

**IN VITRO SEED GERMINATION AND SEEDLING GROWTH OF
STAUROCHILUS RAMOSUM (LINDL.) SEIDENF**

TAPASH KUMAR BHOWMIK*, MINHAJUR RAHMAN AND MD. MAHBUBUR RAHMAN

¹Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

ABSTRACT

A comparative study on *in vitro* seed germination and seedling development of *Staurochilus ramosum* (Lindl.) Seidenf. was carried out *in vitro*. Four basal media namely KC, MS, PM and VW media were used as half strength, full strength and full strength supplemented with 0.5mg/l BAP and 0.5mg/l NAA and solidified with agar. Response like protocorm like bodies (PLBs) initiation, seed germination and seedlings development on different basal media were varied distinctly. Full strength PM medium supplemented with 0.5mg/l BAP and 0.5mg/l NAA showed better response within 6 weeks of culture. The overall result indicates that PM medium was more effective than KC, MS and VW media for initiation of germination, PLBs formation and plantlets development.

Key words: *Staurochilus ramosum*, BAP, NAA, PLBs

INTRODUCTION

Orchids are one of the most striking decorative plants all over the world. They are highly priced in the national and international markets because of their enduring and beautiful flowers. They account for 7% of total flowering plant species which represent one of the most expensive ornamental plants worldwide and dominate the international cut flower trade (Rahman *et al.*, 2005). In addition to high ornamental values, orchids are of considerable importance in medicines as well. They have rich contents of alkaloids, glycosides and other useful phytochemicals (Gutierrez 2010; Pant 2011). Orchids are important aesthetically, medicinally and also regarded as the ecological indicator (Joshi *et al.*, 2009). Due to their varied shape, size, colorful-long lasting flowers, shining green leaves and different shaped pseudobulbs they are very popular around the world. In Bangladesh, orchids are represented by 188 species under 72 genera (Rahman *et al.*, 2017) distributed throughout the country especially Chittagong, Chittagong Hill Tracts,

*Correspondence to Tapash Kumar Bhowmik; E-mail: tapashptc@gmail.com

Cox's Bazar, greater Sylhet, Gazipur and Sundarbans mangrove forest (Bhowmik and Rahman 2020).

Staurochilus ramosum (Lindl.) Seidenf. is an epiphytic monopodial orchid of high ornamental value. It flowers in May (Huda 2008). This orchid is native to Bangladesh and distributed in Cox's Bazar, Chattogram, the Chittagong Hill Tracts districts (Seidenfaden 1988; Huda *et al.*, 1999). It is one of the important epiphytic orchids of high aesthetic value often found as an ornamental plant in gardens, nurseries, hotels, etc. This orchid is highly demanded in the floriculture market because of its exquisite highly intricate beautiful flowers. Indiscriminate collections by orchid lovers, habitat destruction and over exploitation are the main factors that have threatened the survival of this species. Therefore, the conservation of this orchid is now a matter of universal concern (Hoshi *et al.* 1994). Tissue culture technique has been widely used for the *in vitro* mass propagation as well as for the conservation of several commercially important orchids (Malabadi *et al.*, 2005).

Orchid's seeds are very minute, non-endospermic and need mycorrhizal association for germination in nature and require up to 8-10 yrs. for their *in vivo* growth before reaching reproductive maturity (Deb and Pongener 2012). The germination rate of orchid seeds in nature is only 2 to 5% (Hossain 2015); even if they do so, the seeds take a long time to germinate and any disturbance in the habitat may destroy the whole population. The seedlings of *Coelogyne stricta* (D. Don) Schltr. take 12 years to grow to maturity for flowering (Basker and Bai 2006). Vegetative propagation of orchid through the division of clumps of rhizomes, bulbs or by the rooting of off-shoots is slow; so often it is difficult to obtain the desired number of plants. This problem can be overcome by the tissue culture technique. *In vitro* culture of orchid, the seed does not require any fungal association and asymbiotic seed germination can be successfully performed leading to plant regeneration. Modification of traditional tissue culture technique by adding specific plant growth regulators, activated charcoal, peptone and changing culture condition are reported to enhance germination percentage and subsequent development of protocorms in many orchids (Hossain *et al.*, 2009, Bhadra and Bhowmik 2015). The present research highlighted to find out the best medium for *in vitro* mass propagation of ornamentally important orchid *S. ramosum* and successfully adapted in the net house by successive phases of acclimatization.

