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In Vitro Propagation Reviews of Orchid

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Abstract:- *Orchidaceae* is the largest family comes at second place in beautiful as well as sunshine flowering plants. Orchids are used as sources of medicine. Orchid is one of the major flowering plants with high demand in international market due to multifaced powerful aphrodisiac qualities. Orchid's are propagated by standard conventional methods which is very slow process. Vegetative propagation occurs through division of clumps, rhizomes, cutting and off shoots separation. Seed germination requires other supplementary techniques in natural conditions. As compare to natural propagation methods, plant tissue culture technique provides axenic *in vitro* propagation of orchids in large scale with quality plantlets in short period of time. Numerous factors are important for successful micro propagation such as explant collection, pH and temperature, size of explant, environmental conditions, culture medium, PGR, etc. Sequential study of experiments helpful for understanding biological, physical and physiochemical aspect that govern *in vitro* morphogenesis of orchids. Plant tissue culture also aim's in soma clonal variations of orchids for creations of variegated plantlets.

Keywords: *In Vitro, Orchid Species, PGR, Problems and Solutions.*

I. INTRODUCTION

Orchids are the most attractive flowers of god's creations, a exclusive group of plants. They are among the family which is monocotyledons with 25,000-35,000 species [5]. Orchids exhibits a wide range of diversity in size, shape and colouration's of flower petals. They are known for their long lasting and beautiful flowers. Orchids are have simple leaves known as perennial herb. Majority of the cultivated orchids are native to tropical countries such as central America , India, Central America, Australia, Mexico, China, Thailand, etc.

In world, the Chinese were the first to cultivate orchids, Arunachal Pradesh is famous for the cultivation of orchids in India, it also been termed as "Orchid Paradise of India". In India, Sikkim is the largest producer of orchids with about 450 known species. Orchids are also used for medicinal purpose.[24]

In orchids varieties, dendrobium possess medicinal properties that can be used to treat cancer, support the immune system. Orchids have been used as a medicine for the treatment of various diseases and aliments including tuberculosis, paralysis, stomach disorders, cheat pains, acidity, eczema, tumor, piles cholera, inflammation's, menstrual disorders, spermatorrhea, leukoderma, diahorrhea, etc.[4]

Orchid plants require more time to reach the flowering stage for seeds production which used for seed germination and plantlets propagation. But orchid seed are small in size, for seeds germination it depends upon the other microorganisms like mycorrhiza. The seed propagated in nursery bed required more time for germination. Viability of seeds is very less because it contains less amount of nourishing endosperm which supporting embryo for development. These hurdles in the orchids propagation can be overcome by plant tissue culture methods.

In vitro propagation can increase new avenues for large scale, disease free and true to type quality planting material. Numerous factors are important for successful micropropagation such as explant collection, pH and temperature, size of explant, environmental conditions, culture medium, PGR, etc. Sequential study of experiments helpful for understanding biological, physical and physiochemical aspect that govern *in vitro* morphogenesis of orchids. This paper will delve in, understanding various methods for explant selection, surface sterilization of explants, growth condition requirements, Plant growth regulators with their varied concentration, etc. to encounter the problems associated with regeneration.

II. *IN VITRO* ESTABLISHMENT PROBLEMS AND SOLUTIONS

Surface and systemic contamination is a bottleneck for establishment of *in vitro* culture. Surface sterilization treatment is important in establishment of *in vitro* cultures. Sterilant not only removes microorganisms but also causes phyto-toxic effect in plant tissue. After sterilization, immature and small size explants turn to brown and simultaneously dead. Large size explants, contains more microbial count, tolerate harsh sterilants treatment but hinder bud sprouting. To lower-down these contaminants and to improve efficiency of sterilants, newly emerged lateral branches were selected before 6 days of culture initiation and sprayed with solution of Bavistin (0.1%) and streptomycin (100mg/l). The explants used for culturing should newly emerging axillary or apical buds which have less contamination. The treatment of Sodium hypo-chloride can remove the oily and waxy layer on the explant. 70%

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ethanol is a sterilant which have inherent microbial property which denatures the proteins. To reduce contamination in the culture, fungicides used for explant sterilization like Bavistin which can disturb fungus metabolism and reduce fungal infections. Antibiotics can be used to reduce the bacterial load of explants.

