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In Vitro Propagation of *Phaius luridus Thwaites* - A Terrestrial and Endemic Orchid of Western Ghats

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ABSTRACT

Family Orchidaceae constitutes one of the largest families of flowering plants, having around 20,000 species. They are unique in forms, colors and flower structure. The genus *Phaius luridus Thwaites* is the terrestrial orchid, endemic to the Western Ghats and is an endangered species. A rapid *in vitro* seed germination technique is described here. MS, VW, B5 and KC media supplemented with various concentrations of auxins and cytokinins were used in combination for asymbiotic seed germination and plantlet formation. In the evaluation of the media MS medium supplemented with 2 mg BAP/L^{-1}+ 5mg NAA/L^{-1} was found to be suitable with both liquid and solid. Even B5 solid and liquid medium supplemented with 2 mg BAP/L^{-1}+1mg IAA/L^{-1} was found to be suitable. Further, hormonal concentrations of auxins and cytokinins were evaluated for minimal and optimal levels in the medium. Hardened plants were transferred to green house after *ex vitro* rooting technique. Significance of the present work is discussed here.

Keywords: *Phaius luridus Thwaites,* PLB's,MS,VW, B5, BAP, NAA, IAA, CM, *Ex Vitro* Rooting.

I. INTRODUCTION

The terrestrial orchids, the jewel orchids, are grown mainly for their attractively patterned foliage. There are several genera which occur naturally in the deep shade of tropical forests, growing in the leaf litter [(10)].

The genus *Phaius luridus Thwaites* [(1,3)]was established in 1790 by Jao De Loureiro during 1790, in his Flora Cochinchinensis. It is a terrestrial genus represented with about 40 species, distributed in tropical Africa, Madagascar, tropical and subtropical Asia to Oceania; six species are estimated from India [(4,11)]. One such species is *Phaius luridus Thwaites* found in Western Ghats in Shimoga district of Sagar.

Phaius luridius produces large, thin pleated leaves, usually few in number, which grow to about 3' in height. The inflorescence arises from a pseudobulb (a short, fleshy shoot found in most orchids) or rhizome, and consists of an erect four foot raceme of showy, fragrant flowers. Individual flowers of the nun's orchid are large, up to 5" across, rusty brown with a purplish lip.[(5,6)] Flowers are believed to be initiated in response to short day length, mainly late winter and spring. Each inflorescence opens over a period of up to six weeks. The Flowering is between May and June [(8)].

Asymbiotic germination on basal nutrient medium [(9)] and a combination of various growth regulators [(2)] are an efficient and fast method for mass multiplication of orchids. Hence this investigation was undertaken for judicious use of growth regulators [(7)] during *in vitro* seed germination of *Phaius luridius Thwaites.*

II. METHODS AND MATERIAL

Phaius luridus Thwaites was collected from Sagar, Shimoga district and were grown in Green house at St. Joseph's College Post Graduate and Research Centre. The fruit capsules approximately 90 days old were collected for culture. Two protocols were used for surface sterilization of capsules.

Inoculations of disinfected explants and sub-culturing were carried out under aseptic environment, in a

horizontal Laminar Air Flow Unit. Explants were placed on the nutrient medium in culture bottles/tubes with a sterilized forceps. Various basal media like MS, B5, KC and VW were used supplemented with various combinations of Auxins and Cytokinins. PH of the medium was maintained at 5.6 -5.8 the medium with MS and B5 gave good results.

Culture Conditions

- The cultures were incubated at 25± 2^0C temperature
- Photoperiod 16/8 h with 4000- 5000 lux illumination from cool white fluorescent tubes ("Philips", India).
- Humidity level with air condition was between 50-60%.

Maintenance of Cultures

- Cultures were regularly sub-cultured based on the type of cultures, designed in an experiment.
- The sub culturing was done every 2 weeks and observation was made for both solid and liquid medium.
- Each experiment was repeated twice and consisted of 3 replicates of 10 explants for each treatment.

Technique of Hardening Process

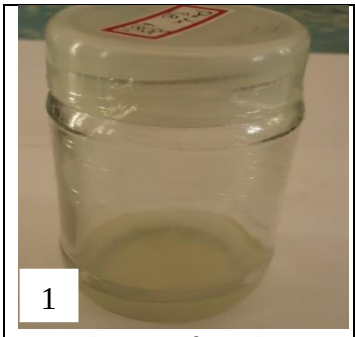
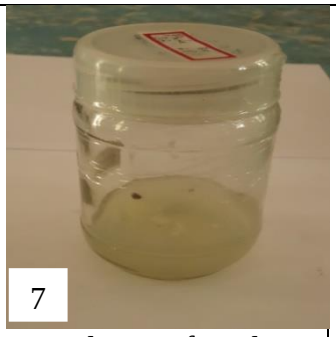
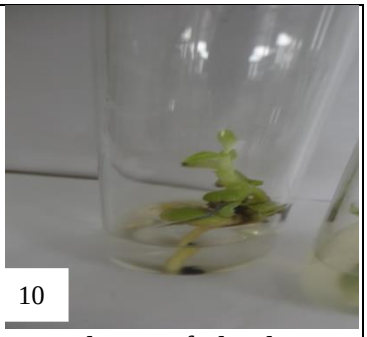
90 days old plantlets with good in vitro rooting and with 3-4 leaf conditions were selected for hardening. Tissue cultured bottles with plantlets were shifted from growth room conditions and were exposed to natural light conditions inside the laboratory area for 4 days. Further plantlets where transferred to the thumb pots containing solrite (a mixture of pearlite and peatmoss).Plants were covered with perforated plastic cup with optimum humidity conditions. Plants were shifted to green house after 10 days.

