IN VITRO SEED GERMINATION OF XENIKOPHYTON SMEEANUM(RCHB.F.) GARAY : A RARE ORCHID OF WESTERN GHATS, KARNATAKA

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ABSTRACT

Xenikophyton smeeanum (Rchb.f.) Garay from high elevation reaches the grasslands grown on standalone trees in the grassland and forest edges. Genus *Xenikophyton smeeanum* is a rare genus of family Orchidaceae with two species distributed along the Western Ghats in South India. No phylogenetic studies have been conducted till date and hence multiplication by in vitro methods can be helpful in doing further research to ascertain more about this orchid. VW and KC was used for plantlet formation. *KC* medium media with 3 mg BAP + 1 mg NAA+ 50 ml CM which did not give good results therefore for asymbiotic seed germination and plantlet formation VW medium supplemented with 2 mg BAP + 1.5 mg NAA + 50 ml CM was found to be most suitable. VW fortified with Basal + 0.5 mg BAP + 1mg IAA + 50 ml CM + 500 mg AC was found to be most suitable for *in vitro* rooting. Hardened plants were transferred to green house after *ex vitro* rooting technique. Significance of the present work is discussed here.

KEY WORDS

Xenikophyton, VW, NAA, IAA, BAP, AC, CM, B5, KC.

ABBREVIATIONS

BAP – Benzyl Amino Purine, NAA–Naphthalene Acetic Acid, IAA–Indole Acetic Acid, CM–Coconut Milk, AC – Activated Charcoal, VW-Vacin and Went medium, B5 - Gamborg B5 medium, KC – Knudson C.

INTRODUCTION

Orchidaceae is one of the largest families of flowering plants. *Xenikophyton smeeanum (Rchb.f.) Garay* is an epiphytic orchid originating in India. (Govaerts, R. (2003). World Checklist of Monocotyledons Database in ACCESS: 1-71827. The Board of Trustees of the Royal Botanic Gardens, Kew)

The two species of Xenikophyton—*X. smeeanum* and *X. seidenfadenianum* M.Kumar, Sequiera & Arditti. J —are limited to southern India. Both species are epiphytes at 900–1350 m in India. No uses have been reported for Xenikophyton; it is rare in cultivation. (Pridgeon, Daws, M. I, Dixon, K.W, Ichihashi, S, Chase, Knudson L (1921), Kulkarni, M.G, Finn N, 2014. Genera Orchidacearum: Volume 6. Epidendroideae (Part Three))

Erect epiphytic herbs. Stem simple or with an occasional short branch, leafy. Inflorescence erect, racemose or paniculate, many-flowered; Flowers about 4 mm long, resupinate, mostly greenish white

streaked with brown, labellum greenish white or white, sometimes with violet around entrance. (Pridgeon, Alec M, Cribb, Phillip J, Chase, Mark W, Rasmussen, Finn N, 2014. Genera Orchidacearum: Volume 6. Epidendroideae (Part Three)) Flowering and fruiting in the month of May-August.

This genus has so far not been included in DNA-based phylogenetic analyses, and therefore its systematic position within the subtribe is still undetermined. *Xenikophyton smeeanum* is having minute flowers with the labellum distinctly projecting beyond the sepals and petals, . (Pridgeon, Alec M, Cribb, Phillip J, Chase, Mark W, Rasmussen, Finn N, 2014. Genera Orchidacearum: Volume 6. Epidendroideae (Part Three))

Asymbiotic germination on basal nutrient medium (Knudson, 1951) VW Medium (vacin and went medium, 1949) and a combination of various growth regulators (Arditti, 1979; Rucker, 1974) are a gift to the Orchid industry. *In vitro* asymbiotic seed germination is thus considered much faster and effective method for conservation and mass multiplication of rare, threatened and endangered orchids. Hence this investigation was undertaken for judicious use of growth regulators during *in vitro* seed germination of *Xenikophyton smeeanum.*

MATERIALS AND METHODS

Plant specimen collection was made from dry deciduous forest around Somwarpet taluk in the north-east of the Coorg district between 1st July 2012 and 6th July 2012. Somwarpet is located at 10.42°N 74.73°E latitude. It has an elevation of 1525 metres (5003 feet). During the visit, the average temperature was about 16°C, Wind NW at 3 km/h and 93% humidity.