Table 1: Globally finding of *In vitro* literature on orchids.

No.	*Varieties*	Medium combinations			Results		Other additives	Culture condition	Reference
		Medium	Cytokinin	Auxin	Cytokinin	Auxin			
1.	*Dendrobium chryseum*	Murashige and Skoog's (MS, 1962)	BAP 0.5 to 2.0 mg/L	IAA 1mg/L IBA 1 mg/L NAA 1 mg/L	BAP 2.0 mg/L	IAA 1.0 mg/L IBA 1.0 mg/L NAA 0.5 mg/L	Gibberellic acid 10%	Temp 24±1ºC Light 16/8 hrs	[20]
2.	*Pholidota imbricata hook*	KC medium	BAP 3 mg /L	NAA 5 mg/L	BAP 3 mg/L	NAA 5 mg/L	-	Temp 25±2ºC Light 8/16 hrs	[21]
3.	*Gastrochilus Matsuran (makino)*	MS medium	IBA 2 µM	NAA 1.5 µM	IBA 2 µM	NAA 1µM IAA 1µM	Coconut water and plant hormone	Light 10µMol $m^{-2}s^{-1}$	[16]
4.	*Vanda orchid*	MS medium	BAP 4.44 to 66.6 µM	NAA 0.27 to 8.06 µM	-	-	Banana homogenete peptone,activated charcoal,coconut water, tomato juice, poato homogenete.	Temp 25C ±1.3ºC Light 12/16 hrs Light intensity 50µMol $m^{-2}s^{-1}$	[35]
5.	*Orchid coelogyna stricta D. Donschltr*	MS medium	BAP 1 Mg/L	NAA 1 Mg/L	BAP 1 Mg/L	NAA 1 Mg/L	-	Temp. 25±2ºC Light 8/16 hrs	[28]
6.	*Cymbidium Goeringii*	MS medium	2,4-D 20 µM	NAA 4 µM	2,4-D 20 µM	NAA 4 µM	TDZ 2µM	Temp 22 ± 2 ºC Light 10µMol $m^{-2}s^{-1}$	[26]
7.	*Dendrobium primulinum Lindl*	MS medium	BAP 1.5 mg/l	NAA 0.5 mg/l	BAP 1.5 mg/l	NAA 0.5 mg/l	-	Temp 25±2ºC Light 12/15 hrs.	[2]
8.	*Dendrobium Orchid*	VW medium	BAP 2.5 mg/l	NAA 0.5 mg/l	BAP 2.5 mg/l	NAA. 0.5 mg/l	-	Temp 25±1ºC Light 000–3000 lux	[3]

