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Regenerative Competence in root explants of *Cattleya* hybrid, an endangered genera: A study in *vitro*

Vishal Sharma
Post Graduate Govt.College for Girls-11, Chandigarh-India

Abstract: The regenerative potential in the root explants of *in vivo* grown Cattleya 'Almakee' plants largely depend on the location of isolated explants from the main root and on the level&type of the growth regulators.Regeneration is affected by polarity all along the root axis of root.The regeneration is of basipetal gradient.The proximal explants responded to the presence of cytokinins(BAP/KN)in Mitra *et al*.,1976 medium.The effect of cytokinins was accentuated in the additional presence of NAA,whereas the distal explants failed to regenerate.The regenerated plantlets were accilimatized &transfered to pots filled with moss, pine bark, brick &charcoal pieces (2:4:1:1) with 90% survival.

Index Terms: Orchid flowering plants root, tissue culture, protocorm like bodies, callus

I. Introduction

Meristem culture has been successfully used to micropropagate a variety of orchids. It is particularly useful in outbreeders like orchids which generate a great deal of heterozygosity in the progenies. Their excision is ,however,is detrimental to the growth and development of mother plant,as it requires the sacrifice of the entire new growth or the only growing point .It is, thus desirable to develop an alternate and equally effective multiplication system by activating adventitious meristems in organs ,whose excision does not endanger the survival of source plant. In order to meet this objective, Beechey(1970) 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 &ease with which they can be planted. Keeping this in view, presently we report the possibility of using root explants from *in vivo* grown endangered plants of *Cattleya* 'Almakee',a perfect quintessence of beauty, progenator of large number of elite interspecific hybrids and favourite of the herbalists for its healing properties, is commonly called "Queen of Orchids" .It extends in distribution from India eastwards to Thailand and is progressively loosing its natural habitat & getting rare with every passage of time due to poor regeneration & extensive commercial collection (IUCN1991)

II. Material and Methods

Cattleya'Almakee'plants were collected in nature from Thailand & Darjelling (900m) and grown under greenhouse conditions at Panjab University, Chandigarh. The roots were harvested from stock plants were used as material for the present study. Subsequently roots were squentially surface sterilized with solutions of Streptomycin (0.1%,20min),Sodium hypochlorite(4%,15min)&dip 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:Mitra *et al* 1976 and its various combinations with NAA(α-naphthalene acetic acid),BAP(6-Benzyl amino purine),KN(Kinetin)& Peptone.

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 five times. All experimental manipulations were done under aseptic conditions & the cultures incubated at 25 ± 2^0c under 12 hr photoperiod of 3500 lux light intensity, were regularly observed.

III. Acclimatization of the Plantlet

After well-developed shoot and root formation the plantlets(3cm tall)were transferred to semi-solid medium containing only half strength macro & micro salts of BM(Mitra *et al*1976 medium;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.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 5 shows an acclimatized plantlet.

IV. Observation & Discussion

The root explants responded to neoformations depending upon their location,maturity level and chemical regime.The distal ones with intact tips with well developed root caps showed an extended growthin 8 wk old cultures regardless of the chemical regime.The neoformations measure 1.5-1.9 cm in length but all efforts to induce shoot development in these failed(Fig.1).It is 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 & reacted positively depending upon the quality and quantity of cytokinins(BAP/KN) in accord with earlier reports in *Cyrtopodium (*Eduardo *et al.*,2011*)*The root segments in 1 mg/l

each of KN and NAA enriched Mitra *et al* 1976 medium initiated small green outgrowths (PLBs) in 3 wks(Fig.2).The benign effect of KN was accentuated in the additional presence of NAA in accord with earlier reports in *Cattleya* hybrid (Kerbauy 1991).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* & *Cymbidium* ,*Doritaenopsis* (Kerbauy 1984,Yasugi,1994,Park *et al*., 2003)