Plant specimens were collected from the natural environment in perforated, clean, polythene bags. Care was taken to ensure to retain the mother plant intact in its natural epiphytic habitat. The plants were taken to the department of Botany, St.Joseph's post-Graduate and Research centre for planting in the green house. Standard floras were referred for authentication of the genus and species. Herbaria were prepared using standard protocol and voucher specimens were deposited in the herbaria of St.Joseph's college Post-graduate and research centre, Bangalore.

Green capsules of wild were collected and then rinsed thoroughly three times with sterile distilled water, followed by dipping them in 70% ethanol for 30s. Sterilized capsules were dried and then split longitudinally with sterile surgical blade. Seeds were inoculated nutrient media. VW medium which was prepared with various concentrations and combinations of phytohormones and other additives gave the best results (2mg BAP +1.5mg NAA +50 ml CM). So VW was standardized for *Xenikophyton smeeanum* for plantlet formation. Seed cultures were placed in growth chamber at 25 ± 20 C and 70 –80% relative humidity under 24h-light and under 16h-light/8h-dark with light provided by cool white fluorescent lamps for 70 days.

Sub-culturing was regularly done every 15 days and observations were made. Each experiment was repeated twice and consisted of 3 replicates of 10 explants for each treatment

***In vitro* rooting**

In vitro rooting was most successful with VW media supplemented with 0.5 mg BAP, 1 mg IAA, 50 ml CM and 500 mg AC.

***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 pm 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 thumb pots filled with solrite medium.

Hardening Process:

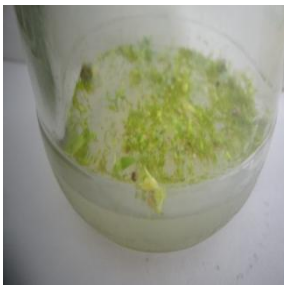
Plantlets were also subjected to high humidity conditions of green house for healthy growth. Well grown shoots from the shoot multiplication medium were directly transferred to small pots containing 1:1:1 - charcoal: sand: peat moss and kept covered with perforated plastic cups at room temperature 32$\pm$2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

1.Xenicophyton Plant with Flowers

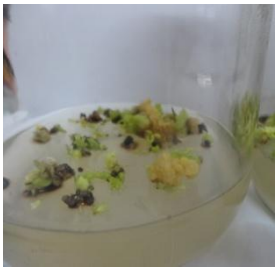
2. Flower

3.Xenicophyton Plant with Fruits

4.Inoculation



5.Slight swelling of seeds



6. Initial stages

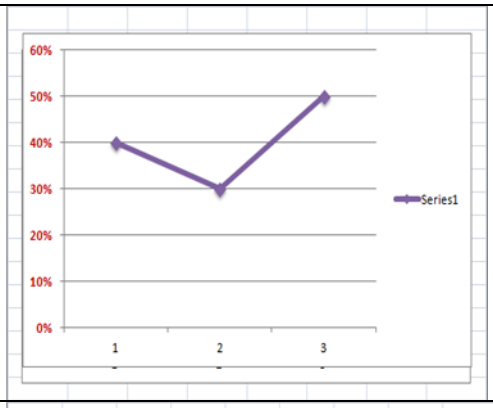
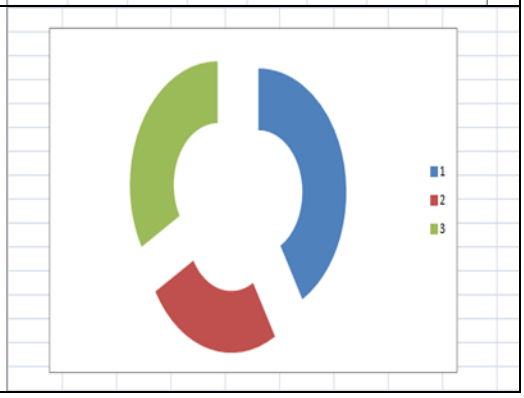
7.Plantlet formation

