International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2018; 6 (1):181-186
ISSN:2320-7817(p) | 2320-964X(o)
UGC Approved Journal No 48951

Original Article Open Access

Micropropagation of the Monopodial Orchid, *Rhynchostylis retusa* (L.)

Sunitibala Devi Y and Neelashree N

Advanced Level Institutional Biotech-Hub, Department of Botany, G.P. Women's College, Imphal, Manipur, India.

Manuscript details:

Received : 23.11.2017
Accepted : 07.03.2018
Published : 13.03.2018

Editor: Dr. Arvind Chavhan

Cite this article as:
Sunitibala Devi Y and Neelashree N (2018) Micropropagation of the Monopodial Orchid, *Rhynchostylis retusa* (L.), *Int. J. of. Life Sciences*, Volume 6(1): 181-186

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Rhynchostylis retusa, a monopodial orchid is important for both its medicinal and economic values. Different parts like shoot, leaf and root were used as explants for micropropagation of *Rhynchostylis retusa*. The explants were cultured in Vacin and Went (VW), Knudson C and Nitsch media supplemented with sucrose (20 g L^{-1}) and growth regulators, viz., NAA (0.1 mgL^{-1}), BAP (1.0 mgL^{-1}) and Kinetin, Kn (0.4-1.0 mgL^{-1}) in different concentrations and combinations. Green protocorm-like bodies (PLBs) and calli which later differentiated into plantlets were induced from shoot-tip, leaf and root-tip explants using VW medium supplemented with NAA (0.1 mgL^{-1}) along with either Kinetin (1.0 mgL^{-1}) or BAP (1.0 mgL^{-1}). Maximum survival as well as differentiation was obtained from shoot-tip explants. Further growth and development of PLBs into plantlets were achieved when cultured in VW medium with gradual removal of NAA and reduction of Kinetin and BAP concentrations. Transplantation in community pots could be done when the plantlets were 3.0-3.5 cm. These results suggest a methodology for *in vitro* mass propagation of *Rhynchostylis retusa*.

Keywords: Micropropagation, *Rhynchostylis retusa*, PLBs (Protocorm-like bodies), *in vitro*, plantlets

INTRODUCTION

Orchid represents one of the highly evolved families among the monocotyledons with a 600-800 genera and 25,000 – 35,000 species. The flowers are most beautiful and have long shelf life which fetches an attractive price in the international markets. Among these, *Rhynchostylis retusa* is a promising orchid as it has both medicinal and economic value. Akhter *et al*. 2017 reported that *Rhynchostylis retusa* have been used by the tribal communities of different parts of south east Bangladesh for treatment of paralysis, rheumatism, piles, irregular menstruation, heavy menstruation, painful menstruation, fracture, fever, allergy and inflammation. The roots of *Rhynchostylis retusa* are also used in treatment of malarial fever (Tiwari *et al*. 2012; Radhika and Murthy, 2013). The juice derived from the roots is also applied to cuts and wounds and dried flowers are used as insect repellent (Subedi *et al*. 2013).

Fig. 1: *Rhynchostylis retusa* in natural habitat

Though it has medicinal importance, but the ornamental usage outweighs its medicinal ones. Nowaday, the propagation of orchids through tissue culture could splendidly provide the increasing demands of orchids. Various tissue culture methods have been widely employed in the clonal propagation of orchids (Murashige 1974, Goh 1989). Shoo-tip and auxillary bud are mostly used explants but in case of monopodial orchids, excision of these explants often results in sacrificing the other plants. Previously many attempts have been made to use alternative explants but the success of these explants in other monopodial orchids are limited to a few, for example, plantlet leaves in *Aranda* (Goh *et al*. 1990), young leaves of mature *Renantanda* (Goh and Tan 1982), floral buds in *Mokara* (Lim Ho *et al* 1986), *Vanda* root and leaf segment in *Rhynchostylis retusa* (Vij *et al.* 1984, 1987), inflorescence-tip explant in hybrid orchid *Aranda* 'Deborah' (Goh and Wang 1990) and shoot-tips of flower stalk buds in *Phalaenopsis* and *Doritaenopsis* (Tokuhara and Masahiro 1993). The present paper describes the successful micropropagation of *Rhynchostylis retusa* using different explants like shoot-tip, leaf and root-tip from *in vitro* raised seedlings.