Table 1: The rates of survival, root elongation & regeneration (%) in Proximal & distal parts of root cultured under light conditions root cultured

Hormones	Rates of (survival %,elongation(mm),regeneration(5) in proximal and distal part of root cultured	
mg/l	Tip(Distal)	Basal(Proximal)
BAP_0 NAA_1	100 , 1.5 , 0	100 , 0.1 , 0
BAP_1 NAA_0	80 , 1.8 , 0	100 , 0.2 , 0
BAP_1 NAA_1	100 , 1.7 , 0	100 , 0.0 , 12.5
BAP_3 NAA_1	100 , 1.2 , 0	100 , 0.0 , 25
KN_1 NAA_1	100 , 1.9 , 0	100 , 0.0 , 37.5
KN_3 NAA_1	100 , 1.9 , 0	100 , 0.0 , 37.5

Highest frequency of callus mediated Plb regeneration from explants(Fig.3) 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 Kerbauy,1984 (Fig.4)The effect of cytokinins(BAP/Kn) on chloroplast development as already indicated by Stelter and Laetsh , 1965.The high survival rate of accilimatized plantlets derived from root explants is in accord with earlier reports(Zelcer *et al* , 1983,Chaturvedi *et al*.,2004)) .

V. Conclusion

In Conclusion, the 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.

References

[1] C.Beechey,Propagation of Orchids from aerial roots?*Am. Orchid Soc.Bull*,1970,pp.85-88

[2] M-E.ChurchilL, E.A.Ball,and J.Arditti, "Tissue culture or orchid II.Method for root tips". *Am. Orchid Soc.Bull*,1972,pp.726-730

[3] F.Eduardo , G .Terada, J. M. Fernández, R. Medina, A .Schinini, H. Rey, and L. Mroginski , Plant regeneration from root- tip culture of *Cyrtopodium brandonianum* Barb. Rodr(Orchidaceae).Propagation of Ornamental plants ,2011,pp.184-88

[4] H.C.Chaturvedi,M.Sharma,A.K.Sharma,M.Jain,B.Q.Agha and P.Gupta,In vitro germplasm preservation through regenerative excised rppt culture for conservation of Phytodiversity,2004,Indian Journal of Biotechnology,pp.305-315

[5] IUCN, "IUCN Directory of Protected Areas in Oceania prepared by the World Conservation Monitering Centre IUCN, Gland, Switzerland and Cambridge,U.K.",1991,pp.447

[6] G.B Kerbauy,Regeneration of protocorm like bodies through in vitro culture of root tips of *Catasetum* (Orchidaceae). *J.Plant Physiol*, 1984,PP.287-291

[7] G.B Kerbauy,*In vitro* conversion of *Cattleya* root tips into protocorm like bodies. *J.Plant Physiol*, 1991,pp.248-251

[8] G.C.Mitra, R.N.Prasadand A.Roy.Chwdhary ,Inorganic salts and differentiation of protocorms in seed callus of an orchid and

[9] correlated changes in its free amino acid content.*Indian J.Exp.Biol*,1976,pp.350-51

[10] S.Y.Park ,H.N.Murthy,K.Y.Paek, Protocorm like body induction and subsequent plant regeneration from root tip culture of *Doritaenopsis.Plant Sci*,2003,pp.919-923

[11] D.A.Stetler , W.M.Laetsh,Chloroplast structure and function in cultured Tobacco tissue.*Science*,1965.pp. 1387-1388

[12] S.K.Yasugi S,K.Sakamoto,K.Onodera and M.Tamashiro, Plantlet regeneration in root culture of *Cymbidium* Kenny 'wine color'.Plant tissue culture letters,1994,PP.150-52

[13] A.Zelcer, Soferman,S.OIzar, Shoot regeneration in root cultures of Solanaceae, *Plant Cell Rep*,1983,pp. 252

Author's Biography:

Dr VISHAL SHARMA(M.Sc ,PhD),Post Graduate Government College For Girls-11,Chandigarh(Panjab University),E-mail-vishal_2370@yahoo.com

