GTRP Botanical Report

Micropropagation of rare orchid, *Eulophia epidendraea* (Retz.) Fischer

M. Maridass[#], G. Raju[#], R. Mahesh* and K. Muthuchelian*

[#]Department of Advanced Zoology and Biotechnology, Pioneer Kumaraswamy College, Nagercoil – 629 003, Tamil Nadu, South India.

*Centre for Biodiversity and Forest Studies, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai – 625 021, Tamil Nadu, India.

Published: 15 September, 2012; Vol. No.1(1):1- 4; www.gbtrp.com;

Abstract

A simple and reliable procedure for *in vitro* clonal propagation of orchid *Eulophia epidendraea* was studied. The explants of pseudo bulbs of *E. epidendraea* were cultured on Murashige and Skoog medium (MS) and media supplemented with different plant growth regulators (BAP, NAA and IBA). In the present study, the highest shoot number formed on the MS medium supplemented with 0.5 mg l^{-1} BAP + 0.25 mg l^{-1} IBA (43.75 shoots / 20 explant) and multiple shoot induction of best auxin combinations of 0.5 IBA mg/L-^{1}+ 0.1 NAA mg/L^{-1} were observed. The plantlets of *E. epidendraea* were acclimatized and transplanted to greenhouse and more than 89% survival rate was observed.

Key words: *Eulophia epidendraea* (Retz.) Fischer, Micropropagation

Abbreviation: BAP: 6-benzylaminopurine; IBA: indole-3-butyric acid; NAA - 1-naphthaleneacetic acid ; MS - Murashige and Skoog Medium.

Introduction

Orchids are the largest and most diverse group among the angiosperms. They are widely known for their economic importance and medicinal value. Several orchids are endemic to India most of the species are ornamental and highly medicine due to demand their natural populations have been over exploited. The several genera are extinct from natural population such as *Arundina, Cymbidium, Coelogyne, Dendrobium, Paphiopedilum, Renanthera, and Vanda and* several orchid species are endangered such as *Acanthephippium sylhetense, Anoectochilus sikkimensis, Aphyllorchis montana, Arachnanthe clarkei, Arundina graminifolio, Cymbidium macrorhizon, Dendrobium densiforum, Didiciea cunninghamii, Eria crassicaulis, Galeola lindleyana, Gastrodia Exilis, Paphiopedilum fairanum, P. druryi, Pleione humilis, Renanthera imschootiana, Vanda coerulea,* and *V. roxburghi (*http://www.orchidsasia.com/orcintro.htm,2012*).* Therefore, there is urgent need for development of new technology of conservation of orchid species.

Plant tissue culture and micropropagation techniques play an important role in conservation of endemic and endangered plants (Engelmann, 2011). Tissue culture allows the rapid clonal propagation of large numbers of plantlets in a short period the conserved several species (Morel, 1960,1970; Stewart and Button,1976; Vajrabhaya,1978; Shimasaki and Uemoto, 1987; Arditti and Ernst,1993; George and Ravishankar,1997; Vij and Kaur,1998; Kanjilal *et al*., 1999; Pyati *et al*.,2002; Decruse *et al*., 2003; Basker and Narmatha, 2006; Martin, 2007, Janarthanam and Seshadri, 2008; Medina *et al*.,2009; Rangsayatorn,2009; Hong *et al*., 2010).

Eulophia epidendraea (Retz.) Fischer is one of the rare species in India (Maridass *et al*., 2005). The tuber of *E. epidendraea* is used for curing several diseases such as tumour, abscess and healing of wound (Maridass *et al*., 2008). The present study made an attempt to mass clonal propagation of *E. epidendraea* (Retz.) Fischer. It is established in MS media with different growth regulators for shoot formation, roots induction and plantlets development.

Materials and Methods

The plant materials of *E. epidendraea* were collected from the scrub jungle of Kambli, Tenkasi Taluka, Tirunelveli District, Tamil Nadu, South India. The explants were surface sterilized and the pseudobulbs were excised. The explants of *E. epidendraea* pseudobulbs were cultured on Murashige and Skoog media (Murashige and

Skoog, 1962) and supplemented with different concentration of BAP, NAA and IBA (Table-1 and 2). After preparing the media, pH was adjusted to 5.8 with digital pH meter adding 0.1N NaOH or 0.1N HCl. Agar powder (10g/l^{-1}) was added to solidify the media. The culture tubes containing the media were autoclaved with 1.16 kg/cm^2 pressure at 121^{O}C for 20 minutes. The isolated pseudobulbs were cultured in MS medium supplemented with different concentration and combinations of auxin and cytokinin (Table -1 and 2). All data were statistically analyzed by Microsoft Excel.