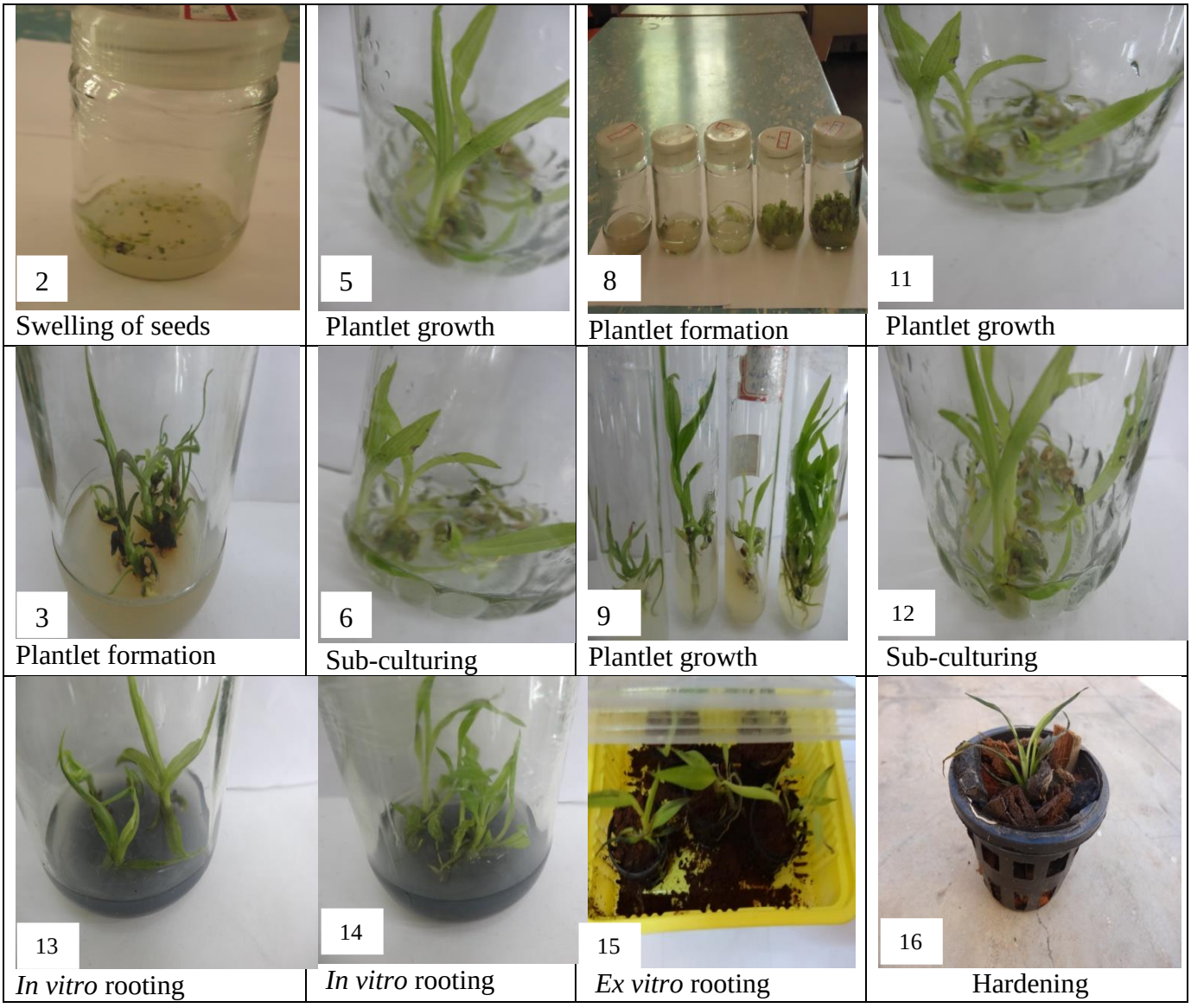
OBSERVATION

MS medium with (2 mg BAP/L^{-1}+ 5mg NAA/L^{-1}) favoured production of maximum number of shoots. Both solid and liquid medium gave good results.

***In vitro* rooting**: *In vitro* rooting was successful with VW medium supplemented with (2.0 mg BAP/L, NAA 5mg/L, 50 ml CM and 500 mg of activated charcoal) induced good rooting. B5 medium with (2 mg BAP/L, 1 mg IAA/L with 500 mg of activated charcoal) also gave good results.