9.	*Anesellia africana*	MS medium	IBA 10 µM	NAA 5 µM	IBA 10µM	NAA 5µM	-	Temp 25±2ºC Light 12/15 hrs	[19]
10.	*Dendrobium nobile*	MS medium	IBA 3.0 mg/l	NAA 3.0 mg/l	IBA 3.0 mg/l	NAA 3.0 mg/l	Coconut water	25 ± 1°C under 16/8 hrs of photoperiod 2000 lux	[6]
11.	*Dendrobium orchid*	MS medium	BAP 1.5 mg/L, IAA 2.0 mg/L	NAA 1.0 mg/L	BAP 2.5 mgl^{-1} IAA 2.0 mg/L	NAA 1.0 mgl^{-1}	-	Temp 25±2ºC Light 12/15 hrs.	[23]
12.	*Phalaenopsis amboinensis*	VW meduim	-	NAA 1 mgL^{-1}	-	NAA 1 mgL^{-1}	Coconut water, banana homogenate	Temp 23 ± 2°C light 16/8 hrs	[40]
13.	*Geodorum densiflorum*	MS medium	BAP 2.0 mg/L	NAA 2.0 mg/l	BAP 2.0 mg/L	IAA 2.0 mg/L	-	Temp 25 ± 2ºC Light 10/14	[11]
14.	*Oncidium sp.*	MS medium	BAP 2.0 mg/l	NAA 1.5 mg/l	BAP 2.0 mgl^{-1}	NAA. 1.5 mgl^{-1}	-	25±1ºC continuous light (3000 lux) with a photoperiod of 12 h daily and 60 – 70% relative humidity	[15]
15.	*Cymbidium eburneum Lindl*	MS medium	BAP 15µM	NAA 15 µM	BAP 15µM	NAA 15µM	-	Temp 25 ± 2 ºC	[18]
16.	*Anoectochilus sikkimensis*	MS medium	BAP 17.6 µM	NAA 2.70 µM	BAP 17.6 µM	NAA 2.70 µM	-	Temp 25 ± 2 ºC Light 12hrs fluorescent light 25-50 µmol $m^{-2}s^{-}$	[14]
17.	*Esmeralda clarkei*	MS medium	BAP 0.2 – 5 mg/L	NAA 0.5 mg/L	BAP 2.0 mg/L	NAA 0.5 mg/l	Coconut milk	Temp 25 ± 2ºC	[30]
18.	*Cymbidium aloifolium*	MS medium	BAP 2.0 mg/L	NAA 0.5 mg/l	BAP 2.0 mg/L	NAA 0.5 mg/l	-	Temp 25 ± 2°C 16/8 hrs	[31]
19.	*Cymbidium iridioide*	MS medium	BAP 0.5 mg/L	NAA 1 mg/L	BAP 0.5 mg/L	NAA 1 mg/L	-	Temp 25 ± 2°C 16/8 hrs	[25]
20.	*Vanda helvola*	MS medium	BAP 0.1 to 4.0 mg l^{-1}	NAA 0.1- 4.0 mg l^{-1}	BAP 2.0 mgl^{-1}	NAA 0.5 mgl^{-1}	-	Temp 25 ± 2_ºC. 14/10 h light	[7]
21.	*Arundina graminifolia (d. Don.)*	MS medium	IAA 0.5 to 1.0 mg/l BAP 0.5 to 3.0 mg/l	NAA 1.0 to 2.0 mg/l	IAA 1.0 mg/l BAP 2.0 mg/l	NAA 1.0 mg/l	Casein hydrolys-ate	Temp 25 ± 2_C. 14/10 h light	[10]

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22.	*Dendrobium Orchid*	MS medium	BAP 0 to 5 mg/l	NAA 0 to 1 mg/l	BAP 2.5 mg/l	NAA 0.5 mg/l	-	Temp 25 ± 2_C. 14/10 h light	[39]
23.	*Dendrobium densiflorum Lindl.*	MS medium	IAA 0.5 to 2.0 mg/l BAP 0.5 to 2.0 mg/l	NAA 0.5 to 2.0 mg/l	IAA 0.5 mg/l BAP 2 mg/l	NAA 0.5 mg/l	-	Temp 25 ± 2°C 16/8 hrs light 350 to 500 lux	[32]
24.	*Calanthe densiflora Lindl.*	MS medium	IAA 1 mg/l BAP 2 mg/l	NAA 1.0 mg/l	BAP 0.5 mg/l	NAA 2.0 mg/l	-	Temp at 25±2ºC under 350-500	[12]
25.	*Dendrobium chrysotoxum lindl.*	MS medium	BA 4.44 µM KIN 4.65 µM	NAA 5.37 µM	BA 4.44 µM KIN 5.6 µM	NAA 5.37 µM	-	Temp 25 ± 2°C 16/8 hrs Light 40µMol $m^{-2}s^{-1}$	[17]
26.	*Catasetum pileatum cv. Alba*	MS medium	KIN 0.00 to 5.00 mg l^{-1}	IBA 0.00 to 5.00 mg l^{-1}	KIN 1.00 mg/l	IBA 1.00 mg/l	-	Temp 25 ± 2°C 16/8 hrs Light 40-60µMol $m^{-2}s^{-1}$	[41]
27.	*Dendrobium fimbriatum*	MS medium	BA 5 mg/L	NAA 0.5 mg/L	BA 5 mg/L	NAA 0.5 mg/L	-	Temp at 25±2ºC under 350-500	[33]
28.	*Paphiopedilum Rothschildianum*	MS medium	2,4-D 4.54 µM	TDZ 4.54 M	2,4-D 0-22.6 M	TDZ 4.54 µM	-	Temp 25 ± 2°C 16/8 hrs	[22]
29.	*Dendrobium barbatum Lindl.*	MS medium	BAP 2 mg/L	NAA 1.0 mg/L	BAP 0.5- 4.0 mg/ L	NAA 1.0 mg/L	-	Temp 25 ± 2°C 16/8 hrs	[1]
30.	*Phalaenopsis*	MS medium	BA 4 mg L^{-1}	NAA 0.1 mg L^{-1}	BA 4 mg L^{-1}	NAA 0.1mg L^{-1}	GA3 1.5 mg L^{-1}	Temp 25 ± 2°C 14 hrs Light of 54–57 µmol m−2 s −1	[42]
31.	*Phalaenopsis amabilis*	MS medium	-	NAA 1 to 5 ppm	-	NAA 1 to 5 ppm	TIBA 5 ppm	Temp 20ºC 16/8 hrs	[13]
32.	*Phalaenopsis amabilis (L.) Blume (Orchidaceae)*	MS medium	BAP 0.5 mg/l	NAA 1.5 mg/l	BAP 0.5 mg/l	NAA 1.5 mg/l	-	Temp 20°C light 2700 Lux	[9]