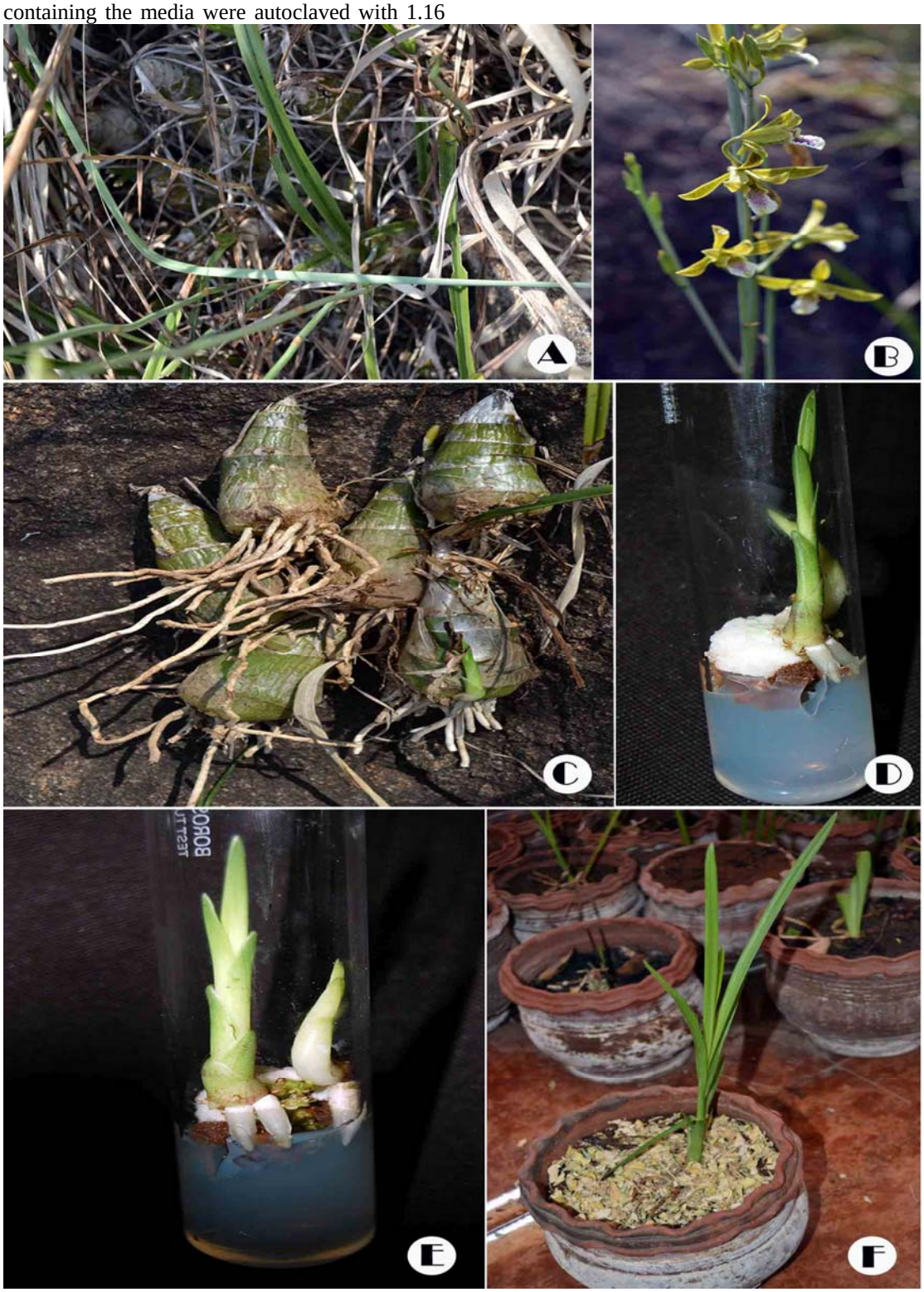

Plate -1: *In vitro* multiple shoots and root induction of *E. epidendraea*

Table-1: Effect of different concentration of BAP and IBA for multiple shoot induction from pseudobulbs explants of *Eulophia epidendraea*