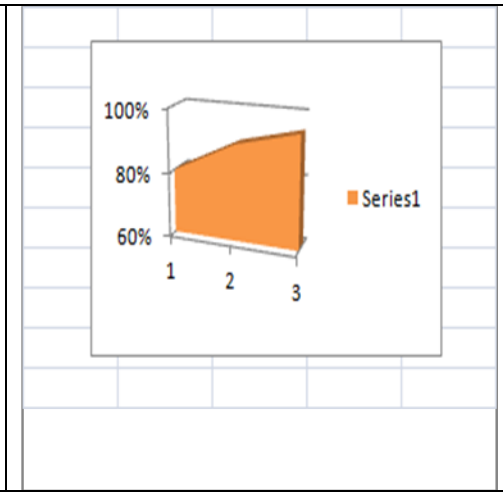
8.Sub-culturing

 **9.Invitro rooting**	 **10. Hardening**	 **11. *Exvitro* rooting**	 **12.Shifted to green house**

RESULTS AND DISCUSSION

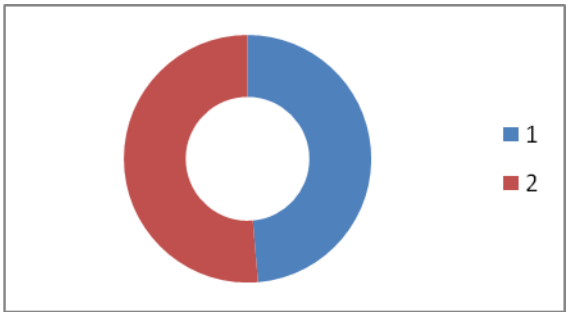
VW, B5 and KC media was used (Table 1)

Media used	**Media composition**	**Average plantlet formation (percentage)**	
B5	2 mg BAP +1.5 mg NAA + 50 ml CM 1 mg BAP + 0.5 mg NAA + 50 ml CM 2.5mg BAP + 2 mg NAA + 50 ml CM	40% 30% 50%	
KC	3 mg BAP + 1 mg NAA+ 50 ml CM 2.5 mg BAP + 1.5 mg NAA + 50 ml CM 2 mg BAP + 0.5 mg NAA + 50 ml CM	50% 30% 40%	

VW	2 mg BAP + 1mg NAA + 50ml CM 1.5mg BAP + 0.5mg NAA+50ml CM 2 mg BAP + 1.5 mg NAA + 50 ml CM	80% 90% 95%	

Basal VW medium supplemented with 2 mg BAP + 1.5 mg NAA + 50 ml CM was found to be most suitable for plantlet formation.

Media Composition For *Invitro* Rooting (Table 2)

Media used	Media composition	Average plantlets showing rooting	
VW	Basal + 1.5 mg BAP + 2mg IAA + 50 ml CM + 250 mg AC Basal + 0.5 mg BAP + 1mg IAA + 50 ml CM + 500 mg AC	90% 95%	

Germination percentage and plantlet formation was most suitable on VW medium supplemented with 2 mg BAP + 1.5 mg NAA + 50 ml CM (Table 1). VW medium supplemented with Basal + 0.5 mg BAP + 1mg IAA + 50 ml CM + 500 mg AC was most suitable for *in vitro* rooting (Ref table 2*). Ex-vitro* rooting was done by dipping the plantlets in IAA solution followed by spraying with Bavistin to prevent fungal infection. Well grown shoots after *ex vitro* rooting were directly transferred to small pots containing 1:1:1 sand: soil:solrite (potting mix) and were allowed to acclimatize in the green house. Successfully established plantlets were subsequently transferred to field conditions.

CONCLUSION

From these studies it can be concluded that the VW medium is most suitable for *Xenikophyton smeeanum* seed germination and plantlet formation. This study also revealed that a low concentration of 2 mg BAP + 1.5 mg NAA + 50 ml CM for good plantlet formation and VW Basal + 0.5 mg BAP + 1mg IAA + 50 ml CM + 500 mg AC gives good results with high percentage for *Invitro* rooting .

SCOPE

1. Use of nano biotechnology in control of bacterial and fungal contaminations.
2. *In vitro* micro propagated plants can be shifted to natural habitats of Western Ghats to facilitate In situ conservation of *Xenikophyton Smeeanum*.
3. Using elicitors (from biological origin) for enhanced plantlet formations.

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