academicJournals

Vol. 15(5), pp. 105-109, 3 February, 2016
DOI: 10.5897/AJB2015.14870
Article Number: DDCD64657185
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

In vitro seed germination and seedling development of the orchid *Coelogyne stricta* (D. Don) Schltr.

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Received 19 July, 2015; Accepted 29 December, 2015

***Coelogyne stricta* (D. Don) Schltr., an orchid of high ornamental and medicinal values, is native to Nepal at 1400 to 2000 m elevations. *In vitro* seed germination and seedling development was carried out on 0.8% (w/v) agar solidified Murashige and Skoog (MS) medium, supplemented with various combinations of α-naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP). MS medium supplemented with 1 mg/L BAP and 1 mg/L NAA was found to be the best condition for the development. The germination started after 7 weeks of culture and complete seedlings were obtained after 23 weeks of culture on the medium supplemented with 1 mg/L BAP and 1 mg/L NAA suggesting the usefulness of both hormones in root induction. In the hormone, free MS medium germination started after 5 weeks, but root initials were not developed even after 32 weeks of culture.**

Key words: *Coelogyne stricta*, *in vitro*, Murashige and Skoog (MS), 6-benzylaminopurine (BAP), α-naphthalene acetic acid (NAA).

INTRODUCTION

Nepal, situated in the lap of Himalaya, harbors 451 species of orchids from 107 genera (Rajbhandari, 2015). Orchids as a whole are cited under Appendix II of CITES except *Paphiopedilum insigne* and *Paphiopedilum venustum* in Nepal. They are important aesthetically, medicinally and also regarded as 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.

A total of 90 species of orchids of Nepal have medicinal value (Pant and Raskoti, 2013). *Coelogyne* represented by 14 plant species (Rajbhandari, 2015), is one of them. It is also the most threatened orchids in Nepal due to its over collection from nature.

Coelogyne stricta (D. Don) Schltr., a native orchid of Nepal, is commonly known as 'The Rigid Coelogyne Pseudobulbs'. It is an epiphyte on tree trunks or lithophytes on mossy rocks at elevations of 1400 to 2000 m in Nepal (Raskoti, 2009; Rajbhandari, 2015). It has high aesthetic value (Figure 1), so it is often used as an ornamental plant in many gardens, nurseries, hotels, etc. Its medicinal value is due to paste of its pseudobulb which is applied to the forehead against headache and

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Figure 1. A flower of *Coelogyne stricta*.

Figure 2. An immature capsule.

fever (Baral and Kurmi, 2006). 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 reasonable short time.

Hence, the present study was undertaken to develop an efficient protocol for *in vitro* propagation of *C. stricta* through seeds and ultimately assist in its conservation.

MATERIALS AND METHODS

Eight weeks old immature capsule of *C. stricta* (Figure 2) collected from the orchid house of National Botanical Garden, Godawari, Kathmandu 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

Immature capsule was selected for this research as it shows better germination response and saves time (Pant, 2006). The most effective germination response for *C. stricta* with complete development of roots and shoots was found to be on MS medium supplemented with BAP (1 mg/L) and NAA (1 mg/L). The quantity and nature of growth regulators have significant effect on the germination of orchid seeds (Arditti, 1979).

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 in five 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 germination after 5 weeks. It was also supported by 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 obtained after 8 weeks of culture in

Table 1. Effect of growth hormones supplemented to MS medium on seed germination and seedling growth of *C. stricta* (D. Don) Schltr.

Medium	Growth hormones	Concentration of hormones (mg/L)	Observation taken in weeks			
			Initiation of germination	Protocorm formation	1st shoot formation	1st root formation
MS	-	-	5	8	13	-
MS	BAP	0.5	5	9	16	-
MS	BAP	1	8	14	19	-
MS	BAP	1.5	8	14	19	-
MS	BAP	2	7	13	21	-
MS	NAA	0.5	5	8	12	25
MS	BAP+NAA	0.5+0.5	5	8	11	24
MS	BAP+NAA	1+0.5	6	9	14	29
MS	BAP+NAA	1.5+0.5	5	9	14	25
MS	BAP+NAA	2+0.5	6	9	13	25
MS	NAA	1	8	10	13	-
MS	BAP+NAA	0.5+1	7	9	12	26
MS	BAP+NAA	1+1	7	9	13	23
MS	BAP+NAA	1.5+1	7	10	13	24
MS	BAP+NAA	2+1	7	10	13	25

Culture conditions: 25± 2°C, 32 weeks, 16 h photoperiod and 6 replicates were used in each combination.

Figure 3. Capsule cut longitudinally into two halves.

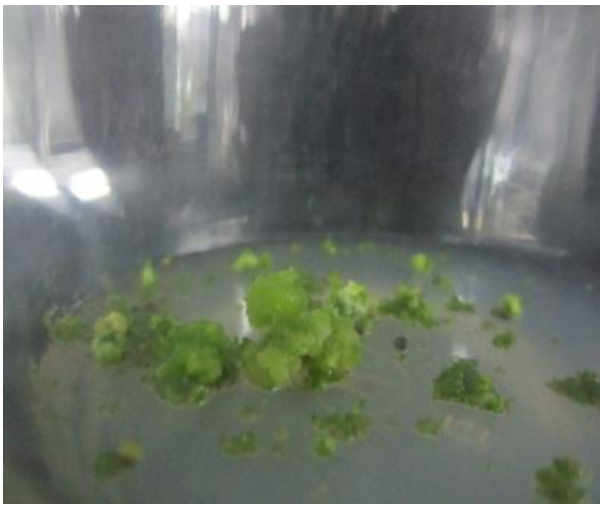

Figure 4. Clumped protocorms on MS medium supplemented with 0.5 mg/L BAP after 10 weeks of culture.