Plant growth regulators BAP+IBA	Explants	No. of explants responses	% Responses of Explants	No of multiple shoots (%)	Length of shoots(mm)
0.1+0.1	20	8	40	25	18.50± 3.33
0.3+0.2	20	13	65	23	21.00± 5.50
0.5+0.25	20	16	80	43.75	20.25± 3.75
1.0+0.5	20	14	70	35.71	20.57± 5.25
2.0+0.1	20	9	45	22.22	19.67± 3.00

Values are expressed mean ± SD

Table-2: Effect of different concentration of BAB and NAA on roots induction from pseudobulbs explants of *Eulophia epidendraea*

Plant growth regulators (mg/$^{L-1}$ BAB + NAA mg/$^{L-1}$)	Explants	No. of explants responses	% Responses of Explants	No of multiple roots (%)	Length of roots (mm)
0.1 +3.0	20	4	20	4	14.25 ± 2.22
0.3+2.0	20	7	35	7	17.85 ± 4.74
0.5+1.0	20	9	45	9	18.11 ± 5.82
1.0+0.5	20	6	30	6	20.50 ± 3.93
2.0+0.25	20	5	25	5	10.80 ± 4.21

Values are expressed mean ± SD

Results and Discussion

The *E. epidendraea* cultured on MS medium supplemented with different combinations of growth regulators and the concentration was observed (Table- 1 and 2). The roots induction and shoot formation are represented in plate -1 A-F. The results indicated that the highest shoot numbers were formed on the MS medium supplemented with 0.5 mg l^{-1} BAP + 0.25 mg l^{-1} IBA (43.75 shoots / 20explant) (Plate-1E). Nine multiple shoot formation in the best auxin combinations of 0.5 IBA mg/L-^{1}+ 0.1NAA mg/L^{-1} was recorded 9 with root length of 18.11 ± 5.82mm. Similar report on clonal culture of orchids through tissue culture of several explants such as leaf (Tanaka, 1987); root tips (Tanka *et al*., 1976) flower stalk (Homma and Asahira, 1985; Lin, 1986) and lateral buds (Ichihashi, 1992) were studied. The established plantlet with well-developed roots (Plate-1D and E) were transferred and acclimatized to greenhouse with 25°C and 90% relative humidity. After 15 days 2% KH_2PO_4 sprayed on leaves of *E. epidendraea* for better development. 89-92% of survival rate of *E. epidendraea* was observed in the nutrient medium of humus: sawdust (1:1). The maximum number of survival rate was recorded after 30 days of transplantation (Plate 1-F). This study produced an efficient regeneration protocol for clonal micropropagation of *E. epidendraea* through pseudo bulbs was established.

Acknowledgement

Authors are grateful to University Grant Commission, New Delhi, for providing financial assistant through Dr. D. S. Kothari Postdoctoral program.

References

http://www.orchidsasia.com/orcintro.htm,2012. Introduction to Orchids. *updated on 15,04,2012.*

Morel, G.M. 1960. Producing virus free cymbidiums. *American Orchid Society Bulletin*, 29: 495–497.

Engelmann, F. 2011. Use of biotechnologies for the conservation of plant biodiversity. *In Vitro Cellular & Developmental Biology*, 47(1):5-16

Morel, G.M.1970. Neues auf dem Gebiet der meristem (in german). *Forschung Die Orchidee*, 21: 435– 443.

Stewart, J. and Button, J. 1976. Tissue culture studies in *Paphiopedilum*. *Proceedings of Eighth World Orchid Conference, Frankfurt*, pp: 372–378.

Vajrabhaya, M.1978. Tissue culture of dormant buds from *Cattleya* back bulbs. *Orchid Review*, 86: 256–257.

Shimasaki, K. and Uemoto, S.1987. Studies on micropropagation of Japanese *Calanthe* species. *Science Bulletin for Faculty of Agriculture. Kyunshu University*, 42: 293–297.

Arditti, J. and Ernst, R.1993. *Micropropagation of orchids*. New York: John wiley and sons.

George, P. S. and Ravishankar, G. A. 1997. *In vitro* multiplication of *Vanilla planifolia* using axillary bud explants. *Plant Cell Reports*, 16: 490–494.

