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In Vitro Propagation of Epiphytic Orchid Pholidota Imbricata Hook. of Western Ghats

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Abstract - This study was conducted to investigate the role of different media fortified with various compositions of hormones for developing a rapid method for *in vitro* seed germination of the epiphytic orchid *Pholidota imbricata Hook*. and to study its morphogenetic responses. MS, VW, B5 and KC media were used, among which KC basal medium supplemented with 3 mg BAP/L^{-1}, 5 mg NAA /L^{-1} and 50 ml CM is found to be most suitable for induction of PLBs. Microscopic seeds germinated directly forming PLBs indicating direct organogenesis. Callogenesis was not noticed in any of the cultures raised. KC medium supplemented with 2mg BAP/L^{-1} and 5mg NAA/L^{-1} along with 50 ml coconut milk and 500 mg activated charcoal was found to be suitable for *in vitro* rooting. 90 days old sub cultured *in vitro* plantlets with pseudobulbs, size measuring 2 cm, were subjected to *ex vitro* rooting followed by hardening. For *ex vitro* rooting induction, roots were treated with 500 ppm bavistin, a systemic fungicide followed by treatment with 200 ppm IAA. Plantlets were transferred to thumbpots filled with solrite medium and were allowed to develop under high humidity conditions of green house for healthy growth.

***Key Words - Pholidota imbricata Hook*. PLB's, KC, BAP, NAA, IAA, CM, pseudobulbs, *in vitro* rooting, *ex vitro* rooting.**

Abbreviations

KC-Knudson C medium, MS-Murashige and Skoog, VW-Vacin and Went, B5 - Gamborg B5 medium, PLB–Protocorm like bodies, BAP – Benzyl Amino Purine, NAA–Naphthalene Acetic Acid, IAA–Indole Acetic Acid, CM–Coconut Milk

INTRODUCTION

Pholidota imbricata Hook.[1] is a pseudobulbous, tropical epiphytic orchid commonly referred to as the 'necklace orchid'. It is found in the moist and dry deciduous forest of Western Ghats at lower altitudes of 1500ft to 2000ft. This orchid is distributed in restricted localities, but occur in abundance in those localities[4]. Many of the distributed localities are easily accessible and hence those orchids are vulnerable to over-exploitation and can be endangered due to excessive habitat destruction [5]. This plant is known for its ethanobotanical purposes and ayurvedic practices [8, 9]. The extract of the plant is found to have good antibacterial and antifungal properties against organisms like *Vibrio cholerae* and *Staphylococcus aureus*[10]. Hence *in vitro* propagation methods are of significance not only for germplasm preservation, but also *in vitro* micropropagated plants can be returned back to nature under suitable environmental conditions.

Asymbiotic germination on basal nutrient medium [7] and a combination of various growth regulators [2] are a gift to the Orchid industry, particularly for raising hybrids. Hence this investigation was undertaken for judicious use of growth regulators during *in vitro* seed germination of *Pholidota imbricata Hook.*

*Pholidota imbricata Hook.*is a peudobulbous epiphyte. Rhizome creeping, rather robust, with many nodes, densely covered with scaly sheaths, with many roots. Pseudobulbs contiguous, suboblong, obscurely obtusely 4-ridged, apex 1-leaved. Leaf blade oblong- oblanceolate, oblong, or nearly broadly oblanceolate, thinly leathery, base cuneate, apex shortly acuminate or acute. Inflorescence arising from young pseudobulbs with nearly mature leaf at anthesis, Densely many flowered; floral bracts persistent, often conduplicate, broadly ovate. Flowers white or slightly tinged with red, lateral sepals free, ovate, cymbiform, dorsally strongly carinate. Petals sublinear-lanceolate veined; lip saccate, slightly 3-lobed; lateral lobes embracing column, erect, nearly broadly oblong, mid-lobe suboblong, 3–4 mm wide, margin slightly undulate, apex emarginate; disk with 2 or 3 longitudinal lamellae or thickened veins near base.Capsule obovoid-ellipsoid[11]. Superposed pollinia is found[6] Flowering is usually between June to August[3]

MATERIAL AND METHODS

Collection:

Pholidota imbricata Hook was collected from Sagar, Shimoga district of Karnataka and was grown in Green house at St. Joseph's College Post Graduate and Research Centre, Bangalore.

