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Short communication

In vitro seed germination in *Cymbidium elegans* Lindl. and *Dendrobium densiflorum* Lindl. ex Wall. (Orchidaceae)

Shreeti Pradhan and Bijaya Pant

Biotechnology Unit, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal; email: shreeti_prd@yahoo.com, bpant@wlink.com.np

Abstract

A comparative study of *in vitro* seed germination of two endangered orchid species, *viz*. *Cymbidium elegans* Lindl. and *Dendrobium densiflorum* Lindl. ex Wall., was carried out on Murashige and Skoog's (MS) medium, supplemented with different concentrations and combination of 6-benzylaminopurine (BAP) and á-Naphthalene acetic acid (NAA). The hormone-free MS medium and MS medium supplemented with various growth hormones were found effective for *in vitro* seed germination of both species. However, the seeds of these two species showed variation in their germination behavior. Hormone-free MS basal medium was found most effective for seed germination of *D. densiflorum*; whereas, basal medium supplemented with BAP (1mg/l) was effective for *C. elegans*. The seeds of *D. densiflorum* showed quick response in earlier germination, protocorm formation and further development into seedlings in comparison to *C. elegans.* In *C. elegans*, germination of immature seeds started after nine weeks of inoculation; whereas in *D. densiflorum,* the initiation of germination started after five weeks of culture. The variations in seed germination, protocorm formation and seedling differentiation in the two orchid species might be due to the differences in their genetic constitution and the presence of different endogenous growth stimulating substances present in their seeds. The present study has provided useful information for *in vitro* clonal mass multiplication of these commercially important orchid species.

Key-words: growth hormone, *in vitro* study, orchid.

Introduction

Cymbidium elegans Lindl. and *Dendrobium densiflorum* Lindl. ex Wall. are important orchid species well known for their horticultural importance as they possess marvelously showy and long-lasting flowers. Therefore, demand of these orchid species is high among local growers for commercial trade. Their exceeding ornamental value and over-exploitation make them a highly threatened species.

Under natural conditions, the seeds of orchid have only 5% germination because of particular fungal requirement (Rao 1997). Vegetative propagation is very slow to produce a large quantity of clone orchids. Hence, tissue culture is the alternative for mass scale propagation and conservation of rare, endangered and threatened orchids. This technique can reduce the length of time required for introduction and conservation of new varieties. The use of exogenous growth hormones stimulates the zygotic embryo to initiate protocorms that develop into plantlets (Pant and Gurung 2005). The present paper highlights the comparative study of the effect of growth hormones on *in vitro* seed germination of *Cymbidium elegans* and *Dendrobium densiflorum*.

Materials and Methods

Immature capsules of *Cymbidium elegans* and *Dendrobium densiflorum* were collected from Conservation Demonstration Garden of ICIMOD (International Centre for Integrated Mountain Development), Lalitpur, Nepal.

Murashige and Skoog's (MS) medium was used as the basal medium for this investigation in combination with different concentrations of growth hormones [6-benzylaminopurine (BAP) and á-Naphthalene acetic acid (NAA)] alone or their combinations. The medium was supplemented with 3% sucrose and solidified with 0.8% agar. The pH of the medium was adjusted to 5.8 before autoclaving. The medium was autoclaved at 15 lb/in^2 for 15 minute.

Before inoculation of seeds, the undehisced capsules were sterilized by using 70% ethyl alcohol (dipped for 2 minutes) and 1% sodium hypochlorite solution (dipped for 15 minutes). Finally, the capsules were rinsed for 5 times with sterile water. The capsules were cut longitudinally into two equal halves and the seeds were inoculated on MS medium alone and in combination with different concentrations and combinations of phytohormones. The cultures were incubated at 25ºC ± 2ºC and subjected to 16 h photoperiods. The observations were taken at regular intervals of one week.

Results and Discussion

Germination of orchid seeds is different from other seeds. Orchid seeds are produced in large number within a capsule or pod. The seeds are very minute, contain undifferentiated embryo and lack endosperm. In certain orchids, self-pollination is not possible and even if possible as in the case of vanda, one has to wait for 4-6 months for pod development (Fitch 1981). Green pod culture, as against mature/dehisced pod culture, is desirable to save time and

to avoid contamination. In the present investigation, immature green pods were taken for *in vitro* culture. Due to non-endospermic nature of seed, the germination in nature is a unique phenomenon and requires fungal infection. Germination is much more successful *in vitro*. The hormone-free MS medium and MS medium supplemented with various growth hormones were found effective for the *in vitro* seed germination of *Cymbidium elegans* and *Dendrobium densiflorum* (Table 1 and 2). However, the seeds of both species showed variation in their germination behavior.

C. elegans showed comparatively better germination in MS medium supplemented with 1 mg/l BAP (Table 1). The seeds started to germinate after nine weeks of culture. The MS medium supplemented with various combination of BAP and NAA also showed better response on seed germination of *C. elegans* which was in accord with the findings of Swar and Pant (2004), who found that MS medium supplemented with BAP (1 mg/l) and NAA (1 mg/l) was most effective for seed germination of *Cymbidium iridioides*.