METHODOLOGY

Fourteen week-old plantlets raised aseptically from 60-80 day-old immature embryos of *Rhynchostylis retusa* were used for taking different explants, viz., shoot-tip, leaf and root-tip. The size of the explants ranges in size from 0.5-1.0 cm in length. Three explants each of shoot-tip, leaf and root-tip were cultured in Vacin and Went (VW), Knudson C and Nitsch media. All these media were enriched with sucrose (20 g L^{-1}) and growth regulators, viz., NAA (0.1 mgL^{-1}), BAP (1.0 mgL^{-1}) and Kinetin, Kn (0.4-1.0 mgL^{-1}) were added singly or in combination. Subculturing of all the cultures were done at an interval of 30 days. Further culture for multiplication of PLBs and calli were done in VW medium supplemented with NAA (0.1 mgL^{-1}) along with either Kinetin (1.0 mgL^{-1}) or BAP (1.0 mgL^{-1}). All the media were adjusted to pH 5.2 before autoclaving at 15 lbs $inch^{-2}$ for 20 min. All the cultures were kept at 4°C in the dark for 48 hr and thereafter, maintained in a culture room at 25±1°C under a photoperiod of 14 hr light. Light of approximately 3000 lux were supplied by the fluorescencent tubes. The experiment was repeated thrice and each treatment consisted of atleast 3 explants.

RESULTS

The different basal media seem to have an effect on the response of the different explants cultured in all the media tested. The explants remained green for about 20 days then became brown and dried up except those cultured in VW medium. As VW is found to be the most suitable medium among those tested, further experiments are done with VW medium only. Different explants exhibit different responses when the combinations and concentrations of growth regulators administered are varied (Table 1).

Shoot explants show maximum response while response is least in root explants. Response rate of leaf explants are also less than fifty percent. The response of shoot, leaf and root explants are either as direct development of protocorm-like bodies (PLBs) or induction of callus. The differential response of these explants in VW medium with different hormone combinations and concentrations are given in Tables 2-4. Shoot explants respond after 2 weeks while leaf explants remain unchanged for 4 weeks. Root explants take the longest time in their response and remain unchanged for as long as 6-10 weeks. The shoot and leaf explants swell while still green in colour, in the areas of the contact with the medium.

PLBs are developed from the swollen areas. In shoot explants, PLBs developed in all the hormone concentrations and combinations tested with the maximum being in VW medium supplemented with NAA (0.1 mgL^{-1}) and Kn (1.0 mgL^{-1}). PLB formation is minimum when NAA (0.1 mgL^{-1}) is added along with BAP (2.0 mgL^{-1}). In leaf explants, PLBs are induced only in VW medium containing NAA (0.1 mgL^{-1}) along with Kn (1.0 mgL^{-1}) or BAP (0.1 mgL^{-1}) but percentage is very low (16%) in comparison with shoot explants. Least PLB induction occurs in case of root explants and only in VW medium containing Kn (1.0 mgL^{-1}).

Table 1: Response of different explants of *Rhynchostylis retusa* in VW medium supplemented with different hormones viz., NAA, Kn and BAP.

Hormones (mgL^{-1})			Response of explants (%)		
NAA	**Kn**	**BAP**	**Shoot**	**Leaf**	**Root**
0.1	1.0	-	90.00	28.00	-
0.1	2.0	-	80.00	-	-
-	1.0	-	90.90	-	10.00
-	0.8	-	76.19	-	-
-	0.4	-	15.38	-	-
0.1	-	1.0	82.61	41.67	-
0.1	-	2.0	20.83	-	13.63

Table 2: Effect of NAA, Kn and BAP supplemented in VW medium on the callus induction and PLB formation of shoot-tip explants of *Rhynchostylis retusa* after 8 weeks of culture.

Hormones (mgL^{-1})			Callus induction (%)	PLB Formation(%)
NAA	**Kn**	**BAP**		
0.1	1.0	-	40.00	50.00
0.1	2.0	-	25.00	25.00
-	1.0	-	54.54	54.54
-	0.8	-	47.61	47.61
-	0.4	-	-	-
0.1	-	1.0	47.83	47.83
0.1	-	2.0	8.33	8.33

Table 3: Effect of NAA, Kn and BAP on callus induction and PLB formation in leaf explants of *Rhynchostylis retusa* with VW as basal medium.

Hormones(mgL^{-1})			Callus induction (%)	PLB formation (%)
NAA	**Kn**	**BAP**		
0.1	1.0	-	12	16
0.1	2.0	-	-	-
-	1.0	-	-	-
-	0.8	-	-	-
-	0.4	-	-	-
0.1	-	1.0	25	16.67
0.1	-	2.0	-	-

Table 4: Response of root-tip explants of *Rhynchostylis retusa* in VW medium supplemented with growth regulators.