Inoculation and culture conditions:

Healthy capsules were harvested from the plants which are approximately three months old. Using a scalpel, dried up tepals and dead tissues of the capsule were carefully cut. The dry capsule was swabbed in 75 % alcohol. Inside laminar air flow cabinet dry capsule was swabbed with 100% alcohol and was quickly flamed using a spirit lamp. Then the dry capsule was cut open and seeds were innoculated on the nutrient media.

KC was used with different composition and supplemented with various combinations of Auxins and Cytokinins. pH of the medium was maintained at 5.8.

The cultures were incubated at 25± 2^0C temperature and with 8-16 hours photoperiod with 4-5000 lux illumination from cool white fluorescent tubes ("Philips", India). Humidity level with air condition was between 50-60%.

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

In vitro rooting:
In vitro rooting was successful with KC medium supplemented with (2 mg BAP/L^{-1} and 5mg NAA/ L^{-1}, 50 ml coconut milk and 500 mg activated charcoal).

Ex vitro rooting:
Plantlets were carefully removed from the culture bottles/ test tubes and were subjected to gentle washing of the root system. Roots were treated with 500 ppm Bavistin, a systemic fungicide for 2-3 minutes. For *ex vitro* rooting induction, shoots were given treatment with 200 ppm IAA. Plantlets were transferred to thumbpots filled with solrite medium.

Hardening Process:
Plantlets were also subjected to high humidity conditions of green house for healthy growth. Well grown shoots from the shoot multiplication medium were directly transferred to small pots containing 1:1:1 - charcoal: sand: peat moss and kept covered with perforated plastic cups at room temperature 32±2 ^{0}C. Successfully established plantlets were subsequently transferred to green house conditions.