D. densiflorum showed quick response in hormone-free MS media than hormone supplemented media. The seed germination started after five weeks of culture (Table 2). This was supported by the findings of Reddy *et al*. (1992), who studied the seed germination and seedling growth in four species of South Indian tropical orchids (*Cymbidium aloifolium, Dendrobium crepidatum, Epidendrum radicans* and *Spathoglottis plicata*). Hoshi *et al*. (1994) also reported similar findings in their study on the germination of four species of *Cypripedium*. Liu and Zhang (1998) also found that ½ MS basal medium was best for the embryo germination and further proliferation of *Dendrobium candidum*. Karanjit (2002) also reported similar findings on *C. iridioides* where the germination rate was vigorous in hormone-free MS medium. The present result is also consistent with the findings of Pant and Gurung (2005) that MS basal medium was most effective for the *in vitro* seed germination and seedling development in *Aerides odorata*.

In vitro germinated seeds further differentiated to form seed clump, protocorm, and finally to plantlets. In case of *C. elegans*, protocorms and initial shoots were developed in 10th and 31st week of culture respectively in MS medium supplemented with 1 mg/l BAP (Table 1). Root differentiation was not observed till 40th weeks of culture. In case of *D. densiflorum*, the protocorms, initial shoot and root were developed in 6th, 8th and 19th week of culture respectively in hormone free MS medium (Table 2). Several authors have suggested different nutrient solutions suitable at different stages of growth for various species (Ernst 1974; Shobana and Rajeevan 1993; Nagaraju and Parthasarathi 1995). The product of orchid seedling from seed involves sequential phases of germination, protocorm formation and seedling development. In the present investigation also same sequence of seedling development was observed when the selected orchids (*D. densiflorum* and *C. elegans*) were grown on the medium.

Several workers have tried various growth regulators under different concentrations to promote seed germination and seedling growth (Arditti 1979, Temjensangba and Chitta 2005). In relation to germination behavior, it was observed that epiphytic orchids germinated more quickly.

In the present study the seeds of *D. densiflorum* germinated and developed into seedlings faster than that of *C. elegans.* The most effective germination of *C. elegans* was found in MS medium supplemented with 1 mg/l BAP, whereas hormone-free MS medium was most effective for the *in vitro* seed germination of *D. densiflorum*. The differences in genetic constitution of the material and the presence of different endogenous growth stimulating substances in seeds are the probable factors which may have differential effect on seed germination and seedling formation in two orchid species studied.

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Table 1. Effect of growth regulators supplemented in MS media on seed germination and seedling growth of *Cymbidium elegans*.

Media	Growth Hormones	Concentration of hormones (mg/l)	Observation taken in weeks				Remarks
			Initiation of germination	Protocorm formation	1st shoot formation	1st root formation	
MS	BM	-	14	17	39	Not observed	Good
MS	BAP	0.5	15	18	38	-	Average
MS	BAP	1.0	9	10	31	-	Best
MS	BAP	1.5	17	22	40	-	Average
MS	BAP	2.0	10	12	31	-	Good
MS	NAA	0.5	18	24	42	-	Poor
MS	BAP+NAA	0.5 + 0.5	10	14	36	-	Average
MS	BAP+NAA	1.0 + 0.5	13	17	30	-	Good
MS	BAP+NAA	1.5 + 0.5	11	18	33	-	Good
MS	BAP+NAA	2.0 + 0.5	12	15	31	-	Good

Culture conditions: MS medium, 25 ± 2°C, 40 weeks, 4 replicates were used in each combination.

Table 2. Effect of growth regulators supplemented in MS media on seed germination and seedling growth of *Dendrobium densiflorum*.

M8edia	Growth Hormones	Concentration of hormones (mg/l)	Observation taken in weeks				Remarks
			Initiation of germination	Protocorm formation	1st shoot formation	1st root formation	
MS	BM	-	5	6	8	19	Best
MS	BAP	0.5	6	8	15	-	Poor
MS	BAP	1.0	7	9	21	-	Poor
MS	BAP	1.5	9	10	20	-	Poor
MS	BAP	2.0	10	12	17	-	Poor
MS	NAA	0.5	6	8	11	22	Good
MS	BAP+NAA	0.5 + 0.5	6	10	15	-	Poor
MS	BAP+NAA	1.0 + 0.5	7	11	15	-	Poor
MS	BAP+NAA	1.5 + 0.5	8	12	18	-	Poor
MS	BAP+NAA	2.0 + 0.5	8	13	20	-	Poor

Culture conditions: MS medium, 25 ± 2°C, 40 weeks, 4 replicates were used in each combination.

Fig.1. Stages of *in vitro* seed germination; a-c: *Dendrobium densiflorum* (a – whitish green globular mass of small protocorms in basal medium at six weeks of culture; b – globular yellowish green mass of protocorms in medium containing BAP 1.5 mg/l + NAA 0.5 mg/l at 12 weeks of culture; c – many green plantlets with small roots in MS basal medium at 20 weeks of culture); d-e: *Cymbidium elegans* (d – brownish white seed clumping in medium containing BAP 1.0 mg/l at 9 weeks of culture; e – small plantlets developed from protocorms in medium containing BAP 1.0 mg/l + NAA 0.5 mg/l at 32nd week of culture).