***Ex vitro* rooting:** The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of IAA (made in ditilled water) then planted in thumb pots containing solrite (mixture of pearlite and peatmoss) and sprayed with bavistin to avoid fungal infection. *In vitro* rooted plants in the portrays containing potting mixture maintained under mist chamber and covered with perforated plastic cups.

MS with	**MS with**	**B5 with**	**B5 with**
 1 Inoculation of seeds	 4 Inoculation of plantelts	 7 Inoculation of seeds	 10 Inoculation of plantlets

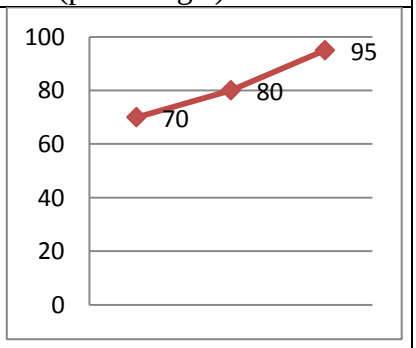


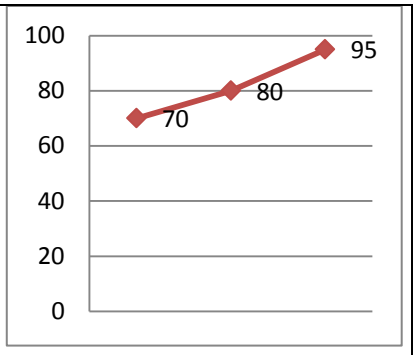
III. RESULTS AND DISCUSSION

MS medium gives good result comparing with B5 medium. Both the mediums are good for the raising of plantlets.

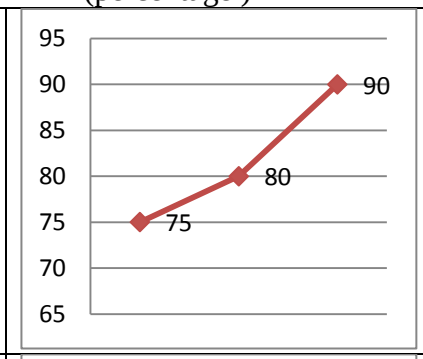
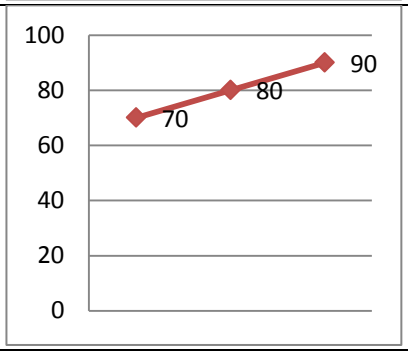
Plantlet formation with MS (solid and liquid) medium

Media Used	Media Composition	Results The average plantlets formation (percentage)
MS (Solid Medium)	Basal MS Media + 1 mg BAP + 1 mg NAA	70%
	Basal MS Media + 2 mg BAP + 2 mg NAA	80%
	Basal MS Media + 2 mg BAP + 5 mg NAA	**95%**

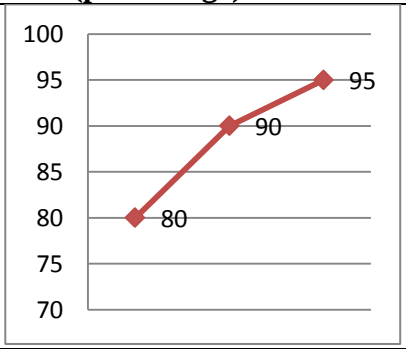


MS (Liquid Medium)	Basal MS Media + 1 mg BAP + 1 mg NAA Basal MS Media + 2 mg BAP + 2 mg NAA **Basal MS Media + 2 mg BAP + 5 mg NAA**	70% 80% **95%**	

Plantlet formation with B5 (solid and liquid) medium

Media Used	Media Composition	Results The average plantlets formation (percentage)	
B5 (Solid Medium)	Basal B5 Media + 1 mg BAP + 1 mg NAA Basal B5 Media + 2 mg BAP + 2 mg NAA **Basal B5 Media + 2 mg BAP + 5 mg NAA**	75% 80% **90%**	
B5 (Liquid Medium)	Basal B5 Media + 1 mg BAP + 1 mg NAA Basal B5 Media + 2 mg BAP + 2 mg NAA **Basal B5 Media + 2 mg BAP + 5 mg NAA**	70% 80% **90%**	

***In vitro* rooting with MS media**

Media Used	**Media Composition**	**Results The average plantlets formation (percentage)**	
MS	Basal MS Media+ 2 mg BAP + 5 mg NAA + 150 ml CM + 500 mg AC Basal MS Media+ 1 mg BAP + 3 mg NAA + 150 ml CM + 500 mg AC **Basal MS Media+ 0.5 mg BAP + 2 mg NAA + 150 ml CM + 500 mg AC**	80% 90% **95%**	

IV. CONCLUSION

It is observed that the present status of *Phaius luridus* is rare in habitat and the natural population in the study regions is very meager. If a regular thread persists in the regions, it will push the species in threatened status in natural habitat. Therefore, conservation of habitat is most necessary for the protection of this species in Karnataka.

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