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RESEARCH ARTICLE

IN VITRO PROPAGATION OF EPIDENDRUM RADICANS OF WESTERN GHATS.

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Abstract

Epidendrum is the largest genus of orchids with about 1500 species, many of which occur in great abundance in the tropics. These sympodial orchids have long, lasting showy flowers which are used commercially as cut flowers and potted plants. A rapid in vitro seed germination technique is described here. VW 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, VW medium supplemented with 0.5 mg BAP, 5 mg NAA and 150 ml CM was found to be suitable for plantlet formation. Further hormonal concentrations of auxins and cytokinins were evaluated for minimal and optimal levels in the medium. In the optimization process for phytohormones, a low concentration of 0.5 mg BAP/L^{-1} with 5 mg NAA/L^{-1} was found to be more suitable for plantlets and multiple plantlets. *In vitro* rooting was successful with VW medium supplemented with 0.5 mg BAP, 2 mg NAA, 150 ml CM and 500 mg AC. *Ex vitro* rooting was most successful with VW media supplemented with 2 mg BAP, 5 mg NAA and 150 ml CM. Hardened plants were transferred to green house after ex vitro rooting technique. Significance of the present work is discussed here.

Abbreviations:-
VW-Vacin and Went, BAP – Benzyl Amino Purine, NAA–Naphthalene Acetic Acid, IAA–Indole Acetic Acid, CM–Coconut Milk, AC – Activated charcoal.

Introduction:-

Epidendrum radicans is the largest genus of orchids with about 1500 species [3], many of which occur in great abundance in Central America. Their origin is not known but it is assumed that they are native to South America and the tropics. The majority of the 1500 species are mainly found on the tropical forest slopes of the Andean and Guiana mountain ranges, including adjacent mountains in Central America [4, 5, 6].

Although terrestrial members are common, the vast majority of *Epidendrum radican*s species are epiphytes, occurring in dry seasonal forests where they grow on Cactaceae and Velloziaceae species or in humid rain forests on mountain slopes where light and moisture are better available in the canopy. These orchids are sympodial orchids that form flowers in clusters on a long inflorescence, which come in various shades of orange, yellow, white, light green, and tan [3]. One important species is Epidendrum radicans, the flowers of which are used commercially as cut flowers and potted plants [2,8,12]. A few critical characters of this genus is the column united to the entire lip length, the rostellum parallel to the column axis and the nectar immersed within the pedicel (cuniculus).

Asymbiotic germination on basal nutrient medium [7] and a combination of various growth regulators [1] 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 Epidendrum radicans [9,10].

Material and methods:-

Epidendrum radicans was collected from Sagar, Shimoga district of Karnataka and were grown in green house at St. Joseph's College Post Graduate and Research Centre, Bangalore. Healthy capsules were harvested from the plants which are approximately 3 months old. Using a scalpel carefully cut the dried up tepals and dead tissues of the capsule. The dry capsule were swabbed with 75 % alcohol and remove the surface dust, and taken to the inoculation room. Inside laminar air flow cabinet, dry capsule were swabbed with 100% alcohol. Then the dry capsule was cut open and seeds were sprayed on the nutrient media.

VW was used with different composition and supplemented with various combinations of Auxins and Cytokinins [11]. pH of the medium was maintained at 5.6 -5.8 the medium with VW gave good results.

The cultures were incubated at 25± 2^0C temperature Photoperiod 16/8 h 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 every 2 weeks and observation was made. Each experiment was repeated twice and consisted of 3 replicates of 10 explants for each treatment.

90 days old plantlets of various stages, were sub cultured for 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.

***In vitro* Rooting:-**

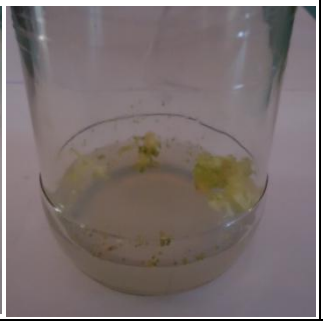
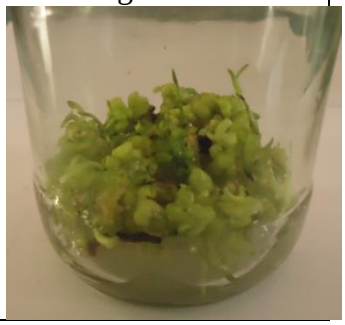
In vitro rooting was successful with VW Media supplemented with 0.5 mg BAP, 2 mg NAA, 150 ml CM and 500 mg AC

***Ex vitro* Rooting:-**

The basal ends of healthy shoots from the shoot multiplication medium were dipped in an auxin solution, 10 ml of IAA (made in tap water) then planted in small pots containing 1:1:1 sand: soil: solrite (potting mix) sprayed with bavistin to avoid fungal infection. *In vitro* rooted plants in the thumbpots containing potting mixture maintained under mist chamber and covered with perforated plastic cups. All these above protocols were followed inside greenhouse conditions and further allowed to acclimatize.