OBSERVATIONS

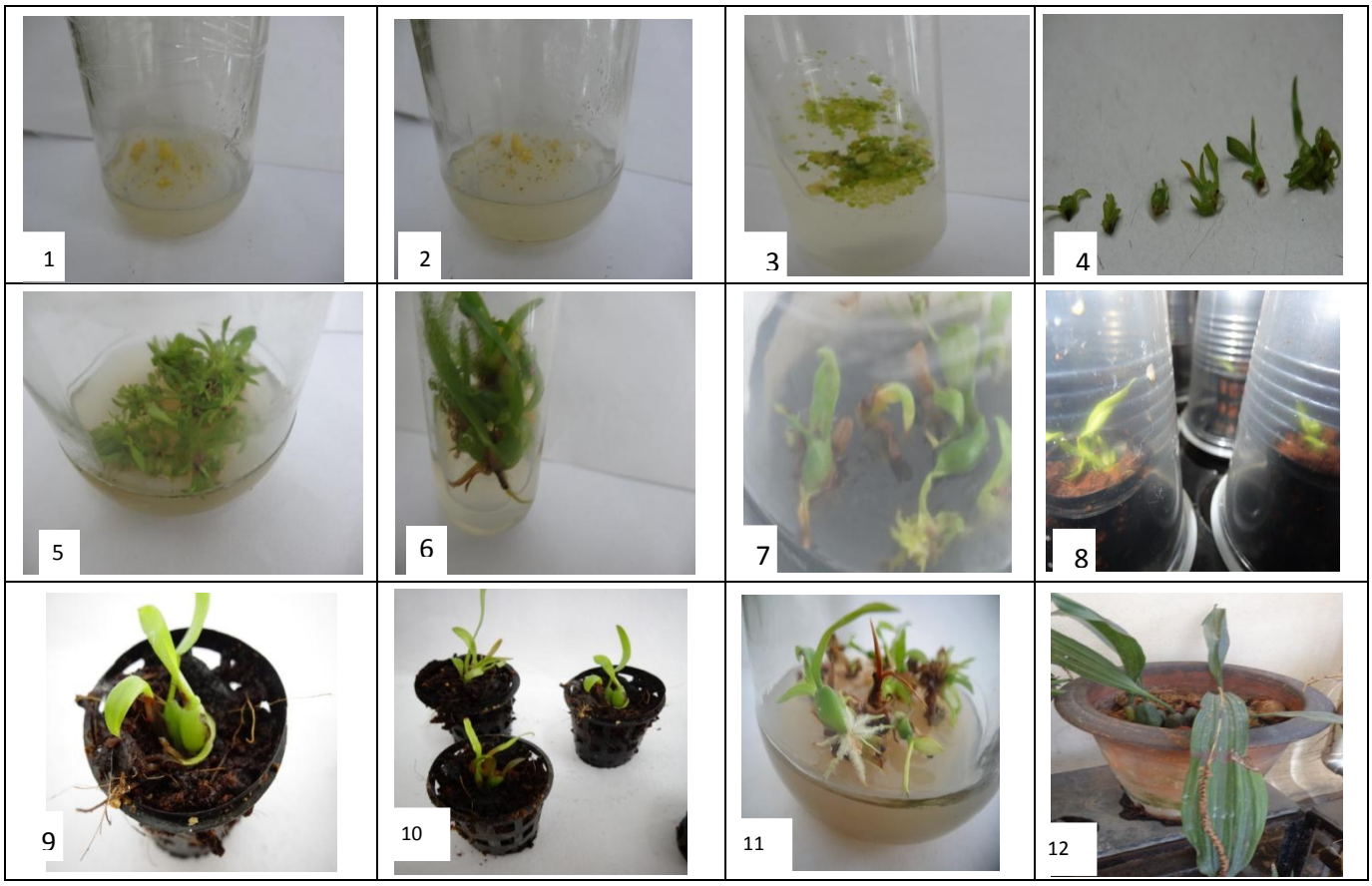


Fig. 1 – Inoculation of seeds
Fig 2 – Swelling of seeds
Fig. 3 – Greenish and yellowish swelling of the seeds
Fig 4 – Morphogenetic stages of pseudobulbs
Fig 5 – Multiple shoot formations - Basal KC Medium + 3mg BAP + 5mg NAA + 50 ml CM
Fig 6 – Sub culturing, transferring of the plantlets to test tube and bottles
Fig 7 – *In vitro* rooting - Basal KC Medium + 2mg BAP + 5mg NAA + 50 ml CM + 500 mg AC
Fig 8– *Ex vitro* rooting
Fig 9 to 10 – Hardening
Fig 11 – Plantlet inside the lab
Fig 12 – Plant inside the green house