Vij, S. P. and Kaur, S.1998. Micropropagation of therapeutically important orchids: *Malaxis acuminata*. *The Journal of The Orchid Society of India*, 12: 89–93.

Kanjilal, B. D., Sarker D. D., Mitra, J., Datta, K. B. 1999. Stem disc culture: Development of a rapid mass propagation method for *Dendrobium moschatum* (Buch. Ham.) Swartz.- an endangered orchid. *Current Science*, 77: 497–499.

Pyati, A.N., Murthy, H.N., Hahn, E.J. and Paek, K.Y. 2002. *In vitro* propagation of *Dendrobium macrostachyum* Lindl. - a threatened orchid. *Indian Journal of Experimental Biology*, 40: 620– 623.

Decruse, S.W., Gangaprasad, A., Seeni, S. and Menon, S. V. 2003. Micropropagation and ecorestoration of *Vanda spathulata*, an exquisite orchid. *Plant Cell Tissue and Organ Culture*, 72: 199–202.

Basker, S. and Narmatha, V. B. 2006. Micropropagation of *Coelogyne stricta* (D.Don) Schltr. via pseudobulb segment culture. *Tropical and Subtropical Agrosystems*, 6: 31–35.

Martin, K.P.2007. Micropropagation of the Bamboo orchid (*Arundina graminifolia* (*D.Don*) HOCHR.) through protocorm-like bodies using node explants. *Propagation of Ornamental Plants*, 7: 97–100.

Janarthanam, B. and Seshadri, S. 2008. Plantlet regeneration from leaf derived callus of *Vanilla planifolia* Andr. *In Vitro Cellular and Developmental Biology-Plant*, 44: 84–89.

Medina, R.D., Flachland, E, A., Gonzalez, A. M., Terada, G., Faloci, M.M. and Mroginski, L.A. 2009. *In vitro* tuberization and plant regeneration from multimodal segment culture of *Habenaria bractescens* Lindl., an argentenian wetland orchid. *Plant Cell, Tissue and Organ Culture*, 97: 91–101.

Rangsayatorn, N. 2009. Micropropagation of *Dendrobium draconis* Rchb.f. from thin cross-section culture. *Scientia Horticulturae*, 122: 662–665.

Hong, P. I., Chen, J. T. and Chang, W. C. 2010. Shoot development and plant regeneration from protocorm-like bodies of *Zygopetalum mackayi*. *In Vitro Cellular & Developmental Biology-Plant*, 46: 306–311.

Maridass, M. Victor, B. Ramesh, U. 2005. Ethnobotanical information of Eulophia epidendraea (Retz) Fischer (Orchidaceae) in the Kambli Malaikovil Forest, Tirunelveli district, Tamil Nadu. Journal Bombay Natural History Society,102(2): 255.

Maridass, M., Thangavel, K. and Raju, G.2008. Antidiabetic Activity of tuber extract of *Eulophia epidendraea* (Retz.) Fisher (Orchidaceae) in alloxan diabetic rats. *Pharmacologyonline*, 3: 606-617.

Murashige, I. and Skoog, F. 1962. A revised medium for rapid growth and bioassys with tobacco tissue culture. *Plant Physiology*,15: 473-497.

Tanaka, M. 1987. Studies on the clonal propagation *Phalaenopsis* through *in vitro* culture. Member, Facultyl of Agriculture, Nagoya University. Japan. 49: 1-85.

Tanaka, M., Senda, Y. and Hasegawa, A. 1976. Plantlet fromation by root tip culture in *Phalaenopsis*. *American Orchid Society Bulletin*,56:1024-1032.

Homma, Y. and Ashira, T. 1985. New means of phalaenopsis propagation with internodal sections of flower stalk. *Journal of Japan. Horticulture Science*,53: 379-387.

Lin, E. E. 1986. *In vitro* culture of flower stalk internodes of *Phalaenopsis* and *Doritaenopsis Lindlayana*, 1:158-163.

Ichihashi, S. 1992. Micropropagation of *Phalaenopsis* through the culture of lateral buds from young flower stalks. *Lindleyana*, 7(4): 208-215.

Manuscript Progress Date
Received : 11.03.2012
Revised : 29.07.2012
Accepted : 30.07.2012