MATERIALS AND METHODS

Explant collection and sterilization: 12 weeks old mature capsule of *S. ramosum* collected from Hill Agricultural Research Centre, Khagrachari, Bangladesh was used in this research. The mature capsule was sterilized by washing under running tap water to remove dust particles and then washed with sterile distilled water four times. The fruits were then rubbed with savlon soaked cotton and washed three times with distilled water. Then they were treated with 0.1% (w/v) HgCl_2 for 10 minutes for surface sterilization and thereafter rinsed three times with double distilled water in a laminar airflow cabinet. Finally, the capsule was treated with 70% ethyl alcohol for one minute and rinsed three times with double distilled water.

Culture medium and culture condition: In the present investigation, half strength (without PGRs), full strength (without PGRs) and full strength with PGRs *viz.* BAP (0.5 mg/l) and NAA (0.5 mg/l) supplemented media of KC (Knudson 1946); MS (Murashige and Skoog 1962); PM-Phytamax (Arditti 1977) and VW (Vacin and Went 1949) were used for *in vitro* seed germination and seedling development. Basal medium was fortified with 30 g/l sucrose for MS and 20 g/l sucrose for KC, PM, VW and with or without different plant growth regulators (PGRs) like BAP & NAA. Agar (0.8% w/v) was used as a gelling agent for all culture media and pH of the media was adjusted at 5.8 in case of MS and 5.4 in KC, PM and VW by using 0.1N NaOH or HCl. Agar was dissolved by boiling the mixture in the water bath and about 50 ml of medium was dispensed into 100 ml of each culture vessel and autoclaved at 121 °C for 30 minutes at 15 lb/cm² pressure. All cultures were maintained at 25±2 °C for a 14h photoperiod per day using white fluorescent tubes.

Inoculation of seeds and data collection: Surface sterilized immature green pods were kept on a sterilized petri dish containing sterilized filter paper for drying. They were cut longitudinally with the help of a sharp sterilized surgical blade. The mature seeds were scooped out with the help of a sterilized spatula and transferred to and spread over the medium. Sub-culturing was carried out every eight weeks into fresh medium. Each treatment was replicated six times. The initiation of seed germination was recorded weekly. Seed germination data was collected, analysed and tabulated in the Table.

RESULT AND DISCUSSION

Mature capsules were selected for this study as they show better germination response (Utami *et al.*, 2017). The immature capsule was also reported to be better in some cases (Pant 2006; Bhowmik and Rahman 2017). The most effective germination response for *S. ramosum* with complete development of roots and shoots was found best on PM medium supplemented with 0.5 mg/l BAP and 0.5 mg/l NAA. The amount and nature of growth regulators have proved to significantly effective on the germination of orchid seeds (Pant *et al.*, 2011; Bhowmik and Rahman 2017).