Hardening Process:-

Well grown shoots from the shoot multiplication medium were directly transferred to small pots containing soil and sand and kept covered with perforated plastic cups at room temperature 32+2^{0}C. Successfully established plantlets were subsequently transferred to field conditions.

EpidendrumRadicans	Inoculation of seeds	Plantlet initiation	Plantlet growth
			

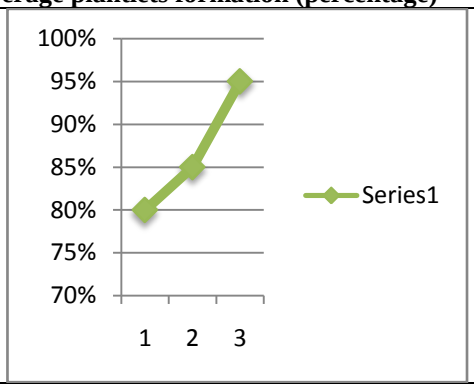
Plantlet formation	Plantlets	Sub culturing	*In vitro* rooting
			
Ex vitro rooting	Hardening	Hardening in Green house	In Green house Epidendrum flowering
			

Result and discussion:-

VW media was used for plantlet formation

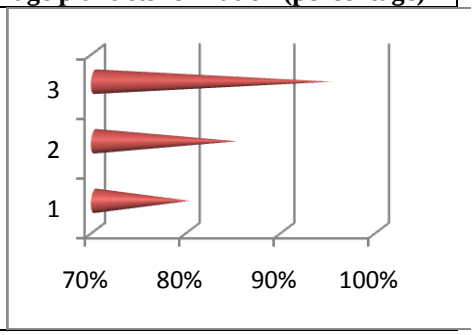
VW - For Plantlet formation (Table 1)

Media Used	Media Composition	Results The average plantlets formation (percentage)
VW	**Basal VW Media+ 0.5 mg BAP + 5 mg NAA + 150 ml CM**	**95 %**
	Basal VW Media+ 1 mg BAP + 10 mg NAA + 150 ml CM	90%
	Basal VW media + 1.5 mg BAP + 5 mg NAA + 150 ml CM	80%



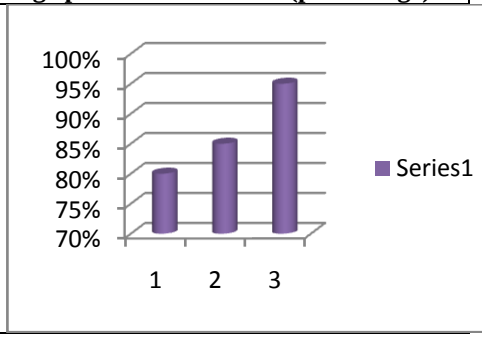
VW for *In vitro* rooting (Table 2)

Media Used	Media Composition	Results The average plantlets formation (percentage)
VW	**Basal VW Media+ 0.5 mg BAP + 2 mg NAA + 150 ml CM + 500 mg AC**	**90 %**
	Basal VW Media+ 1 mg BAP + 3 mg NAA + 150 ml CM + 500 mg AC	85 %
	Basal VW Media+ 2 mg BAP + 5 mg NAA + 150 ml CM + 500 mg AC	80 %



VW - For the *Ex vitro* Rooting (Table 3)

Media Used	Media Composition	Results The average plantlets formation (percentage)
VW	Basal VW Media+ 0.5 mg BAP + 5 mg NAA + 150 ml CM	80%
	Basal VW Media+ 1 mg BAP + 5 mg NAA + 150 ml CM	85%
	Basal VW Media+ 2 mg BAP + 5 mg NAA + 150 ml CM	**95%**



VW media was most suitable for *Epidendrum radicans*. VW medium supplemented with 0.5 mg BAP, 5 mg NAA and 150 ml CM was found to be suitable for plantlet formation (Ref table 1). VW medium supplemented with 0.5 mg BAP, 2 mg NAA, 150 ml CM and 500 mg AC was found to be suitable for the *in vitro* Rooting (Ref table 2). For *ex-vitro* rooting with VW media, 2 mg BAP, 5 mg NAA and 150 ml CM (Ref table 3) was most suited. The plants with good rooting were transferred to community pots and then to greenhouse conditions.

Conclusion:-

From these studies it can be concluded that the VW medium is most suitable for *Epidendrum radicans* seed germination. VW medium supplemented with 0.5 mg BAP, 5 mg NAA and 150 ml CM was found to be suitable for plantlet formation. This study also revealed that a low concentration of 0.5 mg BAP/L^{-1} with 5 mg NAA/L^{-1} was found to be more suitable for plantlets and multiple plantlets.

Scope:-

1. Use of nano biotechnology in control of bacterial and fungal contaminations.
2. In vitro micropropagated plants can be shifted to natural habitats of Western Ghats to facilitate in situ conservation of *Epidendrum radicans*.
3. Rapid *in vitro* method to enhance production of flowers for floriculture.
4. Using elicitors (From biological origin) for enhanced plantlet formations.

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