Correspondence Author: Dr VISHAL SHARMA;E-mail-vishal_2370@yahoo.com;Contact Number:09988150150

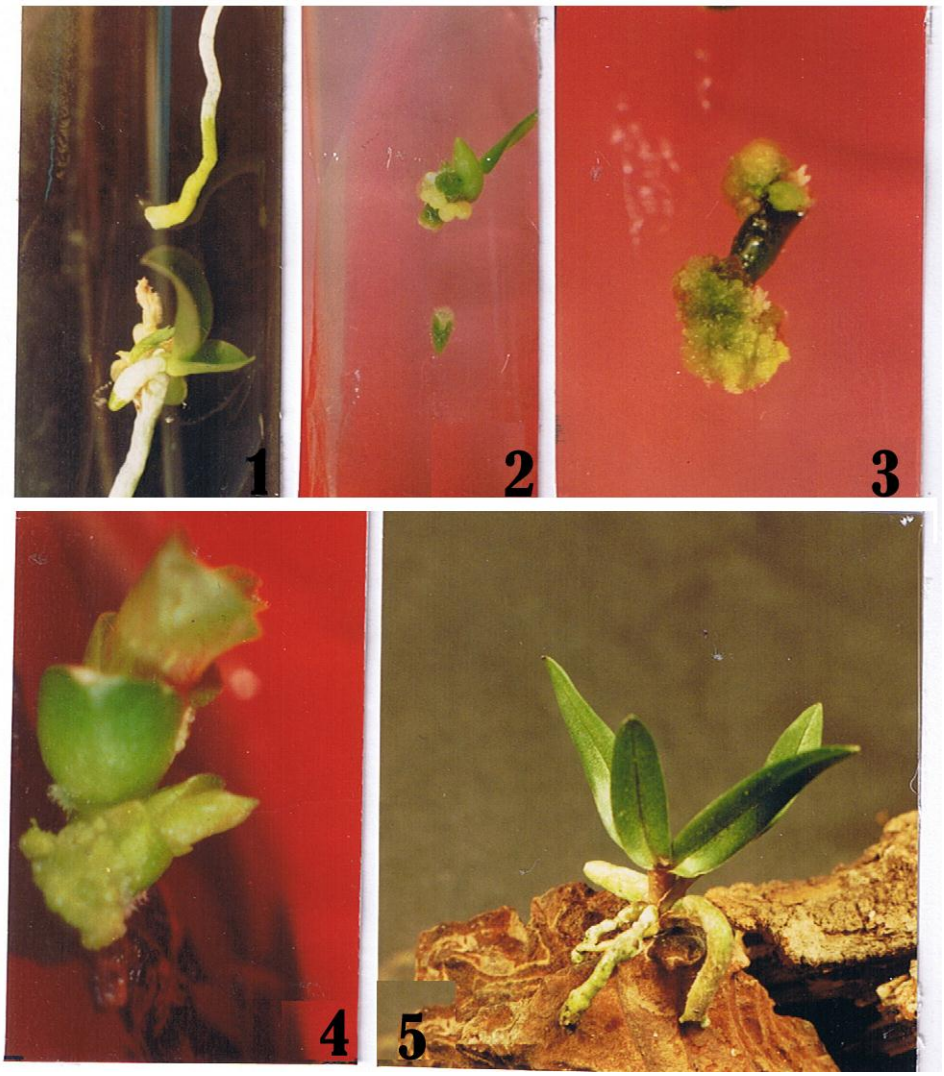

Fig1: Extended root growth in BM+KN_1+NAA_1+AC;
Fig2: Plbs formed in rings in BM+KN_1+NAA_1;
Fig.3: Profused callusing in BM KN_1+NAA_1+AC;
Fig4: Callusing of shoots along the base in BM+ KN_1+NAA_1+AC;
Fig5: Accilimatized Plantlet