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REGENERATIVE COMPETENCE IN ROOT EXPLANTS OF RHYNCHOSTYLIS GIGANTEA, AN ENDANGERED GENERA: AN IN VITRO STUDY

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Abstract

The neoformations in the in vivo root explants of Rhynchostylis gigantea depend upon their location, maturity level and chemical regime.The regeneration is affected by polarity showing basipetal gradient. The distal ones with intact tips with well developed root caps showed an extended growth with sub-apical formation of globular structure whereas,the proximal explants responded to the presence of cytokinin(Kn) in Mitra et al.,1976 medium.The effect of cytokinins was accentuated in the additional presence of NAA and the higher organogenetic responses are observed in explants when BAP,Kn was used in dose double than that of NAA.The regenerated plantlets were accilimatized &transfered to pots filled with moss, pine bark, brick and charcoal pieces (2:4:1:1) with 90% survival.

Keywords*: Orchid; Flowering plants; Root; Tissue culture; Protocorm-like-bodies; Callus.*

Introduction

Orchids constitutes one of the largest and diverse family of angiosperms families with 30,000-35,000 species in 600-800 genera, still in an evolutionary flux [1]. They have out-smarted and out-numbered their counterparts due to their long - lasting flowers of myraid shapes, sizes and colours. Their latter utility accounts for a highly lucrative trade in floriculture. Tissue culture technique have been exploited as a mean of *ex situ* conservation, particularly in outbreeders like orchids which generate a great deal of heterozygosity in the progenies

C.N. Beechey [2] suggested possibility of using aerial roots in micro-propagating orchids.The utility of roots as explant source is being increasingly realized due to their easy availability, low oxidation rate and ease with which they can be planted. Keeping this in view, presently we report the pioneer attempt to use root explants from *in vivo* grown fox tail Orchid, *R. gigantea* (Lindl.) Ridl., a native of Thialand. *R. gigantea* exhibit free fertility within and beyond the taxonomic limits and has been used as breeding material for raising floriculturally significant hybrids [3]. Besides being victim of its own beauty & utility *R. gigantea* is progressively losing its natural habitat and is getting rarer with every passage of time and figures prominently in Appendix II of the Convention on International Trade in Endangered species of Wild fauna and flora [4].

Material and Methods

R. gigantea Lindl. plants are procured from nursery in Thailand and grown under greenhouse conditions at Panjab University, Chandigarh.The root were harvested from stock plants were used as material for the present study.The roots were squentially surface sterilized

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with solutions of Streptomycin (0.1%, 20min), Sodium hypochlorite (4%, 15min) in Ethanol (70%, 3sec) before rinsing with sterilized distilled water. Excised root segments were segmented into 0.5 cm large explants and inoculated on sucrose (2%) supplemented and agar (0.9%) gelled basal medium (BM) [5] and its various combinations with NAA (α-naphthaleneacetic acid), BAP (6-Benzylaminopurine), KN (Kinetin).

The pre-inoculation medium pH was adjusted at 5.6. In parallel set of experiments 0.2% activated charcoal (AC) was used in the medium.Thirty two replicates for each treatment &the experiments were repeated a four times. All experimental manipulations were done under aseptic conditions and the cultures incubated at 25±2ºC under 12h photoperiod of 3500lx light intensity, were regularly observed [6-10].

Acclimatization of the Plantlet

After well-developed shoot and root formation the plantlets (3cm tall) were transfered to semi-solid medium containing only half strength macro- and micro-salts of BM medium [5], sucrose and vitamins were elimated. The plantlets were kept in this condition until they are 4-5cm tall, andwashed with luke warm water before transfering to moss, pinebark, brick and char - coal pieces (1:1:1:1) mixture. Humidity was maintained by covering each pot with transparent polythene bag. Holes of increasing size were made in the bags to reduce the humidity level gradually [11-13]. The bags were removed after 4 weeks and small plants in the pots were transferred from 90% shade to the sunlight. Survival rate was 90%. Spraying with fungicide (Bavistin 1%) twice a week was necessary to keep fungus off from the young plants. Figure 1 shows an acclimatized plantlet.