Hormones(mgL^{-1})			Callus induction (%)	PLB formation (%)	Weeks before browning
NAA	**Kn**	**BAP**			
0.1	1.0	-	-	-	8
0.1	2.0	-	-	-	6
-	1.0	-	-	10	6
-	0.8	-	-	-	10
-	0.4	-	-	-	8
0.1	-	1.0	-	-	9
0.1	-	2.0	13.63	-	6

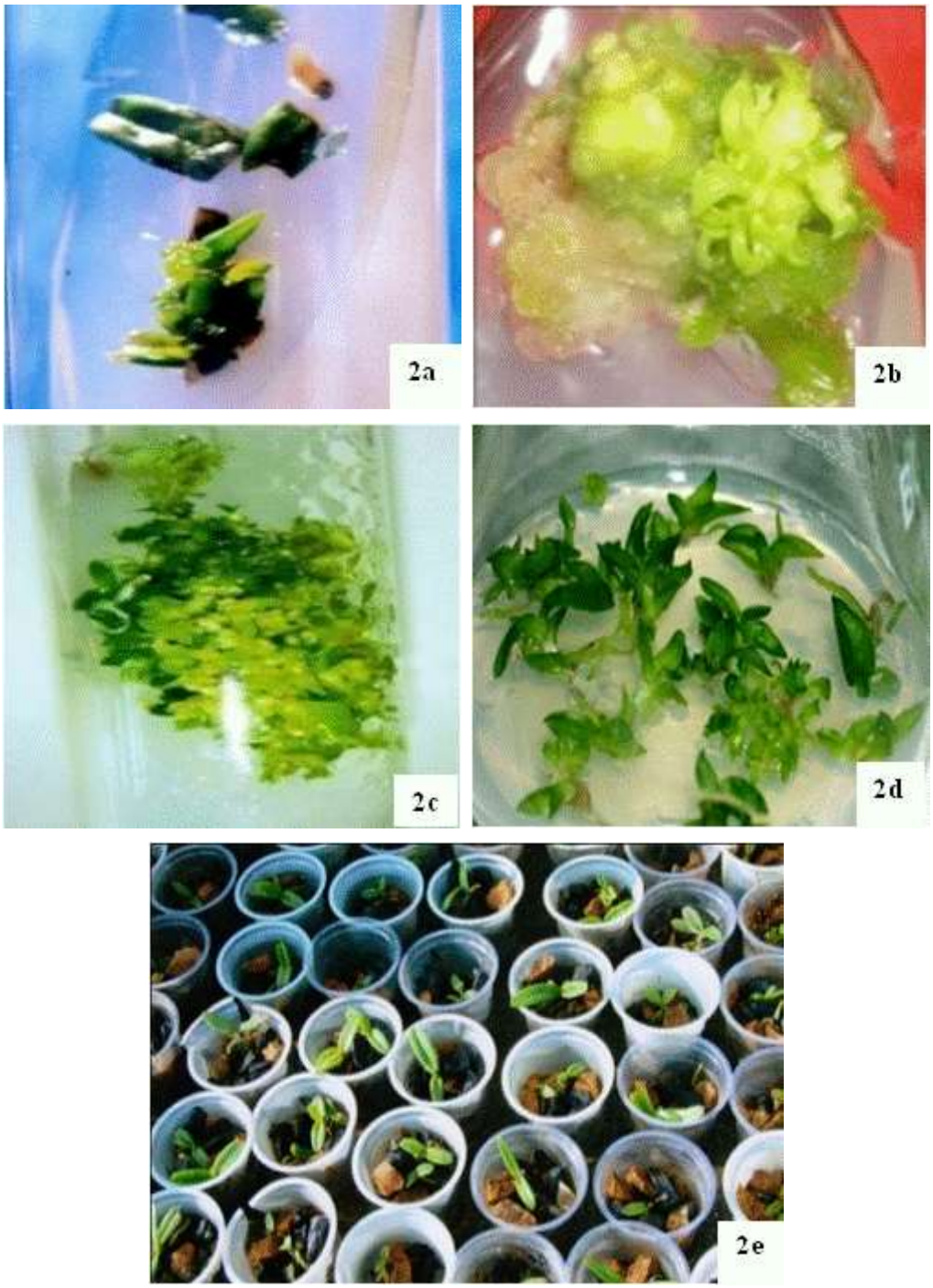

Fig. 2: (2a) Induction of callus from leaf; **(2b)** development of PLBs and plantlets from callus; **(2c)** plantlet development with appearance of leaf and root; **(2d)** regenerated plantlet of orchid under multiplication; **(2e)** transplanted seeding of *Rhynchostylis retusa*