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33.	*Dendrobium fimbriatum Hook.*	MS medium	BAP 1 mg/L	NAA 1 mg/L	BAP 2 mg/L	NAA 1 mg/L	-	Temp 25 ± 2°C 16/8 hrs	[27]
34.	*Dendrobium bensoniae*	MS medium	BA 0.5 to 2.0 mg/l	IBA 0.5 to 2.0 mg/l	BA 1.0 mg/l	IBA 1.5 mg/l	-	Temp 25 ± 2°C 16/8 hrs	[34]
35.	*Phalaenopsis amabiliscv*	MS medium	IAA 1 mgL^{-1}	NAA 1 mgL^{-1}	IAA 1 mgL^{-1}	NAA 0.5 mg/l	-	Temp 25 ± 2°C 16/8 hrs	[8]
36.	*Eulophia andamanensis*	MS medium	BAP 0.5 mg/l KIN 1 mg/l	NAA 0.5 mg/l	BAP 2.0 mg/l	NAA 0.5 mg/l	-	Temp 25 ± 2°C 16/8 hrs	[37]
37.	*Dendrobium Transparens L.*	MS medium	IAA 1 mgL^{-1}	NAA 2 mgL^{-1}	IAA 1 mg L^{-1}	NAA 2 mgL^{-1}	-	Temp 25 ± 2°C 16/8 hrs	[38]
38.	*Coelogyne flaccida Lindl.*	MS medium	BAP 0.5 mg/L	NAA 0.5 mg/L	BAP 0.5 mg/L	NAA 0.5 mg/L	-	Temp 25 ± 2°C 16/8 hrs	[29]
39.	*Coelogyne cristata Lindl*	MS medium	BAP 10 mg/l KIN 10 mg/l	NAA 5 mg/l	BAP 10 mg/l, KIN 10 mg/l	NAA 5 mg/l	-	Temp 25 ± 2°C 12/8 hrs 3500 lux light intensity	[36]

III. CONCLUSION

In conclusion, the outcome of various researches indicates that endogenous level and exogenously supplied plant that growth regulators play an importance role during regeneration of plantlets and these PGR's are species specific. Although there is contamination problems during establishments, multiplication rate can be increases by manipulating PGR plant growth regulator concentration. During establishments of culture, initially all cultures initiated on 0.25mg/l of BAP supplemented medium. When a huge mother culture initiated, then PGR combinations employed to carry out the experiment regarding manipulation of organogenic responses, higher multiplication rate and root formation. From various studies of scientists, it is concluded that, for multiplications of clumps, medium supplemented with combinations ranging from 0.25 mg/L BAP to 5 mg/L BAP.

Next problematic steps of *in vitro* propagation of orchids is rooting of propagules, as increased level of endogenous cytokinin in previous steps of multiplication. To increase rooting, it is need to lower down endogenous level of cytokinin, for these few subcultures carried-out on lower concentration of auxins or on growth regulators free medium. For rooting induction combinations ranging from 0.25 mg/IBA/IAA/NAA to 2.0 mg/L may use. Best results for rooting as done on medium supplementing NAA (0.25 to 5.0 mg/l). During hardening temperature, light, humidity with respect to species maintained. The most effective culture conditions is 25ºC temperature and 16/8 light/dark photoperiod.

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