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In Vitro Propagation of *Phaius Tankervilleae* (Banks) Blume

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Abstract:- *Phaius tankervilleae* (Banks) Blume is one of the most widely valued ornamental orchids of the world. It is collected unsustainably for trade and consumption, the species has been decreased fast in natural habitats. Seeds of *Phaius Tankervilleae* were inoculated on Murashige and Skoog (MS) medium with various concentrations of auxin and cytokinin either alone or in combination. The germination and multiplication of seeds was successfully established in 30-45 days in MS medium fortified with 3% sucrose,0.8% agar supplemented with 2.5mg/L Benzyl Amino Purine (BAP) combination with 0.5 mg/L Naphthalene Acetic Acid(NAA). Subculturing on MS medium with 2.0 mg/l Benzyl Amino Purine (BAP) alone favored production of maximum number of shoots. Elongated shoots were separated and rooted on ½ strength MS medium with 1.5 mg/L Indole Butric Acid (IBA). Plantlets, thus developed were established in soil with 65% survivability

Keywords:- *In Vitro Propagation, Phaius Tankervilleae, MS Medium, Activated Charcoal, Plant Lets.*

I. INTRODUCTION

Phaius tankervilleea (Blank) Blume commonly known as Nun's hood Orchid is a robust perennial which produces large, multicoloured flowers that blooms during April to July. It is mainly native to the tropics and distributed throughout Asia to Australia which includes India (Cheng et al., 2012). *P. tankervilleae* is a terrestrial perennial orchid with slender leaves and pseudobulbs as storage organs (Change and Jian, 2010 and Wildlife of Hawaii, 2015).

P. tankervilleae is valuable ornamental plant in India and is collected unsustainably from the wild for ornamental purpose and other economic uses (Kanwal, 2014). *P. tankervilleae* is well known for ethnobotanical reasons among tribal communities of Arunchal Pradesh, India (Kanwal, 2014). The pseudobulbs, roots and leaves are used to extract natural dyes (Mahanta and Tiwari, 2005 and Kanwal, 2014). Paste prepared from the pseudobulb of this species is used to cure swellings of hands and legs (Buragohain et al., 2016; De and Singh, 2016). Smoked flowers of P. tancarvilleae are eaten as a contraceptive in Papua New Guinea (Orchid Species, 2016).

Orchid's seeds are small, numerous and without endosperm. Germination rate is very slow and some needs fungal association for germination (Deb and Pongener, 2011). The germination of orchid seeds is affected by several factors including embryo stage, green pod and nutrient media with growth hormones (Arditti, 1979). Because of its high ornamental and medicinal values, and poor natural propagation the plant is experiencing a steady decline. This orchid species has been declared endangered, under the Conservation Act of Environmental Protection and Biodiversity (Briggs and Leigh, 1996). Hence, it is vital to take necessary actions to conserve and propagate these orchids. It is propagated on a large scale by micropropagation (Malemnganba et al., 1994 and Pradhan et al., 2013).

Considering the above fact in mind, the current study was carried out to standardise an *in vitro* propagation method for production and conservation of *Phaius tankervilleae*.

II. MATERIALS AND METHODS

The capsules of the *Phaius tankervalleae* was collected in Gene Pool Garden, Gudalur, the Nilgiris and were used as a source of explants.

A. *Sterilization*

The fresh capsules collected were carefully washed with running tap water and then with 0.1% teepol detergent. The capsules were disinfected by surface sterilization with 3% sodium hypochlorite for 20 mins, and then washed for 4-5 times in sterile distilled water. The capsules were dipped for a minute in ethyl alcohol (80%) and finally rinsed thoroughly 5-6 times with distilled water. The capsules were then spitted longitudinally with sterilized scalpel and the seeds were collected and used for inoculation.

B. Culture Medium

In the present study, MS medium (Murashige and Skoog) with or without plant growth hormones was used. MS nutrient media supplemented with various concentration of BAP alone and with combination of NAA used for seed germination. 0.8% agar was added to the medium and the pH was adjusted to 5-8. The medium id then sterilized at 121°C for 20 minutes. Culture were maintained at 25±2°C below a 16 h photoperiod supplied all the way through white fluorescent tubes (Philips, India).