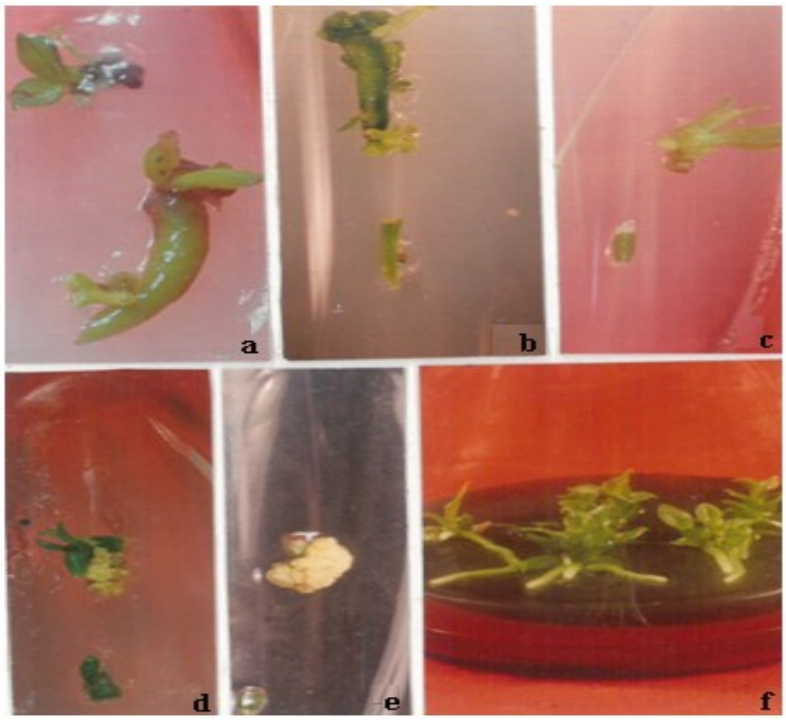

Fig. 1. Acclimatization plantlet procedure: a - Swollen Extended root tip with embryogenesis in BM+BAP(1mg/L) + NAA(1mg/L); b - Direct somatic embryogenesis in BM + Kn(1mg/L) + NAA (1mg/L); c - Direct shoot formation in BM + BAP (3mg/L) + NAA(1mg/L); d - Plbs proliferation in rings in BM + KN (3mg/L)+NAA(1mg/L); e - Indirect somatic embryogenesis in BM + BAP (3mg/L); f - Acclimatized Plantlet

Results

The root explants responded to depending neoformations upon their location, maturity level and chemical regime. The distal ones with intact tips with well developed root caps showed an extended growth,the root tips become greenish and swollen at one end after 3 weeks of inoculation.The sub apical swollen portion is protruded out with globular structures in 5 weeks old cultures in auxin (NAA; 1mg/L) enriched medium. The neo-formations measure 1.2 - 1.9cm in length (Fig. 1). On the other hand, the segments from the proximal segments reacted positively depending upon the quality and quantity of cytokinins (BAP/KN). The root segments (43.75±6.25) in 1mg/L each of KN and NAA, enriched by *G.C. Mitra et al* [5], medium initiated small green outgrowths (PLBs) in 3 weeks (Fig. 2), however, when KN is replaced with BAP (1mg/L), the response is indirect somatic embryogenesis mediated in 12.5% explants (Fig. 3).The higher concentration (3mg/L) of kinetin impaired the regeneration response in explants and number of plantlets obtained,31.25±6.25 (Table1). The increased concentration of BAP shows marginal increase in regeneration and % age of response (Table 1). The plantlet complete with 2-3 leaves and 1-2 roots were harvested after 15 weeks of subculture in 1 - 3 mg/L of KN with 1mg/L NAA, whereas it is obtained in 17 weeks and 16 weeks respectively in 1 and 3 mg/L of BAP and NAA (1mg/L).

Discussion

Juvenility of the tissue emerged as the major factor controlling the activation of proliferative loci in root explants due to fact that the younger tissues with less rigid cell walls are physiologically and biochemically more active and show better morphogenetic potential has already been demonstrated in several plant groups including Orchid [14-16].