The seeds were inoculation on half strength (without PGRs), full strength (without PGRs) and PGRs (0.5 mg/l BAP and 0.5 mg/l NAA) supplemented full strength KC, MS, PM and VW media and gave different responses at different times. The most suitable medium was assessed based on the percentage of seeds germination and duration of time taken for germination, growth and development. The germination of seeds as well as growth and development of seedlings were markedly influenced by the presence of different basal medium and plant growth regulators (PGRs) in the medium (Table 1). Seedlings' development showed different developmental stages, *viz.*, seed germination, protocorms formation, shoot development and root formation (Figs. 1A-F). Seed germination started after six weeks of inoculation on PM medium supplemented with 0.5mg/l BAP and 0.5mg/l NAA followed by eight weeks on same PGRs fortified MS medium. Many studies revealed a beneficial effect of PGRs supplemented medium in promoting germination frequency, protocorms multiplication and healthy growth of seedlings in a large number of orchid species (Reddy *et al.*, 1992; Hoshi *et al.*, 1994; Pradhan and Pant 2009). Half strength KC medium without PGRs gave poor responses of seeds germination (50%) and required the maximum time (19 weeks). Half strength KC medium contained low amounts of nutrients than the other three media. On the other hand, half strength MS medium was more effective for germination of *Dendrobium chrysotoxum* Lindl. (Kaur and Bhutan 2011). PGRs supplemented PM medium was best for the development of protocorms followed by PGRs fortified MS medium after 8 and 10 weeks of inoculation respectively; whereas, the maximum time required (24 weeks) in PGRs free half strength KC medium. Such types of similar findings were also reported in *Dendrobium densiflorum* Lindl. (Pradhan and Pant 2009), *Eria bambusifolia* Lindl. (Basker and Bai 2010), *Cymbidium aloifolium* (L.) Sw. (Nongdam and Chongtham 2011), *Phaius tancarvilleae* (Banks) Blume (Pant *et al.* 2011), *Cymbidium eburneum* Lindl. (Gogoi *et al.* 2012), *Cymbidium*

IN VITRO SEED GERMINATION AND SEEDLING GROWTH OF *STAUROCHILUS RAMOSUM* (LINDL.) SEIDENF

TABLE 1: EFFECTS OF DIFFERENT STRENGTH OF MEDIUM AND PGRs ON SEED GERMINATION OF *STAUROCHILUS RAMOSUM* (LINDL.) SEIDENF.

Medium	Culture condition	Time taken in weeks					% of seed germinated per vessels	Remarks
		Initiation of germination	Development of protocorm	Differentiation of				
				1st leaf primodia	1st root primodia	Seedling		
KC	Half without PGRs	19	24	-	-	-	50	+
	Full without PGRs	14	18	23	30	37	60	+
	Full with *PGRs	11	14	19	26	33	80	+
MS	Half without PGRs	15	19	-	-	-	60	+
	Full without PGRs	11	14	19	26	32	80	+
	Full with *PGRs	8	10	14	20	26	100	+
PM	Half without PGRs	11	15	-	-	-	70	+
	Full without PGRs	8	12	16	24	31	90	+
	Full with *PGRs	6	8	12	18	23	100	++
VW	Half without PGRs	16	21	-	-	-	60	+
	Full without PGRs	12	16	21	27	35	70	+
	Full with *PGRs	9	11	15	21	27	90	+

*PGRs (BAP 0.5mg/l + NAA 0.5mg/l); + denotes Germination favoured, ++ denotes Germination (Best); Six replicates were used in each treatment.



A. Mature seeds turned into PLBs on $\frac{1}{2}$ PM medium B. Mature seeds turned into PLBs on KC medium C. PLB's with small shoot developed on full strength of VW medium



D. Development of small shoot on full strength MS medium E. Development of small root on full strength PM medium F. Plantlets formed on PM with 0.5 mg/l BAP and 0.5 mg/l NAA

FIGURE 1. DIFFERENT STAGES OF *IN VITRO* SEED GERMINATION AND SEEDLING DEVELOPMENT OF *STAUROCHILUS RAMOSUM* (LINDL.) SEIDENF.

aloifolium (Pradhan *et al.*, 2013), *Coelogyne flaccida* Lindl. (Parmar and Pant 2015), *Coelogyne stricta* (D. Don) Schltr. (Parmar and Pant 2016), *Cymbidium aloifolium* (Bhowmik and Rahman 2017) and protocorms formation took place after 6, 7, 5-6, 9, 9, 7, 6, 5 and 8 weeks of culture respectively.

