic composition, nitrogen and iron nutrition on the growth of seedling *In vitro*. *J. Korean Soc. Hort. Sci.*, **24**: 169-74.

Mitra, G. C., R. N. Prasad, and A. R. Chowdhury. 1976. Inorganic salts and differentiation of protocorms in seed callus of an orchid (*Dendrobium fimbriatum*) and correlated changes in its free amino acid content. *Ind. J. Exp. Biol.*, **14**: 350-51.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, **15**: 473-97.

Nath, M., J. Devi, B. Borthakur, J. Sharma, and P. C. Deka. 1991. Embryo culture of *Rhynchostylis retusa* and *Vanda coerulea*. *J. Orchid Soc. India*, **5**: 97-101.

Nitsch, J. P. 1969. Experimental androgenesis in *Nicotiana*: *Phytomorpholgy*, **19**: 389-404.

Oliva, A. P. and J. Arditti. 1984. Seed germination of North-American Orchids II. Native California and related species of *Aplectrum*, *Cypripedium*, and *Spiranthes*. *Bot. Gaz.*, **145**: 495-501.

Pathak Promila., K. C. Mahant, and Ashish Gupta. 2001. *In vitro* propagation as an aid to conservation and commercialization of Indian orchids: Seed culture. *In: Orchids: Science and Commerce* (eds. Promila Pathak, R.N. Sehgal, M. Sharma, and A. Sood) pp. 319-62. Bishen Singh Mahendra pal Singh, Dehra Dun, India.

Pyati, A. N. and H. N. Murthy. 1995. *In vitro* seed germination and seedling development of *Dendrobium ovatum* (W) Krunzl. *J. Orchid Soc. India*, **9**(1&2): 69-74.

Seeni, S. and P. G. Latha. 2000. *In vitro* multiplication and ecorehabilitation of the endangered Blue *Vanda*. *Plant Cell Tiss. Organ Cult.*, **61**: 1- 8.

Sharma, S. K. and P. Tandon. 1986. Influence of growth regulators on asymbiotic germination and early seedling development of *Coelogyne punctulata* Lindl. *In: Biology, Conservation, and Culture of Orchids* (ed. S. P. Vij) pp. 441-51. Affiliated East-West Press, New Delhi, India.

Sharma, S. K. and P. Tandon. 1987. Axenic germination of some epiphytic orchid of Meghalaya, India. *J. Orchid Soc. India*, **1(1&2)**: 85-90.

Van Waes, J. M. and P. C. Debergh. 1986. *In vitro* germination of some Western European orchids. *Physiol. Plant.*, **67**: 253-61.

Vij, S. P., Promila Pathak, and K. C. Mahant. 1995. Green-pod culture of a therapeutically important species *Dactylorhiza hatagirea* (D. Don) Soo. *J. Orchid Soc. India*, **9** (1-2): 7-12.

Withner, C. L. 1959. The Orchids physiology. *In: The Orchids: A Scientific Survey* (ed. C. L. Withner) pp. 351-60. Ronald Press, New York, US.

Yam, T. W. and M. A. Weatherhead. 1989. Germination and seedling development of some Hong Kong orchids. *Lindleyana*, **3**:156-60.