In the present study,distal root segments from *in vivo* grown plants failed to respond whereas proximal one responded, this differential response of the explants indicate the role of their source and physiological age [17]. The distal ones with intact tips with well developed root caps showed an extended growth in 8 weeks old cultures regardless of the chemical regime.The neoformations measure 1.5-1.9cm in length with low conversion frequency, due to the fact that the distal zone has limited number of competent responding cells. On the other hand, the segments from the proximal segments retain their plasticity longer than those the distal segments and reacted positively depending upon the quality and quantity of cytokinins (BAP/KN) in accord with earlier reports in *Aerides*, *Cyrtopodium*, Dendrobium, *Oncidium* [18-22]. Presently,auxins containing medium showed an elongation and induce globular structure at sub-apical part of root segments in compliance to earlier reports in C. paludicolum [18, 22, 23, 24], however, in contrary to studies in *Catasetum fimbriatum* and *C. pileatum* [25, 26], which responded in PGRs bereft medium. This indicates that the nutritional requirement required in conversion of root to shoot meristem is species specific.

However, in the present study higher organogenetic responses are observed in explants when BAP,Kn was used in dose double than that of NAA in compliance to earlier reports *Aerides*, *Cyrtopodium*, *Dendrobium*, *Oncidium* [9, 10, 11-14], however, a higher dose (10:1; BAP:NAA) is required for root meristem conversion into shoot meristem in *Holostemma annulare* [27].The benign effect of KN was accentuated in the additional presence of NAA in accord with earlier reports in *Cattleya* hybrid *Aerides Cyrtopodium* [14, 18, 19, 22, 28].The efficacy of BAP was obligatory to the presence of NAA ,and it was required at 3 mg/L to elicit response in the explants (Table 1). A similar BAP related autonomy was reported in *Catasetum*, *Cattleya*, *Cymbidium*, *Cyrtopodium*, Dendrobium, *Doritaenopsis*, *Vanda coerulea* [15, 17, 23, 24, 29, 30].

Highest frequency regeneration from explants was observed on a medium with BM+KN (1mg/L) + NAA (1mg/L). The dark green color of regenerants in cytokinin (BAP/KN) supplemented media is in accord with similar earlier reports *G.B. Kerbauy*, in 1984 [23].

Table 1. The rates of survival, root elongation and regeneration (%) in Proximal and distal parts of *R. retusa* root explants

Hormones	Rates of survival (%), elongation (mm), regeneration (%) in proximal and distal part of root cultured		No of Plantlet (weeks)	Pathway
mg/L	Tip (Distal)	Basal (Proximal)		
BAP_0 NAA_1	100, 1.6, 0	100, 0.2, 12.5	10(18)	Indirect SE (Callus)
BAP_1 NAA_0	80, 1.8, 0	100, 0.2, 0	-	-
BAP_1 NAA_1	100, 1.6, 0	100, 0.0, 12.5	18(17)	Indirect SE (Callus)
BAP_2 NAA_1	100, 1.6, 0	100, 0.0, 12.5	24(16)	Indirect SE (Callus)
BAP_3 NAA_1	100, 1.2, 0	100, 0.0, 18.75±6.25	26(16)	Indirect SE (Callus)
BAP_4 NAA_1	100, 1.2, 0	100, 0.0, 0	-	-
BAP_5 NAA_1	80, 1.2, 0	100, 0.0, 0	-	-
KN_1 NAA_1	100, 1.6, 0	100, 0.0, 43.75±6.25	22(15)	Direct SE (PLBs)
KN_2 NAA_1	100, 1.8, 0	100, 0.0, 43.75±6.25	22(15)	Direct SE (PLBs)
KN_3 NAA_1	100, 1.9, 0	100, 0.0, 31.25±6.25	18(15)	Direct SE (PLBs)
KN_4 NAA_1	100, 1.9, 0	100, 0.0, 0	-	-
KN_5 NAA_1	100, 1.9, 0	100, 0.0, 0	-	-

The effect of cytokinins (BAP/KN) on chloroplast development as already indicated by *D.A. Stelter and W.M. Laetsh* [31, 32]. The high survival rate of accilimatized plantlets derived from root explants is in accord with earlier reports [33, 34]. The efficiency of regeneration lies in the success rate obtained in the acclimatization stage. Presently, the survival rate is 90% in compliance with earlier reports [22, 34].

Conclusions

Root segment culture proved as an reliable method for clonal propagation as it prevents somaclonal variations and no phenotypic variations were observed in accilimatized plantlet. In conclusion, the results clearly indicate that the root segments as an reliable method of clonal propagation bereft of somaclonal as the the root explants is an effective alternative an effective alternative to shoot meristem for micropropagation due to their easy availability and does not require the sacrifice of mother plant and provide exciting opportunities to raise large numbers of true-to type plantlets.

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