The first shoot initial was obtained from germinated protocorms after 12 weeks of seed inoculation on PM medium supplemented with BAP (0.5 mg/l) and NAA

IN VITRO SEED GERMINATION AND SEEDLING GROWTH OF *STAUROCHILUS RAMOSUM* (LINDL.) SEIDENF

(0.5 mg/l) subsequently required 14 weeks on the same PGRs fortified MS medium. The result agrees with Pant *et al.*, (2011) in *P. tancarvilleae*; Bhowmik and Rahman (2017) in *C. aloifolium* and those took the same time (12 weeks). While it was obtained after 8 weeks of culture in *D. densiflorum* (Pradhan and Pant 2009), *C. flaccida* (Parmar and Pant 2015), *C. stricta* (Parmar and Pant 2016) and 10 weeks were required in *C. aloifolium* (Pradhan *et al.*, 2013).

The first root initial was initiated on PGRs fortified PM medium descended by MS medium after 18 and 20 weeks of culture accordingly. Similar results were achieved in *D. densiflorum* (Pradhan and Pant 2009), *P. tancarvilleae* (Pant *et al.*, 2011), *C. aloifolium* (Pradhan *et al.*, 2013), *C. flaccida* (Parmar and Pant 2015), *C. stricta* (Parmar and Pant 2016) and *C. aloifolium* (Bhowmik and Rahman 2017) after 19, 18, 14, 10, 11 and 18 weeks respectively.

Complete plantlets of *S. ramosum* were obtained after 23 weeks on PGRs supplemented PM medium followed by 26 weeks on MS medium. Similar findings were noted by Pant *et al.*, (2011) in *P. tancarvilleae*; Paudel *et al.*, (2012) in *Esmeralda clarkei* Rchb.f; Pradhan *et al.*, (2013) in *C. aloifolium*; Parmar and Pant (2015) in *C. flaccida*; Parmar and Pant (2016) in *C. stricta*; Bhowmik and Rahman (2017) in *C. aloifolium*. Those orchids were developed complete plantlets from seeds after 24, 25, 27, 22, 24, 24 weeks of inoculation respectively. Whereas, MS medium supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA was found to be the best for seed germination and seedlings development of *Cymbidium iridioides* D. Don (Pant and Swar 2011) and *C. stricta* (Parmar and Pant 2016).

This study revealed that the PM medium supplemented with 0.5 mg/l BAP and 0.5 mg/l NAA was found to be the optimum for *in vitro* seed germination, shoot initiation and seedlings development of *S. ramosum*.

CONCLUSION

This simple and efficient protocol for regenerating a large number of plantlets from the seed culture of *S. ramosum* could be used for large-scale propagation and *ex situ* conservation.

ACKNOWLEDGEMENT

The authors would like to express sincere gratitude to the Ministry of Science and Technology, Government of the Peoples Republic of Bangladesh for providing financial support under grants for Advanced Research in Sciences. We would also like to thank Hill Agricultural Research Centre, Khagrachari for providing the orchid capsules.