Fig 13 (A) – The Morphogenetic stages of pseudobulb development

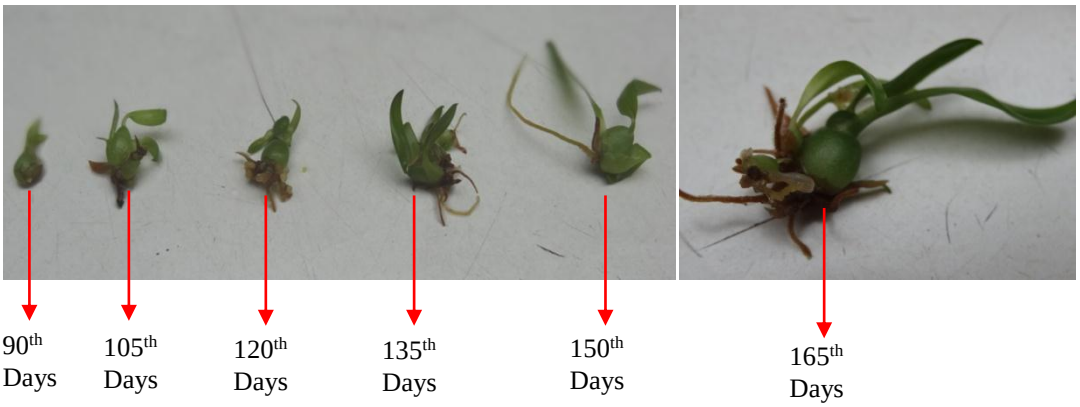
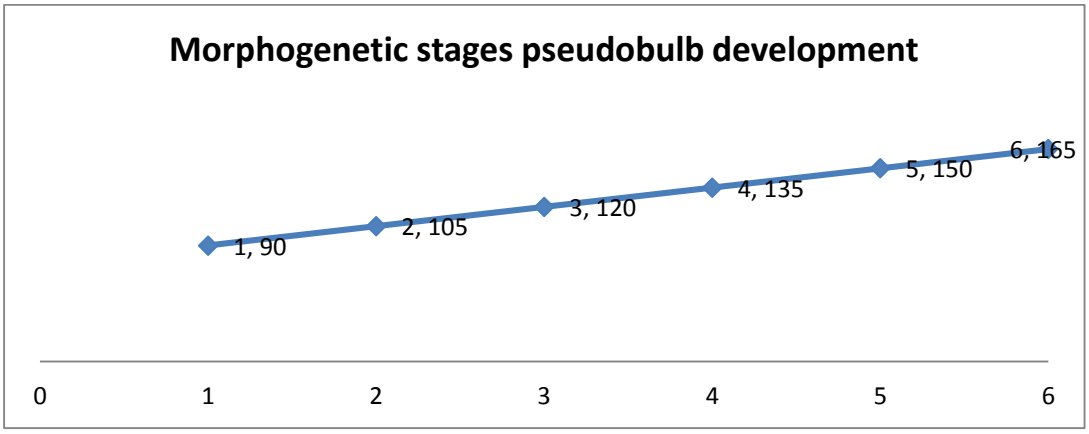
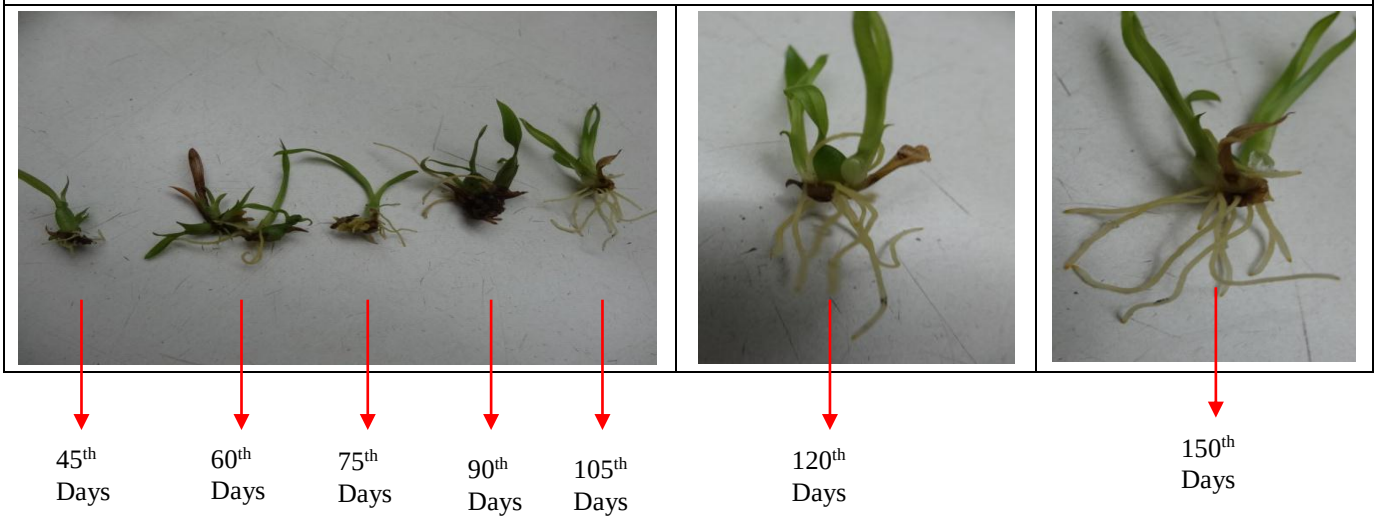


Fig 13 (B) – The Morphogenetic stages of pseudobulb development



After 45 days of sub-culturing for *in vitro* rooting, the following were observed.

