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Full length Research paper

In vitro seed germination of endangered Nepalese orchid species: *Dendrobium fimbriatum* Hook.

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Dendrobium fimbriatum **Hook., a native epiphytic orchid of high ornamental and medicinal values, is found at an altitude of 200 to 2100 m in Nepal. *In vitro* seed germination and shoot development of this orchid was carried out on 0.8% (w/v) agar solidified Murashige and Skoog (MS) medium and the medium supplemented with various combinations of α- naphthalene acetic acid (NAA) and 6- benzylaminopurine (BAP). The germination of seed started after five weeks of culture in three different hormonal combinations of the medium viz. hormone free MS medium, MS medium supplemented with 0.5 mg/L NAA and MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA. First shoot initials were observed after 11 weeks of culture on MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA; and the medium combination was found to be the best condition for the development of shoots followed by hormone free MS medium and the MS medium supplemented with 0.5 mg/L NAA which both gave shoots after 12 weeks. Under different tested combinations of the medium, the germination percentage was found to vary from 90 to 100%.**

Key words: *Dendrobium fimbriatum*, *in vitro*, seed germination, MS medium.

INTRODUCTION

Nepal, situated in the central Himalaya, harbors 451 species of orchids belonging to 107 genera (Rajbhandari, 2015). Orchids as a whole are cited under Appendix II of CITES except *Paphiopedilum insigne* and *Paphiopedilum venustum* in Nepal (DPR, 2012). They are important aesthetically, medicinally and also regarded as an ecological indicator (Joshi et al., 2009). Due to their varied shape, size, colourful- long lasting flowers, shinning green leaves and variously shaped pseudobulbs, they are very popular around the world. *Dendrobium* is represented by 30 plant species in Nepal (Rajbhandari, 2015). It is one of the most threatened orchid genus in Nepal due to deforestation and over exploitation in nature.

Dendrobium fimbriatum Hook., a native orchid of Nepal, is commonly known as 'The fringe-lip Dendrobium'. It is an epiphytic herb found on tree trunks at elevations of 200 to 2100 m in the central and eastern Nepal (Raskoti, 2009; Rokaya et al., 2013).

It has high aesthetic value (Figure1), so it is often used as an ornamental plant in many gardens, nurseries, hotels, etc. It is highly medicinal orchid; whole plant is used against liver upsets and nervous debility (Baral and Kurmi, 2006; Pant and Raskoti, 2013). Owing to its high demand in the national and international markets, over collection from its natural habitat and slow growth rate in nature, this species is restricted only to narrow pocket areas in the nature.

Orchid seeds lack functional endosperm, so the germination of the seeds requires an aid of suitable fungus. The germination rate of orchid seeds in nature is only 2 to 5% (Rao, 1977); even if they do so, the seeds take a long time to germinate and any disturbance in the habitat may destroys the whole population. The seedlings take 12 years to grow to maturity (Basker and Narmatha Bai, 2006). Vegetative propagation of this orchid through division of clumps of rhizomes, bulbs or by the rooting of off -shoots is slow; so often, that it is difficult to obtain the desired number of plants. These difficulties in natural germination and slow vegetative propagation may drive this species to extinction. *In vitro* propagation of orchids through seeds can produce large number of orchids in reasonably short time.

Hence, the present study was undertaken to develop an efficient protocol for *in vitro* propagation of *D. fimbriatum*

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Table 1. Effect of growth hormones supplemented to MS medium on seed germination of *Dendrobium fimbriatum* Hook.

Medium	Growth hormones	Concentration of hormones (mg/L)	Observation taken in weeks			
			Initiation of germination	Protocorm formation	1st shoot formation	% seed germination
MS	-	-	5	8	12	100
MS	BAP	0.5	6	10	15	100
MS	BAP	1	7	12	17	100
MS	BAP	1.5	7	12	17	95
MS	BAP	2	7	13	20	90
MS	NAA	0.5	5	8	12	100
MS	BAP+NAA	0.5+0.5	5	8	11	100
MS	BAP+NAA	1+0.5	6	9	14	100
MS	BAP+NAA	1.5+0.5	7	12	18	100
MS	BAP+NAA	2+0.5	7	12	19	100
MS	NAA	1	7	10	15	100
MS	BAP+NAA	0.5+1	7	11	15	100
MS	BAP+NAA	1+1	7	12	16	100
MS	BAP+NAA	1.5+1	7	12	16	100
MS	BAP+NAA	2+1	7	13	19	100

Culture conditions: 25± 2°C, 25 weeks, 16 h photoperiod and 6 replicates were used in each combination

through seeds and ultimately assist in its conservation.