REFERENCES

- ARDITTI, J. 1977. Clonal propagation of orchids by means of tissue culture: A manual *In: J Arditti (ed.). Orchid Biology: Reviews and Perspectives*, Ithaca University Press, Ithaca, New York. pp. 203-293.
- BASKER, S., AND BAI, VN. 2006. Micropropagation of *Coelogyne stricta* (D. Don) Schltr. *via* pseudobulb segment cultures. *Tropical and Subtropical Agroecosystems*, **6(1)**: 31-35.
- BASKER, S., AND BAI, VN. 2010. *In vitro* propagation of an epiphytic and rare orchid *Eria bambusifolia* Lindl. *Res. Biotechnol*, **1**: 15-20.
- BHADRA, S.K., AND BHOWMIK, T.K. 2015. *In vitro* seed germination and micropropagation of *Dendrobium lindleyi* Steud. an indigenous orchid of Bangladesh. *J Asiatic Soc Bangladesh (Science)*, **41(2)**: 163-171.
- BHOWMIK, T.K., AND RAHMAN, M.M. 2017. *In vitro* seed germination and rhizome based micropropagation of *Calanthe densiflora* Lindl: An indigenous terrestrial orchid of Bangladesh. *Int J Bot Studies*, **2(1)**: 110-116.
- BHOWMIK, T.K., AND RAHMAN, M.M. 2020. *In vitro* mass scale propagation and seedlings development of medicinal orchid *Dendrobium transparens* Wall. ex Lindl. *J Med Plant Stu*, **8(3)**: 141-146.
- DEB, C.R., AND PONGENER, A. 2012. Studies on the *in vitro* regenerative competence of aerial roots of two horticultural important *Cymbidium* species. *J Plant Biochem Biotechnol*, **21**: 1-7.
- GOGOI, K., KUMARIA, S. AND TANDON, P. 2012. *Ex situ* conservation of *Cymbidium eburneum* Lindl.: a threatened and vulnerable orchid, by asymbiotic seed germination. *Biotechnol*, **2(4)**: 337-343.
- GUTIERREZ, P.M.P. 2010. Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. *J Med Plants Res*, **4**: 592-638.
- HOSHI, Y., KONDO, K., AND HAMATANI, S. 1994. *In vitro* seed germination of four Asiatic taxa and notes on the nodal micropropagation of American *Cypripedium montanum*. *Lindleyana*, **9**: 93-97.

IN VITRO SEED GERMINATION AND SEEDLING GROWTH OF *STAUROCHILUS RAMOSUM* (LINDL.) SEIDENF

- HOSSAIN, M.M. 2015. *Orchid: Biology and Biotechnology*. Protiva Prokash, Dhaka-1217. pp 1-250.
- HOSSAIN, M.M., SHARMA, M., AND PATHAK, P. 2009. Cost effective protocol for *in vitro* mass propagation of *Cymbidium aloifolium* (L.) Sw. a medicinally important orchid. *Eng Life Sci*, **6**: 444-453.
- HUDA, M.K. 2008. *Orchidaceae*. In: AHMED, Z.U., HASSAN, M.A., BEGUM, Z.N.T., KHONDKER, M., KABIR, S.M.H., AHMAD, M., AHMED, A.T.A., RAHMAN, A.K.A. AND HAQUE, E.U. (eds.). *Encyclopedia of Flora and Fauna of Bangladesh*, Vol. 12. *Angiosperms: Monocotyledons*. Asiatic Society of Bangladesh, Dhaka. pp. 1-149.
- HUDA, M.K., RAHMAN, M.A., AND WILCOCK, C.C. 1999. A preliminary checklist of orchid taxa occurring in Bangladesh. *Bangladesh J Plant Taxon*, **6(1)**: 69-85.
- JOSHI, G.C., TEWARI, L.M., LOHANI, N., UPRETI, K., JALAL, J.S., AND TEWARI, G. 2009. Diversity of orchids in Uttarakhand and their conservation strategy with special reference to their medicinal importance. *Report and Opinion*, **1(3)**: 47-52.
- KAUR, S., AND BHUTAN, K.K. 2011. *In Vitro* Propagation of *Dendrobium chrysotoxum* (Lindl.) Floriculture and Ornamental Biotechnology, *Global Science Books*, **5(1)**: 50-56.
- KNUDSON, L. 1946. A new nutrient solution for germination of orchid seed. *Am. Orc. Soc. Bul.* **15**: 214-217.
- MALABADI, R.B., MULGUND, S.G., AND KALLAPPA, N. 2005. Micropropagation of *Dendrobium nobile* from shoot tip sections. *J Plant Physiol*, **162**: 473-478.
- MURASHIGE, T., AND SKOOG, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473-497.
- NONGDAM, P., AND CHONGTHAM, N. 2011. *In vitro* rapid propagation of *Cymbidium aloifolium* (L.) Sw.: a medicinally important orchid via seed culture. *J Bio Sci* **11(3)**: 254-260.
- PANT, B. 2006. Biotechnology in orchid conservation. Proceeding of Natural Resource Management, *Nepal Bio Soc*, (NBS).
- PANT, B. 2011. Orchids of Nepal with their medicinal properties. *Orchideen Journal*, **18(3)**: 92-98.
- PANT, B., AND SWAR, S. 2011. Micropropagation of *Cymbidium iridioides*. *Nepal J Sci Technol*, **12**: 91-96.

