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GREEN POD CULTURE IN *DENDROBIUM CHRYSANTHUM* LINDL.: A STUDY *IN VITRO*

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Abstract

Dendrobium chrysanthum, an epiphytic orchid of North Eastern India finds extensive utility in traditional system of medicine for its antipyretic and immunomodulatory effects. Unregulated collection and habitat destruction pressures have detrimently affected the size and frequency of its natural populations. It is getting rare and needs to be conserved. With a view to enlarging its population base, the germination competence of immature seeds was presently tested using green pod culture technique. The seeds positively responded to germination on Mitra *et al.* medium but the germination process leading to seedling development was markedly influenced in the additional presence of growth additives While, the protocorms multiplied rapidly in cultures treated with either of Peptone and Yeast extract, early development of healthy seedlings was achieved on KN and AC supplimented media.

Introduction

THE ORCHID seeds are poorly organized; they lack endospern and have undifferentiated embryos that require a fungal stimulus for germination in nature. Their ability to germinate *in vitro*, prior to reaching maturity (Sagawa, 1963) laid the foundation of green pod culture technique, which has added new dimensions to orchid propagation.

Dendrobium chrysanthum is a widely distributed species extending from India, Nepal, Bhutan, China, Thailand, and Laos to Vietnam. It finds extensive utility in traditional system of Chinese medicine for its antipyretic and immunomodulatory effects. The species is also effective in treating stomach pains, mouth sores, sunstrokes and other conditions caused by dry weather and pollution. According to Bensky and Gamble (1993), *D. chrysanthum* is also used to enhance skin quality. In India, this epiphytic species is met with in North Eastern Region where it dwells in tropical climates within an altitudinal range of 300-1200 m. Unregulated collection and habitat destruction pressures have detrimently affected the size and frequency of its natural populations. It is getting rare and needs to be conserved. With a view to enlarging its population base, the germination competence of immature seeds was presently tested using green pod culture technique. Some of the salient results are presented in this paper.

Material and Methods

Explant Sterilization

The green and undehisced capsules of *Dendrobium chrysanthum*, harvested 48 wks after pollination (wap), served as source of immature seeds. The freshly harvested capsules were scrubbed with 'Teepol' (0.01%), and washed thoroughly under running tap water for 15-20 min. They were then dipped in 70% ethyl alcohol for 30 sec, flamed and subjected to 8 min, surface sterilization with $HgCl_2$ solution (0.1%) prior to washing with sterilized distilled water. 'Teepol' was used as a wetting agent. The surface sterlized capsules were also treated with Streptomycin (0.01%) and Bavistin (0.1%) solutions. The capsules, thus, prepared were split opened with a sterilized blade to scoop out the immature seeds.

Culture Media and Incubation Conditions

The germination potential of immature seeds was assessed on Mitra *et al.* (1976, M) medium and its different combinations with growth additives [peptone ($2gl^{-1}$), yeast extract ($2gl^{-1}$); activated charcoal ($2gl^{-1}$); coconut water (10%); auxin (IAA, $1mgl^{-1}$); and cytokinin (KN, $1mgl^{-1}$)]. The cultures were maintained at 25 ± 2^0 C temperature and exposed to 12 hr, illumination of 3500 lux intensity. These were subcultured at regular intervals. The physical conditions were kept constant for initiation, multiplication and maintenance of cultures.

Acclimatization

Healthy seedlings with 2-3 well grown leaves and 1-2 roots were gradually hardened *in vitro*, by sequential elimination of growth additives, vitamins, sucrose and minor salts from the nutrient matrix at 15 days interval. The hardened seedlings were washed thoroughly with lukewarm water to remove agar and potted in clay pots (6 cm dia.), using charcoal, moss clippings, pine bark, and brick-bats (1:1:1:1) as the potting media.

Statistical Analysis

One way analysis of variance was performed with respect to each response (average ± standard error against each additive is mentioned in Table 1). As ANOVA results showed the non significant difference of additives at 5% level of significance, various groups of additives showing identical / similar response were

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formed statistically. To this end, Tukey Test was performed at 5% level with respect to each response.

Results

The immature seeds from green capsules germinated readily on Mitra *et al.* (1976, M) medium and its various combinations with growth additives (see Table 1). In the basal medium, nearly 65% seeds germinated and developed into protocorms. Chlorophyll development was a post-protocorm phenomenon and the differentiation of 1st leaf primordium preceded that of 1st root primordium. Seedlings complete with 2-3 leaves and 1-2 roots were obtained within 190 days. The morphogenetic changes leading to seedling development were somewhat accelerated in the additional presence of AC.

