IN VITRO MICROPROPAGATION OF DENDROBIUM PEGUANUM LINDL. – THE SMALLEST ORCHID OF WESTERN GHATS OF KARNATAKA

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ABSTRACT: *Dendrobium is an epiphytic orchid it is one of the most important genera with distribution of eleven species in the Western Ghats, belonging to the family Orchidaceae. In the micro propagation of Dendrobium peguanum helps to conserve measures of this orchid species. VW, MS, B5 media supplemented with various concentrations of auxins and cytokines were need in combination for a symbiotic seed germination and plantlet formation. There was good plantlet formation of the media MS mediums comparing with VW & B5. MS medium supplemented with 2mg of BAP+5mg NAA gave excellent growth for plantlet formation. MS medium for fortified with 2mg of BAP+1.5mg of IAA used for In-vitro rooting. Hardened plants were transferred to green house after Ex-vitro rooting technique.*

Keywords: Dendrobium peguanum, VW, *B5, MS, NAA, IAA, BAP, AC, CM.*

ABBREVIATIONS*:* VW - Vacin and Went medium, B5 - Gamborg B5 medium, MS- Murashige and Skoog medium, NAA–Naphthalene Acetic Acid, IAA–Indole Acetic Acid, BAP – Benzyl Amino Purine, AC – Activated Charcoal, CM–Coconut Milk.

INTRODUCTION:

Dendrobium peguanum Lind:

The genus Dendrobium is the largest genus belonging to orchidaceae, which is one of the largest flowering families. Most of the members are epiphytic. Dendrobium peguanum Lindl is the smallest orchid it is an endangered species so there is a greater need to protect the species. Dendrobium peguanum Lindl is a delightful miniature from India, Thailand and Burma. It grows as a lithophyte or epiphyte and is characterized by short, stout pseudo bulbs with 2 to 4 leathery, deciduous leaves. Many small, fragrant flowers are produced on leafless canes in winter.

Dendrobium peguanum Lindl is an epiphytic orchids in a small population size, which is encountered in growing of many different species of host trees. *Dendrobium peguanum* Lindl. in J. Proc. Linn. Soc. 3: 19 (1859); Pearce et Cribb, Orch. Bhutan, 420 (2002); Misra, Orch, 431 (2004). Flowering occurs during the month between Dec-Feb and fruiting is in the month of April-May.

MATERIALS AND METHODS:

Dendrobium peguanum plants with fruits were collected from Sagar Shimoga district during month of May , was maintained in the green house as a part of germplasm collection. 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 on different nutrient media like MS medium, B_5 medium, VW medium which were prepared with various concentrations and combinations of phytohormones and other additives. MS medium gave the best results in comparison to all other media. So MS medium was

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standardized for *Dendrobium peguanum*. MS medium with 2mg BAP +5 mg NAA has given good results 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. Comparing with VW, B5, KC Medium. The Ex-plants were obtained from the callus formation of Dendrobium Peguanum seeds. 60 days old culture of Dendrobium peguanum with 3- 4 leaved healthy plantlets were subjected to sub-culturing.

In-vitro rooting

The matured sub-cultured plants were subjected to rooting using Rooting Culture Medium.

In-vitro rooting was successful with MS media supplemented with 2 mg BAP, 1.5 mg IAA, 50 ml CM and 500 mg AC.

Ex-vitro rooting

The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of IAA(200 PPM)for 15 mints then planted in small pots containing solrite (Garden Soil, Rock Dust and Peat Moss) sprayed with bavistin to avoid fungal infection. *In-vitro* rooted plants in the pot trays containing potting mixture maintained under mist chamber and covered with perforated plastic cups.

Hardening

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 NAA. Plantlets were transferred to thumb pots containing soil, and solrite and were kept in the green house. Plantlets were also subjected to high humidity conditions of green house for healthy growth. Successfully established plantlets were subsequently transferred to field condition.

Dendrobium peguanum Flowering in Green House

Callus formation in Dendrobium peguanum

Sub – culture of Dendrobium peguanum

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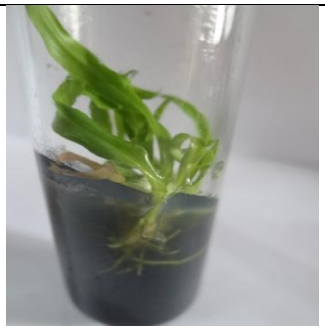
In vitro rooting of Dendrobium peguanum