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 7 weeks for protocorms formation, Pant et al. (2011) on *Phaius tancarvilleae* which took 9 weeks and Gogoi et al. (2012) on *Cymbidium eburneum* which also took 9 weeks. Clumped protocorms were observed in this case on MS medium supplemented with 0.5 mg/L BAP after 10 weeks of culture (Figure 4). 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 13 weeks of culture on hormone-free MS medium (Figure 5). This was supported by the findings of Pant et al. (2011) on *P. tancarvilleae* which took 12 weeks for first shoot formation. Dense shoot formation was common under various hormonal combination of the medium (Figure 7) except on MS medium supplemented with 0.5 mg/L BAP (Figure 6). The first root initial was obtained after 23 weeks of culture on MS

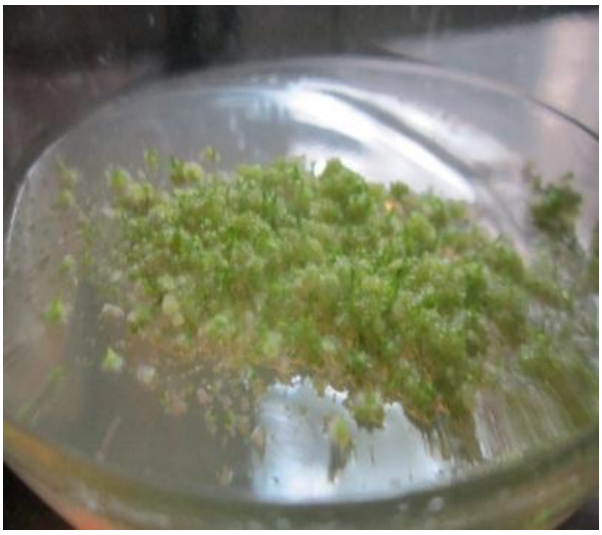

Figure 5. Initiation of shoots on hormone-free MS medium after 13 weeks of culture.

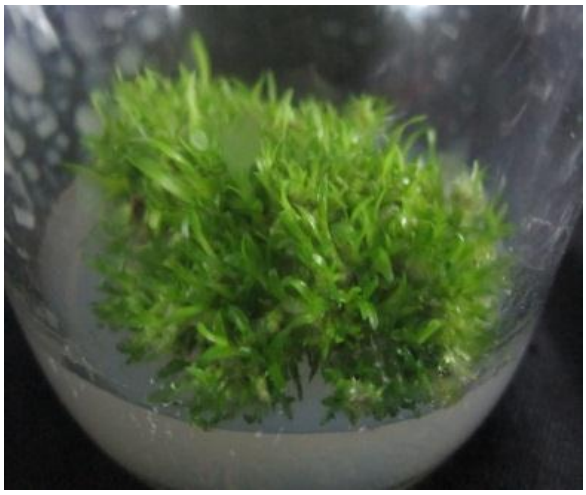

Figure 7. Shoots on MS medium supplemented with 1 mg/L BAP and 1 mg/L NAA after 20 weeks of culture.

Figure 6. Formation of shoots on MS medium supplemented with 0.5 mg/L BAP after 20 weeks of culture.

Figure 8. Initiation of root on MS medium supplemented with 0.5 mg/L NAA after 25 weeks of culture.

medium supplemented with BAP (1 mg/L) and NAA (1 mg/L) while on MS medium supplemented with 0.5 mg/L NAA it was observed after 25 weeks of culture (Figure 8). Pradhan and Pant (2009) in the seed germination of *D. densiflorum* found 19 weeks needed for the first root formation.

Complete plantlet of *C. stricta* was obtained after 23 weeks of culture. This was supported by the findings of Pant et al. (2011) on *P. tancarvilleae* which took 24 weeks to develop into complete plantlets and Paudel et al. (2012) on *Esmeralda clarkei* which took 25 weeks. MS medium supplemented with BAP (1 mg/L) and NAA (1 mg/L) was found to be the best for seed germination of *C. stricta*. This was supported by the findings of Pant and Swar (2011) in the study of seed germination of *Cymbidium iridioides* who found MS medium supplemented with BAP (1 mg/L) and NAA (1 mg/L) to be best for the protocorms formation and seedlings growth.

Phytohormone NAA was found to be essential for root initiation as the medium combination lacking NAA did not develop root even after 32 weeks of culture (Table 1). It may be due to genetic constitution of explants and the endogenous growth regulators present in them. According to Yam et al. (1989), the nutritional requirements of germinating orchid seeds vary with their physiological state and this may be species specific. This also revealed that the addition of root hormone NAA might be essential in the nutrient medium for the

successful root growth and development. 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).

Conclusion

The phytohormones BAP and NAA are both necessary for *in vitro* seed germination and seedlings growth of *C. stricta.* MS medium supplemented with BAP (1 mg/L) and NAA (1 mg/L) was found to be the best for this purpose.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to express sincere gratitude to Prof. Dr. Pramod K. Jha, Head, Central Department of Botany, Tribhuvan University and Dr. Sushim Ranjan Baral, former Chief, National Herbarium and Plant Laboratories, Godawari for providing necessary laboratory facilities for the research. We would also like to thank National Botanical Garden, Godawari for providing the orchid capsule.

Abbreviations

MS, Murashige and Skoog medium; **BAP,** 6-benzylaminopurine; **NAA,** α-naphthaleneacetic acid.

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