The germination frequency was impaired and morphogenetic change leading to seedling development was accelerated when growth additives were used in the medium (Table 1). IAA favoured early development of leaf and root primordia and its efficacy was further improved in AC suplemented medium. KN induced early seedling development and it was better used with AC. The protocorms multiplied rapidly and these followed an accelrated development in to seedlings in Peptone (P) treated cultures. Multiple protocomes were likewise obtained in Yeast Extract (YE) supplimented medium; the combination favoured early rooting in the germinating entities. Small sized protocorms were observed in CW enriched combination.

A medium containing KN and AC proved best for germination and early seedling development. Seedlings complete with 2-3 leaves and 1-2 roots were transferred to green house with 70% survival. A treatment with Peptone was beneficial for protocorm multiplication.

Table 1. *In vitro* seed germination and seedling development on Mitra *et al.* (1976, M) medium, in *Dendrobium chrysanthum.*

Additives	Germination frequency (%)	Time taken in days for						Remarks
		Onset of germination	Spherule formation	Protocorm formation	Emergence of first leaf primordium	Emergence of first root primordium	Formation of complete seedling	
-	65.75 ± 0.85^{a}	11.50 ± 0.65^{a}	23.25 ± 0.85^{a}	41.50 ± 0.65^{a}	88.25 ± 0.48^{a}	120.00 ± 0.82^{a}	190.25 ± 1.03^{a}	Healthy seedlings
AC	69.00 ± 0.41^{a}	9.00 ± 0.41^{b}	22.25 ± 0.85^{c}	36.00 ± 1.08^{b}	82.00 ± 0.82^{b}	112.00 ± 1.08^{b}	190.25 ± 1.03^{a}	Healthy seedlings
IAA	75.25 ± 0.85^{b}	9.00 ± 0.41^{b}	22.25 ± 0.85^{c}	39.00 ± 0.41^{a}	81.00 ± 0.70^{b}	96.00 ± 0.82^{c}	198.50 ± 1.32^{b}	Better leaf and root growth
IAA + AC	79.00 ± 0.41^{ci}	8.00 ± 0.41^{c}	20.00 ± 0.41^{c}	37.00 ± 0.82^{b}	77.00 ± 0.41^{c}	92.00 ± 0.82^{d}	196.00 ± 0.82^{b}	Better leaf and root growth
KN	92.00 ± 0.82^{dfg}	9.00 ± 0.41^{b}	21.00 ± 0.41^{c}	36.00 ± 1.08^{b}	75.00 ± 0.41^{c}	88.75 ± 1.03^{e}	159.00 ± 0.41^{c}	Better root growth
KN + AC	94.0 ± 0.41^{d}	8.00 ± 0.41^{c}	18.00 ± 0.82^{b}	31.50 ± 0.65^{c}	66.00 ± 0.82^{def}	88.00 ± 0.41^{e}	155.00 ± 0.41^{d}	Early seedling development
P	88.0 ± 1.01^{egh}	8.00 ± 0.41^{c}	18.0 ± 0.41^{b}	28.0 ± 0.82^{c}	68.00 ± 0.82^{dg}	92.00 ± 0.82^{d}	186.00 ± 0.41^{e}	Protocorm multiplication; luxuriant growth
P + AC	90.00 ± 0.41^{e}	7.25 ± 0.25^{d}	17.00 ± 0.41^{bd}	26.0 ± 0.82^{d}	64.0 ± 0.41^{de}	88.00 ± 0.82^{e}	170.00 ± 1.08^{f}	Good for germination and subsequent developmental stages
YE	94.00 ± 0.41^{d}	9.00 ± 0.41^{b}	19.00 ± 0.41^{b}	32.00 ± 0.82^{c}	72.00 ± 1.08^{cf}	89.00 ± 0.41^{e}	163.00 ± 1.08^{c}	Protocorm multiplication
YE + AC	95.00 ± 0.41^{d}	7.00 ± 0.41^{d}	18.00 ± 0.82^{b}	30.00 ± 0.82^{c}	84.00 ± 0.41^{b}	88.00 ± 1.22^{e}	160.00 ± 0.82^{c}	Healthy seedlings
CW	82.00 ± 0.82^{f}	11.00 ± 0.41^{c}	21.50 ± 0.65^{c}	36.00 ± 0.82^{b}	78.50 ± 1.32^{bc}	98.00 ± 0.41^{c}	173.00 ± 0.70^{f}	Small sized protocorms
CW+AC	86.0 ± 0.82^{eg}	11.00 ± 0.41^{a}	20.02 ± 0.71^{c}	31.50 ± 0.65^{c}	69.00 ± 0.41^{dfg}	96.00 ± 0.82^{e}	170.50 ± 1.25^{f}	Healthy Seedlings

Entries in column nos. 2 to 7 are Mean ± S.E.; same alphabetical letter in the superscript denotes that the corresponding means are in the same group using Tukey test at 5%.