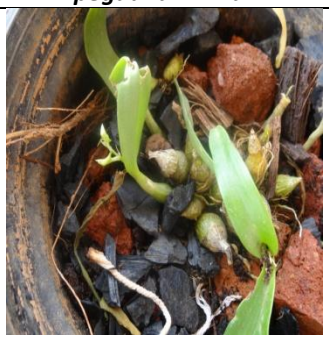


Well grown roots of Dendrobium peguanum Lindl

Ex-vitro Rooting of Dendrobium peguanum Lindl

Hardening of Dendrobium peguanum Lindl

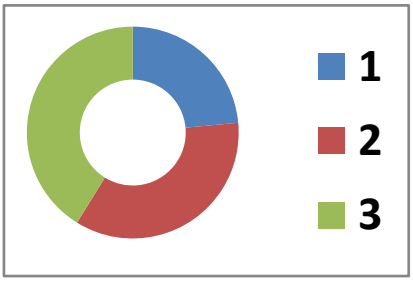
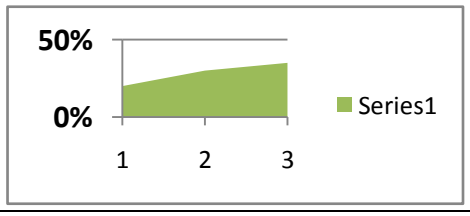
Hardening of Dendrobium peguanum Lindl in Green House



Dendrobium peguanum Lindl Flowering in Green House

RESULTS AND DISCUSSION

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

Media used	Media composition	Average plantlet formation (percentage)	
B_5	Basal B_5 media + 1mg BAP + 3mg NAA Basal B_5 media + 2mg BAP + 2mg NAA Basal B_5 media + 3mg BAP + 1mg NAA	25% 30% 40%	
VW	Basal VW media + 1mg BAP + 1 mg NAA Basal VW media + 2mg BAP + 1.5 mg NAA Basal VW media + 3mg BAP + 2 mg NAA	20% 30% 35%	

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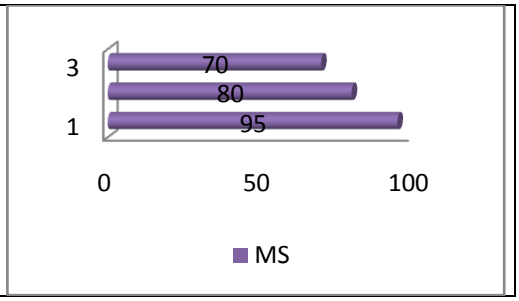
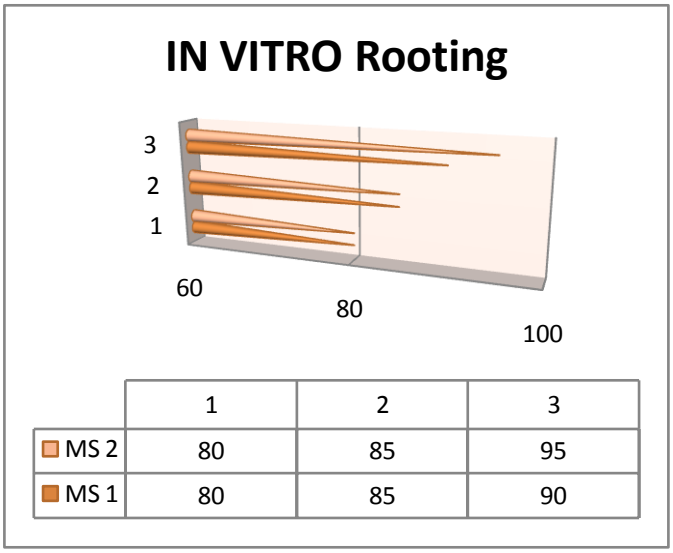
MS	**Basal MS Media + 2 mg BAP + 5 mg NAA**	**95%**
	Basal MS Media + 1.5 mg BAP + 3 mg NAA	80%
	Basal MS Media + 1 mg BAP + 2 mg NAA	70%



Media Composition For *Invitro* Rooting (Table 2)

Media used	Media composition	Average plantlets showing rooting
MS	Basal MS Media + 1.5 mg BAP + 3 mg IAA + 500 ml CM + 200 AC	80%
	Basal MS Media + 1 mg BAP + 4 mg IAA + 50 ml CM + 250 AC	85%
	Basal MS Media + 0.5 mg BAP + 5 mg IAA + 50 ml CM + 500 mg AC	90%
MS	Basal MS Media + 3 mg BAP + 2.5 mg IAA + 50 ml CM + 150 mg AC	80%
	Basal MS Media + 2.5 mg BAP + 2 mg IAA + 50 ml CM + 250 mg AC	85%
	Basal MS Media + 2 mg BAP + 1.5 mg IAA + 50 ml CM + 500 mg AC	**95%**



CONCLUSION

From these studies it can be concluded that the MS medium is most suitable for *Dendrobium peguanum Lindl* seed germination. This study also revealed that a low concentration of 2mg BAP and 5 mg NAA was found to be more suitable for plantlets and multiple plantlets. MS medium supplemented with 2 mg BAP, 1.5 mg IAA, 50 ml CM and 500 mg AC was found to be suitable for *In-vitro* Rooting.

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