All explants which do not form PLBs turn brown and calli are induced from the brown explants. Callus formation of shoot explants occur in varying frequencies in all hormone concentrations and combinations tested. Leaf explants induced callus only in NAA (0.1 mgL^{-1}) and BAP (1.0 mgL^{-1}) or Kn (1.0 mgL^{-1}) containing medium (Fig: 2a) while root explants form callus in medium with NAA (0.1 mgL^{-1}) and BAP (2.0 mgL^{-1}). Calli are fragile and white in colour in the initial stage of induction. Later, after 2-3 subcultures, calli turn creamish and PLBs are induced from the superficial portion of the cream-coloured calli (Fig: 2b). Calli growth is very slow

in root explants when compared with those derived from shoot and leaf explants. Calli production is more in explants taken from 14 week-old plantlets when compared with explants from 16 week-old plantlets. When the PLBs derived directly or differentiated from calli induced from all the three explants are subcultured, continuous production of PLBs are obtained for more than one year. The older and upper portion of the clusters of PLBs first developed into plantlets and new PLBs are induced from the basal portion (Fig: 2c). The developing plantlets while growing in hormone containing medium for 7-8 passages turn brown and the medium also become blacken. Growth of plantlets in such condition is slow but is enhanced when NAA and Kn or BAP are reduced sequentially at every subculture and finally transfer to hormone-free medium. Roots are developed when the shoots attained 1.5-2.0 cm in height in hormone free medium (Fig: 2d). The plantlets 3.0-3.5 cm in height thus produced survive when transplanted to pots containing a mixture of charcoal and brick pieces with *Sphagnum* moss (Fig: 2e).

DISCUSSION

The results showed that VW medium was superior to the other media cultured. Reports on *in vitro* clonal propagation of any *Rhynchostylis sp.* is limited (Vajrabhaya and Vajrabhaya, 1970, Vij *et al.* 1984, Sood and Vij 1986, Bui *et al.*1999, Kumar *et al.* 2003). This experiment showed that the different explants had differential regenerative potentials. The shoot bud explants showed the highest rate of regeneration similar to as recorded in case of *Vanda* (Valmayor *et al.*1986) using NAA and BAP. The explants formed PLBs which corresponded developmentally to one of the embryonal stages in orchid seed germination as well as callus which later differentiated into plantlets. The responses as well as the regenerative potential were even more in leaf explants than the root explants which showed least response. Formation of callus was also reported earlier in the leaf and root segment culture of *Rhynchostylis retusa* (Vij.1984, Sood *et al.* 1986, Kumar *et al.* 2003, Parab *et al.*2012). The age of the explants and different concentration of plant growth regulator seemed to have an effect on the growth of the explants and calli differentiation (Dhar and Joshi 2005; Zhang *et al*. 2017). The relationship between brown exudation and browning of the plantlets while culturing continuously in hormone containing medium could not be defined. The brown exudation in the medium was less and browning of plantlet could be avoided when transferred to medium with reduced hormone concentration and then hormone-free medium. This rapid and continuous production of plantlets from different explants particularly, shoot-bud could be used as a means of propagation of this rare orchid. Calli growth is very slow in root explants when compared with those derived from shoot and leaf explants. The use of other explants like leaf and root for micropropagation could also be increased and should be applicable to other cultivars of *Rhynchostylis* also.

CONCLUSION

Rhynchostylis retusa is an orchid which has medicinal properties as well as economic value. But, lack of proper cultivation practices, destruction of plant habitats and illegal and indiscriminate collection of plants from natural habitats pose a great threat to many medicinal (Devendra *et al*. 2011; Bhattacharjee *et al*. 2014). Thus, micropropagation offers an alternate method for effective *in vitro* conservation and mass propagation of *Rhynchostylis retusa* .

Acknowledgement

The authors are thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for providing working facility by giving financial assistance for the establishment of Institutional Level Biotech Hub.

REFERENCES

Akhter M, Hoque MM, Rahman M and Huda MK (2017) Ethnobotanical investigation of some orchids used by five communities of Cox's Bazar and Chittagong hill tracts districts of Bangladesh. *Journal of Medicinal Plants Studies* 5: 265-268.

Arditi, J (1977) Orchid Biology: Reviews and Perspective I, Cornell Univ. Press, New York, p203-293.