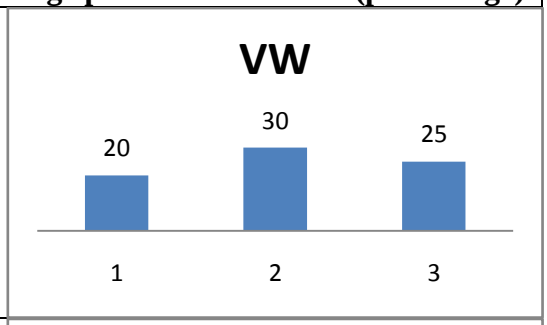
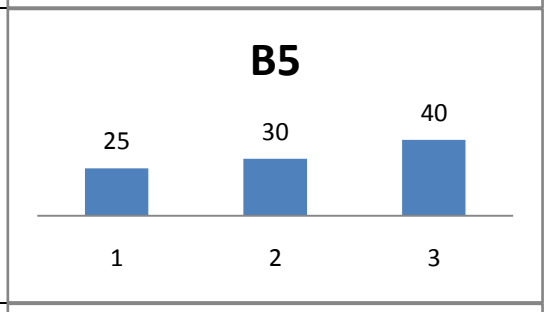
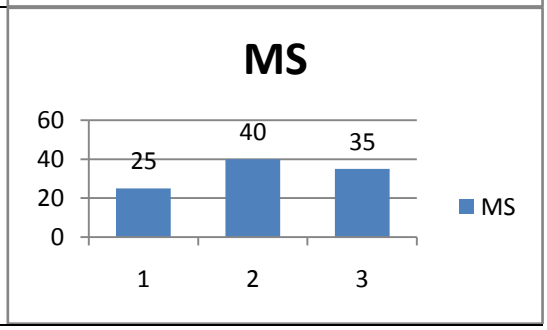
Fig 14 : In vitro rooting



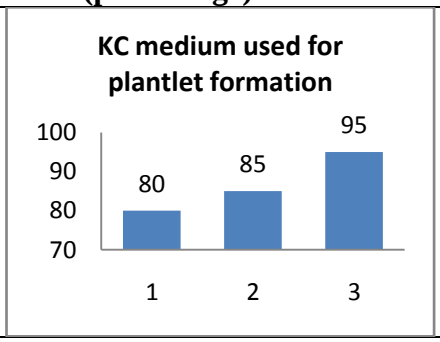
RESULTS AND DISCUSSION

In comparison with VW, B5 and MS medium, KC medium gives good result

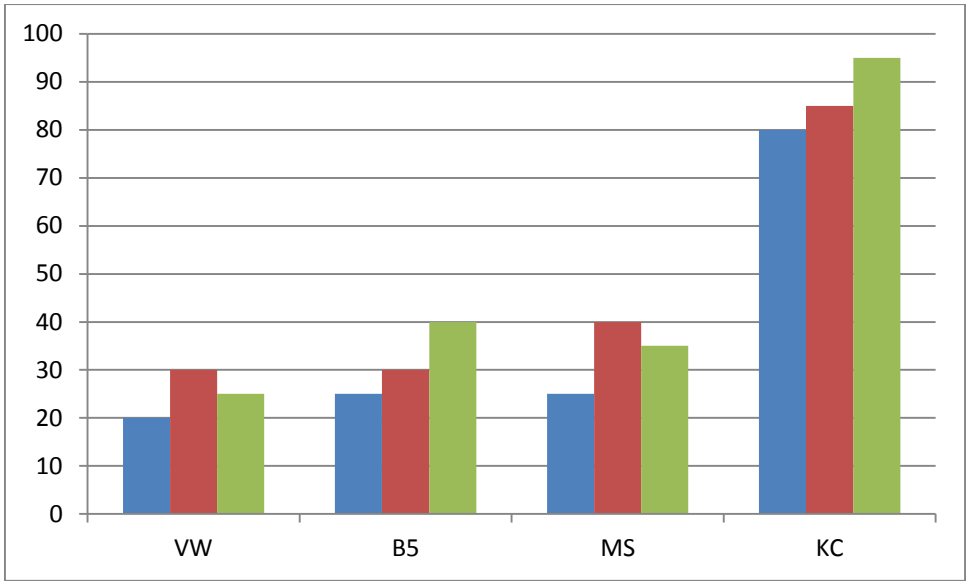
VW, B5 and MS Media used for plantlet formation (Table 1)