MATERIALS AND METHODS

An immature capsule of *Dendrobium fimbriatum* Hook. (Figure 2) collected from Pokhara, Kaski district, central Nepal was used in this research.

The capsule was sterilized by washing under running tap water besides 2 to 3 drops of tween 20 solution (Qualigens Fine Chemicals Pvt. Ltd.) for 50 min until the water became totally clear and transparent. The capsule was then rinsed in 70% ethyl alcohol for 2 min and in 1% solution of sodium hypochlorite for 10 min. Finally, it was rinsed with sterile water five times.

Murashige and Skoog (MS) medium was used alone and in different combinations of 6-benzylaminopurine (BAP) and α-naphthalene acetic acid (NAA) (Table 1). The medium was supplemented with 3% sucrose. The pH of the medium was adjusted to 5.8 before autoclaving and solidified with 0.8% (w/v) agar. The medium was autoclaved at 15 psi for 15 min.

The sterilized capsule was then dissected longitudinally into two halves (Figure 3) using sterile surgical blade inside pre-sterilized laminar air flow cabinet. The seeds were then inoculated on the surface of MS medium alone and in different combinations of BAP and NAA using sterile forceps.

The cultures were incubated at 25± 2°C under photoperiod of 16/8 h light/dark cycle.

RESULTS AND DISCUSSION

An immature capsule was used in this research as Pant (2006) reported that immature capsules show better germination response and saves time. The most effective germination response of *Dendrobium fimbriatum* besides shoots development was found to be on MS medium supplemented with BAP (0.5 mg/L) and NAA (0.5 mg/L). The quantity and nature of growth regulators play significant role in the germination of orchid seeds (Arditti, 1979). According to Yam et al. (1989), the nutritional requirements of germinating orchid seeds vary with their physiological state and this may be species specific. The percentage of seed germination was found to vary from 90 to 100% under different tested combinations of the medium (Table 1). The nutrient requirement of orchid seeds in terms of quantity as well as form may vary at different stages of development for various species (Ernst, 1974; Arditti and Ernst, 1984).

The most appropriate medium was selected on the basis of time taken for germination of seeds and their growth and development.

Initiation of seed germination was observed after five weeks of culture (Figure 4) in three different hormonal combinations of the medium (Table 1). This was supported by the findings of Reddy et al. (1992), who studied the seed germination and seedling growth in four different species of orchids (*Cymbidium aloifolium, Dendrobium crepidatum, Epidendrum radicans and Spathoglottis plicata*) and found the seed

Figures: (1) Flowers of *Dendrobium fimbriatum* Hook.; (2) A sterilized capsule of *D. fimbriatum*; (3) Longitudinally dissected capsule exposing seeds; (4) Initiation of seed germination on hormone free MS medium after five weeks of culture; (5) Development of shoots from protocorms on MS medium supplemented with 0.5 mg/L NAA after 12 weeks of culture; (6) Well developed shoots on MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA after 16 weeks of culture.

germination after five weeks. It was also supported by the similar findings of Hoshi et al. (1994) on the seed germination of four species of *Cypripedium* and Pradhan and Pant (2009) on *Dendrobium densiflorum*. Protocorms were observed after eight weeks of culture in three different hormonal combinations of the medium (Table 1). Similar findings were also reported by Basker and Narmatha Bai (2010) in the seed germination of *Eria bambusifolia* which took seven weeks for protocorms formation. Similarly, Pant et al. (2011) on *Phaius*

tancarvilleae and Gogoi et al. (2012) on *Cymbidium ebuneum* took nine weeks for the formation of protocorms. The first shoot initial was obtained after 11 weeks of culture on MS medium supplemented with BAP (0.5 mg/L) and NAA (0.5 mg/L) while it was observed after 12 weeks of culture on hormone free MS medium and the MS medium supplemented with 0.5 mg/L NAA (Figure 5). This was supported by the findings of Pant et al. (2011) on *P. tancarvilleae* which took 12 weeks for first shoot formation.

Formation of dense shoots (Figure 6) was very common under various hormonal combinations of the medium in this research. It may be due to genetic constitution of explants and the endogenous growth regulators present in them.

CONCLUSIONS

MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA was found to be the optimum condition for the seed germination and shoot development of *Dendrobium fimbriatum* Hook. suggesting the usefulness of both phytohormones, BAP and NAA, for their *in vitro* propagation.

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