Bhattacharjee B and Islam SMS (2014) Effects of plant growth regulators on multiple shoot induction in *Vanda tessellata* (Roxb.) Hook. Ex G.Don an endangered medicinal orchid. Int. *J. Sci. Nat*, 5: 707-712.

Bui VL, Hang Phuong NT, Anh Hong LT and Van KTT (1999) High frequency shoot regeneration from from *Rhynchostylis gigantea* (Orchidaceae) using thin cell layer. *Plant Growth Reg*., 28: 179-185

Devendra BN, Srinivas N and Naik GR (2011) Direct somatic embryogenesis and synthetic seed production from *Tylophora indica* (Burm.f.) Merrill an endangered, medicinally important plant. *Int. J. Bot*, 7: 216-222.

Dhar U and Joshi M (2005) Efficient plant regeneration protocol through callus for *Saussurea obvallata* (DC) Edgew (Asteraceae): effect of explants type, age and plant growth regulators. *Plant Cell Reports*, 24: 195-200

Goh CJ (1989) Orchids-monopodials. In PV Amirato, DA Evans, WR Sharp and YPS Bajaj (Eds), Handbook of plant cell culture, Vol.5, Ornamental Species, Mc-Graw Hill, New York, p598-637.

Goh CJ and Tan H (1982) Clonal Propagation from leaf explants in *Renantanda* orchid hybrid, Orchid Rev 90, p295-296.

Goh CJ and Wang PF (1990) Micropropagation of the monopodial orchid hybrid *Aranda* Deborah using inflorescence explants. *Scientia Hort*., 44: 315-321.

Kumar A, Palni LMS and Nandi SK (2003) The effect of light and gelling agent on micropropagation of *Rosa damascena* Mill. and *Rhynchostylis retusa* (L.) Bl. *J. Hort. Sci. Biotechnol.,* 78: 786-792

Lim Ho CL, Teo-Lee GC and Phua LK (1986) Clonal propagation of orchids from flower buds. In proceeding of the Fifth Asian Orchid Congress(Ed: AN Rao), Singapore, p98-101.

Murashige T (1974) Plant propagation through tissue culture. *Ann.Rev.Plant Physiol.* 25:135-166.

Parab GV and Krishnam S (2012) Rapid *in vitro* mass multiplication of orchids *Aerides maculosa* Lindl. and *Rhychostylis retusa* (L) Bl. from immature seeds *Indian Journal of Biotech.* Vol 11: 288-294

Radhika B and Murthy N (2013) Preliminary phytochemical analysis and *in vitro* bioactivity against clinical pathogens on medicinally important orchid of *Rhynchostylis retusa Blume. Am. J. Pharm. Tech. Res*, 3: 510-520.

Sood A and Vij SP (1986) *In vitro* root segment culture of *Rhynchostylis retusa* Bl, In Biology, Conservation and Culture of Orchids(Ed: SP Vij) Affiliation East-West Press, New Delhi, p463-468.

Subedi A, Kunwar B, Choi Y, Dai Y, Andel TV, Chaudhury RP, Boer HJD and Gravendeel B (2013) Collection and trade of wild-harvested orchids in Nepal. *J. Ethnobio*. E*thnomed*, 9: 64-74.

Tiwari AP, Joshi B and Ansari AA (2012) Less known ethnomedicinal uses of some orchids by the Tribal inhabitants of Amarkantak Plateau, Madhya Pradesh, India. Nat. Sci, 10: 33-37.

Tokuhara K and Masahiro M (1993) Micropropagation of *Phalaenopsis* and *Doritaenopsis* by culturing shoot tips of flower stalk buds. *Plant Cell Rep*., 13:7-11.

Vajrabhaya M and Vajrabhaya T (1970) Tissue culture of *Rhynchostylis gigantean*, A monopodial orchid.Am. *Orchid Soc. Bull*., 39:907-910.

Valmayor HL, Pimentel ML and Martinez MT (1986) Callus formation and plantlet morphogenesis in *Vanda.* Malay Orchid Rev (Singapore) 20: 22-30.

Vij SP, Pathak P and Sharma M (1987) On the regeneration potential of *Rhynchostylis retusa* root segments: A study *in vitro*. *J Orchid Soc. India*., 1:71-74.

Vij SP, Sood A and Plaha KK (1984) Propagation of *Rhynchostylis retusa* Bl. by direct organogenesis from leaf segment culture. *Bot. Gaz*., 145: 210-214.

Zhang Y, Sun D and Hu S (2017). *In vitro* propagation of medicinal and ornamental plant *Lysimachia davurica*. *European Journal of Horticultural Science*, 82: 54-60.