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C. *Inoculation*

The seeds which were sterilized were then placed on the surface of the medium supplemented with or without, either in combination of BAP alone and in combination with NAA. The seeds maintained on the medium produced protocorn like bodies (PLBs). Those PLBs were sub-cultured in MS basal medium containing sucrose (3% w/v) and agar (0.8%) with BAP at (0.5 – 2.5mg/L) for plantlets and mass multiplication. Shoots of length 2-3cm, were separated and transferred to half MS medium with 0.5-2.5 mg/ml of IBA for root initiation. The residual medium was removed from well rooted shoots and transferred to plastic cups containing nutrient medium. The nutrient medium is prepared by the mixture of soil, sand, charcoal and brick pieces (1:1:4:4). The pots were then maintained in the green house at temperature 28±2°C and 80-90% humidity. Cultures were sub-cultured into fresh medium once in every eight weeks and were replicated thrice. The rate of seed germination was noted every week. To prevent microbial contamination, the complete experiment was done under laminar air flow in aseptic condition.

III. RESULTS AND DISCUSSION

The current studies on *P. tankervilleae*, seed germination and mass multiplication mainly depend on the age of the seeds, culture media and plant growth regulators (PGRs). The nodule like structure formed from the seeds after 40 days was the initial stage of seeds germination. In most of the cases mature seeds failed to germinate and in some case only nodule like structure was observed after a long period of culture. The seeds of 7 weeks after pollination failed in germination. The time taken for the germination of seeds after pollination varies with species. The seed propagation of *P. tankervilleae* was difficulty, hence rarely been cultivated. The storage of seeds for long term was also a difficult task (Hirano et al., 2009).

The rate of seed germination of *P. tankervilleae* was found to be variable in MS medium without growth regulators and MS medium with various concentrations of BAP and NAA. Of the different concentration of medium used for inoculation, medium with BAP (2.5 mg/l) in combination with NAA (0.5 mg/l) were most suitable for germination of seeds (72 ±5.1) (Table:1; Fig:A) when compared to those basal medium supplemented with hormones. In this study, MS medium with and without plant growth hormones were found to be proficient for seed germination. This might be due to the MS medium enriched with both macronutrients, micronutriients and vitamins that favored seed development. MS basal medium fortified with different plant growth hormones improved the nutritional status of the basal medium and favored earlier seed germination and protocorm formation (Rocky Thokchom et al., 2017).

MS+Growth Regulators		seed germination %	Average No. of seed germination
BAP	NAA		
0	0	0	0
0.5	0.5	0	0
1.0	0.5	36± 2.4	1.3± 0.4
1.5	0.5	44.5± 4.3	1.4± 0.6
2.0	0.5	59±3.1	2.3± 0.6
2.5	0.5	72 ±5.1	3.4± 0.5
3.0	0.5	64± 6.8	2.9± 0.6
1.0	1.0	12± 7.2	3.0± 0.4
2.0	1.0	30± 2.1	3.8± 0.6
2.5	1.0	36 ±0.5	3.7± 0.5

Table 1:- Effect of various growth hormones for seed germination of *Phaius tankervilleae*
(Values are Mean ±Standard Error (SE) after culture of six weeks)

The protocorm like body thus obtained from seed germination were sub-cultured in MS medium with different concentration of plant growth hormones. The medium with BAP with concentration 2.0 mg/L showed production of the shoot proliferation (64.4±1.0) in 8-10 weeks (Table: 2; Fig:B&C). Sheelavantmath *et.al* (2000) reported that in Vanda, BAP and NAA in the culture medium showed better shoot proliferation from rhizomes than BAP alone.