- PANT, B., SHRESTHA, S., AND PRADHAN, S. 2011. *In vitro* seed germination and seedling development of *Phaius tancarvilleae* (L'Her.) Blume. *Sci World*, **9(9)**: 50-52.
- PARMAR, G., AND PANT, B. 2015. *In vitro* seed germination and seedling development of *Coelogyne flaccida* Lindl. (Orchidaceae). *Adv For Sci Cuiabá*, **2(4)**: 85-88.
- PARMAR, G., AND PANT, B. 2016. *In vitro* seed germination and seedling development of the orchid *Coelogyne stricta* (D. Don) Schltr. *African J Biotechnol*, **15(5)**: 105-109.
- PAUDEL, M., PRADHAN, S., AND PANT, B. 2012. *In vitro* Seed Germination and Seedling Development of *Esmeralda clarkei* Rchb.f. (Orchidaceae). *Plant Tiss Cult Biotechnol*, **22(2)**: 107-111.
- PRADHAN, S., AND PANT, B. 2009. *In vitro* seed germination in *Cymbidium elegans* Lindl. and *Dendrobium densiflorum* Lindl. ex Wall. (Orchidaceae). *Bot Orient J Plant Sci*, **6**: 100-102.
- PRADHAN, S., REGMI, T., PARMAR, G., AND PANT, B. 2013. Effect of Different Media on *in vitro* Seed Germination and Seedling Development of *Cymbidium aloifolium* (L.) Sw. *Nepal J Sci Technol*. **14(1)**: 51-56.
- RAHMAN, A.R.M., ISLAM, M.O., PRODHAN, A.K.M.A., AND SYOICHI, I. 2005. Effects of arbohydbrates on callus growth and callus derived plantlet regeneration in *Doritaenopsis* orchid. *Biotechnol*, **4**: 126-131.
- RAHMAN, M.A., HUDA, M.K., AND RASHID, M.E. 2017. Orchid species diversity in Bangladesh and their revised nomenclatural updates. *Biod Bull Bangladesh*, **10**: 1-63.
- REDDY, P.V., NANJAN, K., AND SHANMUGAVELU, K.G. 1992. *In vitro* studies in tropical orchids: seed germination and seedling growth. *J. Orchid Soc. India*, **6(1, 2)**: 75-78.
- SEIDENFADEN, G. 1988. Orchid genera in Thailand XIV. Fifty nine *Vandoid* genera. *Opera Bot*, **95**: 1-398.
- UTAMI, E.S.W., HARIYANTO, S., AND MANUHARA, Y.S.W. 2017. *In vitro* propagation of the endangered medicinal orchid, *Dendrobium lasianthera* J.J. Sm through mature seed culture. *Asian Pac J. Tropical Biomedicine*,
- VACIN, E. AND WENT, F. 1949. Some pH change in nutrient solution. *Bot Gard Cons News*, **110**: 605-613.

Manuscript received on 14.08.2019; Accepted on 21.09.2021

The Chittagong University Journal of Biological Sciences, Vol. 10 (1 & 2). Page No.181-190.