Figs. 1- 12. *In vitro* seed culture in *Dendrobium chrysanthum*: 1, Immature seeds at the time of inoculation (×10); 2, Multiple embryos emerging out of bursted seed coat (×10); 3, Multiplication of protocorms (M); 4, Rapid multiplication of protocorms and differentiation of leaf primordia (M+IAA); 5-6, Formation of leaves (M+P+AC, M+IAA); 7-8, Differentiation of protocorms and leaf formation (M+KN+AC; M+P); 9, Differentiation of protocorms (M+CW+AC) Healthy seedlings (M, M+YE, M+KN)

Discussion

The immature seeds from green capsules of *Dendrobium chrysanthum* were successfully germinated under asymbiotic conditions *in vitro*. Literature studies reveal that, in orchids, the immature seeds germinate better than the mature ones. However, the stage at which the embryos can be cultured successfully varies with the species, genus, hybrid, nutrient medium, and culture conditions (Arditti *et al.*, 1982). Presently, the seeds were obtained 48 wap, and vitamin enriched Mitra *et al.* (1976) medium was used has the nutrient pool as done in several earlier investigations (Mitra, 1986; Pathak *et al.*, 1992, 2001, 2011; Piri *et al.*, 2013; Vij and Pathak, 1988).

The type and concentration of growth substances plays an important role during *in vitro* propagation of orchids (Arditti and Ernst, 1993). Presently, IAA supported better germination frequency and early differentiation in the germinating entities but seedlings took longer to develop in its presence in earlier studies, it was shown to improve germination frequency in *Calanthe discolor* and *Laeliocattleya* (Kano, 1965), promote protocorm formation in *Rhynchostylis retusa*, *Saccolabium calceolare*, and *Vanda testacea* (Vij *et al.*, 1981), and favour organogenesis in *Cattleya* (Withner, 1959); it was, however, inhibitory to germination in *Dendrobium nobile* (Miyazaki and Nagamatsu, 1965) and *Dactylorhiza purpurella* and *Coelogyne viride* (Hadley, 1970), besides delaying seedling development in *Cymbidium lowianum* (Sood, 1984), *Dendrobium chrysanthum*, *Rhynchostylis retusa* and *Vanda testacea* (Pathak, 1989). In our study, KN proved beneficial for inducing early germination and seedling development in contrast to its detrimental effect on the processes in several orchids (Arora, 1990; Hadley, 1970; Sharma and Tandon, 1986). Presently, YE supported better germination frequency and protocorm multiplication besides accelerating seedling formation in accord with its similar efficacy in many orchids representing diverse habits and habitats (Krishna Mohan and Jorapur, 1986; Mahant, 1991; Mead and Bulard, 1979; Pathak, 1989; Prasad and Mitra, 1975; Vij *et al.*, 1981). It however, impaired germination in *Aerides multiflora* (Vij *et al.*, 1981) and *Dactylorhiza maculata* cultures (Van Waes and Debergh, 1986); CW is used extensively in orchid micropropagation (Arditti, 1967, 2008; Arditti and Ernst, 1984). Presently, CW though proved beneficial for enhancing the germination frequency but it delayed seedling development. It favoured germination in *Cattleya* (Kerbauy and Handro, 1981), *Cleisostoma racemiferum* (Temjensangba and Deb, 2006), *Dendrobium* spp. (Devi *et al.*, 1990), and *Paphiopedilum purpuratum* (Yam and Weatherhead, 1988), and seedling growth in *Dendrobium* spp. (Devi *et al.*, 1990). High adsorption affinity of activated charcoal to excessive and inhibitory compounds in the culture media may be responsible for maximum germination of seed and production of significantly large protocorms in this species. AC promoted germination frequency, protocorm multiplication, chlorophyll development, and healthy growth of seedlings in a large number of orchid species (Arditti and Ernst, 1984; Hossain *et al.*, 2008, 2009; Pathak *et al.*, 2001; Vij and Pathak, 1988; Yam *et al.*, 1989).

Present results indicate that green pod culture is a feasible method for *Dendrobium chrysanthum* propagation and can be successfully used for orchid conservation.

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