MS + BAP (mg/ltr)	Germination %	Average No. of shoots/ plant
0	0	0
0.5	0	0
1.0	36.4±0.5	2.3±0.6
1.5	50.6±0.6	3.0±0.6
2.0	64.4±1.0	3.0±0.6
2.5	42.6±1.2	2.7±0.6
3.0	30.6±1.0	2.1±0.5

Table 2:- Effect of various growth hormones for shoot multiplication of *Phaius tankervilleae*
(Values are Mean ±Standard Error (SE) after culture of six weeks)

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The shoots were transferred in the half MS basal media containing 1.5 mg/L IBA (72.6± 1.3) alone favored for roots after 4-5 weeks (Table:3; Fig:D). The multiplication medium with activated charcoal (AC) influenced production of shoots, but the culture in medium with higher concentrations of Activated Charcoal (0.3%) turned brown and later showed no growth. Seeds could not undergo differentiation in MS medium without plant growth hormones because terrestrial orchids require more plant growth regulators for immature seeds to develop into seedlings (Pant et al., 2011). The results from the above findings showed that MS basal medium supplemented with plant growth hormones were found to be more effective for growth and development of seeds into seedlings.

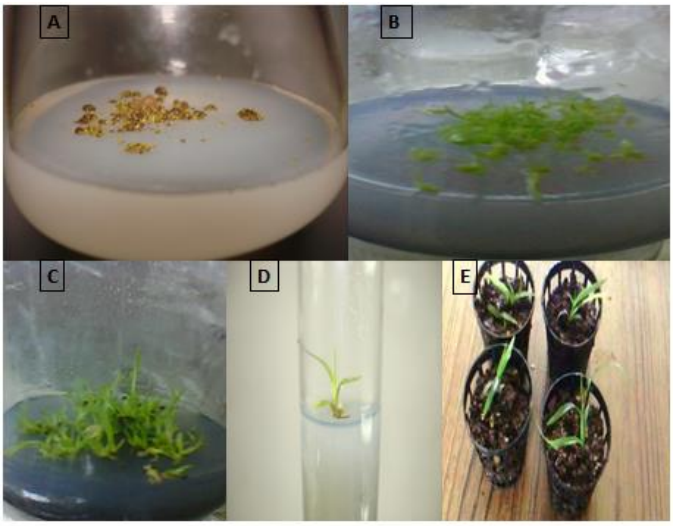
½ MS+ IBA (mg/l)	% of cultures with roots	Average No. of roots/ plant
0.5	0	0
1.0	62.4±1.3	2.3±0.6
1.5	72.6± 1.3	3.3±0.6
2.0	68.6±1.4	2.8±0.6
2.5	50.6±1.0	1.7±0.5

Table:- 3 Effect of growth hormones for root initiation of *Phaius tankervilleae*
(Values are Mean ±Standard Error (SE) after culture of six weeks)

The development of plants that took long term in normal environment was considerably reduced by the use of in-vitro propagation methods. Here emerges the importance of *in vitro* propagation and consequent ex- vitro cultivation methods of rare and endangered orchids and even in reintroducing the species. The rooted plantlets with minimum of 3-5 roots per plants developed on medium under controlled condition were taken to hardening after 8-9 week (Fig:E). During hardening the plantlets were placed in culture vessels under control environment. The hardened plants were transferred to pots and then to natural environment. Finally 65% of the transplanted plantlets were survived. Similar results were reported for several other orchids using (chang and chang et al 2000) and Teng et al (1997).

IV. CONCLUSION

The current investigation reports proficient and effortless procedure for *in vitro* seedling development of *Phaius tankervilleae* using immature seeds as explant. Optimal use of BAP and NAA along with the MS medium was found to be effective for germination of seeds, formation of nodule like structures or protocorms, growth and seedlings development. The rooted plantlets in vitro can be effectively hardened ex vitro. This procedure may be useful for propagation and conservation of this most valued and rare orchid species.



Figs A-E
(A) PLBs development from immature seeds on MS medium with BAP (2.5mg/l) and NAA (0.5 mg/l).
(B) Proliferation of shoot buds on MS medium with 2mg/l of BAP.
(C) Multiple shoots formation on MS medium.
(D) Well differentiated plantlet with roots on ½ strength MS medium with IBA (1.5 mg/l).
(E) *In vitro* raised plants established inside pot after 30 days of transfer.

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