Media Used	Media Composition	Results The average plantlets formation (percentage)	
VW	Basal KC Medium+ 1 mg BAP + 3 mg NAA Basal KC Medium + 2 mg BAP + 5 mg NAA Basal KC Medium + 3 mg BAP +10 mg NAA	20% 30% 25%	
B5	Basal B5 Medium + 1 mg BAP + 1 mg NAA Basal B5 Medium + 2 mg BAP + 1 mg NAA Basal B5 Medium + 3 mg BAP + 2 mg NAA	25% 30% 40%	
MS	Basal MS Medium + 1 mg BAP + 1 mg NAA Basal MS Medium + 2 mg BAP + 2 mg NAA Basal MS Medium + 2 mg BAP + 5 mg NAA	25% 40% 35%	

KC medium composition for plantlet formation (Table 2)

Media Used	Media Composition	Results The average plantlets formation (percentage)	
KC	Basal KC Medium + 1mg BAP+ 3mg NAA+ 50 ml CM Basal KC Medium + 2mg BAP+ 10mg NAA+ 50 ml CM **Basal KC Medium + 3mg BAP+ 5mg NAA + 50 ml CM**	80% 85% **95%**	

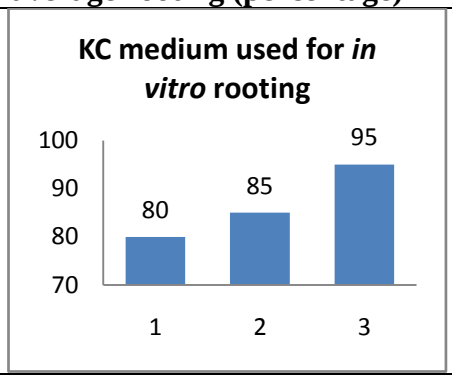
Comparative plantlet formation based on different media used



Germination was faster on the KC medium when compared to the VW, B5 and MS medium (Table 1). KC medium fortified with different concentrations of hormones were used which gave good results. KC medium supplemented with 3mg BAP/L^{-1} and 5mg NAA/L^{-1} and 50 ml Coconut milk was however found to be the most favourable for plantlet formation (Table 2). The percentage of germination was found to be higher with KC medium showing multiple shoots and pseudobulbs of various stages. Thus it can be concluded that KC medium is most suitable for *Pholidota imbricata* seed germination .This study also revealed that a high concentration of 3mg BAP/L^{-1} and 5 mg NAA/L^{-1} was found to be more suitable for plantlets and multiple plantlets.

KC - For the *In vitro* Rooting (Table 3)

Media Used	**Media Composition**	**Results The average rooting (percentage)**
KC	Basal KC Medium + 0.5mg BAP+ 3mg NAA+ 50 ml CM +250 mg AC	80%
	Basal KC Medium + 1mg BAP+ 10mg NAA + 50 ml CM+750 mg AC	85%
	Basal KC Medium + 2mg BAP+ 5mg NAA + 50 ml CM + 500 mg AC	**95%**



KC medium was also suitable for *in vitro* rooting. KC medium supplemented with 2mg BAP/L^{-1}, 5mg NAA/L^{-1} along with 50 ml coconut milk and 500 mg activated charcoal was found to be suitable for *in vitro* rooting (Table 3). *Ex vitro* rooting was done by dipping the healthy shoots in test tubes containing 10 ml of 200 ppm IAA solution, along with bavistin to avoid fungal infection. The plants with good rooting were transferred to community pots and then to greenhouse conditions.

The different stages of psuedobulbs were recorded. After 90 days, plants with pseudobulbs were produced inside the bottles. The pseudobulbs were found to have phytochemical significance. Experimental work is under progress.

SCOPE

- The pseudobulbs have medicinal and phytochemical significance which can be further investigated.
- The pseudobulbs have high water content and nutrients and hence can be of nutritional significance.
- The plant can be preserved by cryopreservation techniques.
- Plant tissue culture of *Pholidota imbricata* can immensely aid conservation and it can be returned to the original habitat.

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