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REGENERATION COMPETENCE OF AN ORNAMENTALLY IMPORTANT EPIPHYTIC ORCHID, *RHYNCHOSTYLIS GIGANTEA* (LINDL.) RIDL. THROUGH LEAF SEGMENTS: A STUDY *IN VITRO*

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Abstract

This paper elucidates the possibility of using leaf segments for micropropagating *Rhynchostylis gigantea*. Mitra *et al*. (1976, M) medium supplemented with KN (1.5 mgl^{-1}) proved optimal nutritional combination for initiation, multiplication, and early plantlet formation in *Rhynchostylis gigantea* leaf culture. The plantlets, thus raised were subjected to hardening procedure (*in vitro* and *ex vitro*) and were established with about 70% survival frequency.

Introduction

RHYNCHOSTYLIS GIGANTEA (Lindl.) Ridl., commonly known as *Fox tail Orchid* is widely distributed in India (tropical Himalayan valleys from Sikkim Westwards to Garhwal and Eastward to Bhutan), Myanmar, Thailand, Malaysia, Vietnam, China, Bangladesh and the Philippines. The species is well known for its beautiful white flowers in long compact and pendant inflorescences. Due to extensive habitat destruction and commercial collection pressure, its natural populations are on decline (Rittirat *et al*., 2011). The species of economic importance, figures prominently on the list of rare plants of India. Hence, propagation of the species is urgently required with a view to conserving the species. Earlier, though the regeneration potential of various explants (seeds, stem, leaf, root, inflorescence *etc.*) has been tested *in vitro* in different species (Anuprabha and Pathak, 2012; Arora *et al*., 2014, 2016; Bhattacharjee and Hossain, 2015; Borah *et al*., 2015; Chauhan *et al*., 2010, 2015; Hegde, 2012; Hoque *et al*., 2016; Kaur and Pathak, 2014; Laqishram and Devi, 1999; Pathak *et al*., 1992, 2011, 2012, 2016; Sibin and Gangaprasad, 2016; Sibin *et al*., 2014; Verma *et al*., 2013; Vij *et al*., 1987,1989, 1994, 1995) so as to develop protocols for their *in vitro* propagation, the data is, however, meager in terms size of the orchid family.

Presently, an attempt was made to develop an appropriate *in vitro* propagation method for *Rhynchostylis gigantea,* using entire leaf segments. Some of the important features of the study are documented in this paper.

Materials and Methods

Whole leaf segments (0.5-1 cm long) obtained from 26 wks old *in vitro* grown cultures were assessed using Mitra *et al*. (1976, M) medium without and with different concentrations and combinations of PGRs [(IAA, NAA, BAP, KN, IAA+KN, IAA+KN, NAA+BAP) see table1]. The cultures were incubated in the ambience of 25±2^{0}C and 12 hr photoperiod of 3,500 Lux light intensity. The results were analyzed using one way analysis of variance performed with respect to each response (average ± standard error) against each additive. As ANOVA results showed the non significant difference of additives at 5% level of significance, various groups of additives showing identical/similar response were formed statistically. To this end, Tukey Test was performed at 5% level with respect to each response.

Results

In the present study, entire leaves from axenic sources were used for regeneration of *Rhynchostylis gigantea*. The regenerative competence of foliar explants of the species was significantly influenced by the nutrient medium, quality and quantity of PGRs, segmentation of leaf explants and orientation of explants on the medium. Similar results were obtained earlier by Deb and Pongener, 2013 and Pathak (1989). According to Wimber (1965), PLBs from the leaves of cymbidiums were successfully developed which opened up an effective alternative to apical shoot meristem cultures. The regeneration competence of leaf explants was positively tested for more than 60 orchid species and success is restricted mostly with epiphytic orchids and only a few species from terrestrial orchids have responded for he purpose (Deb and Sungkumlong, 2010).

Preently, the regeneration potential of whole leaf segments (0.5-1 cm long) procured from 26 wks old *in vitro* raised cultures was successfully tested using Mitra *et al*. (1976, M) medium with and without different

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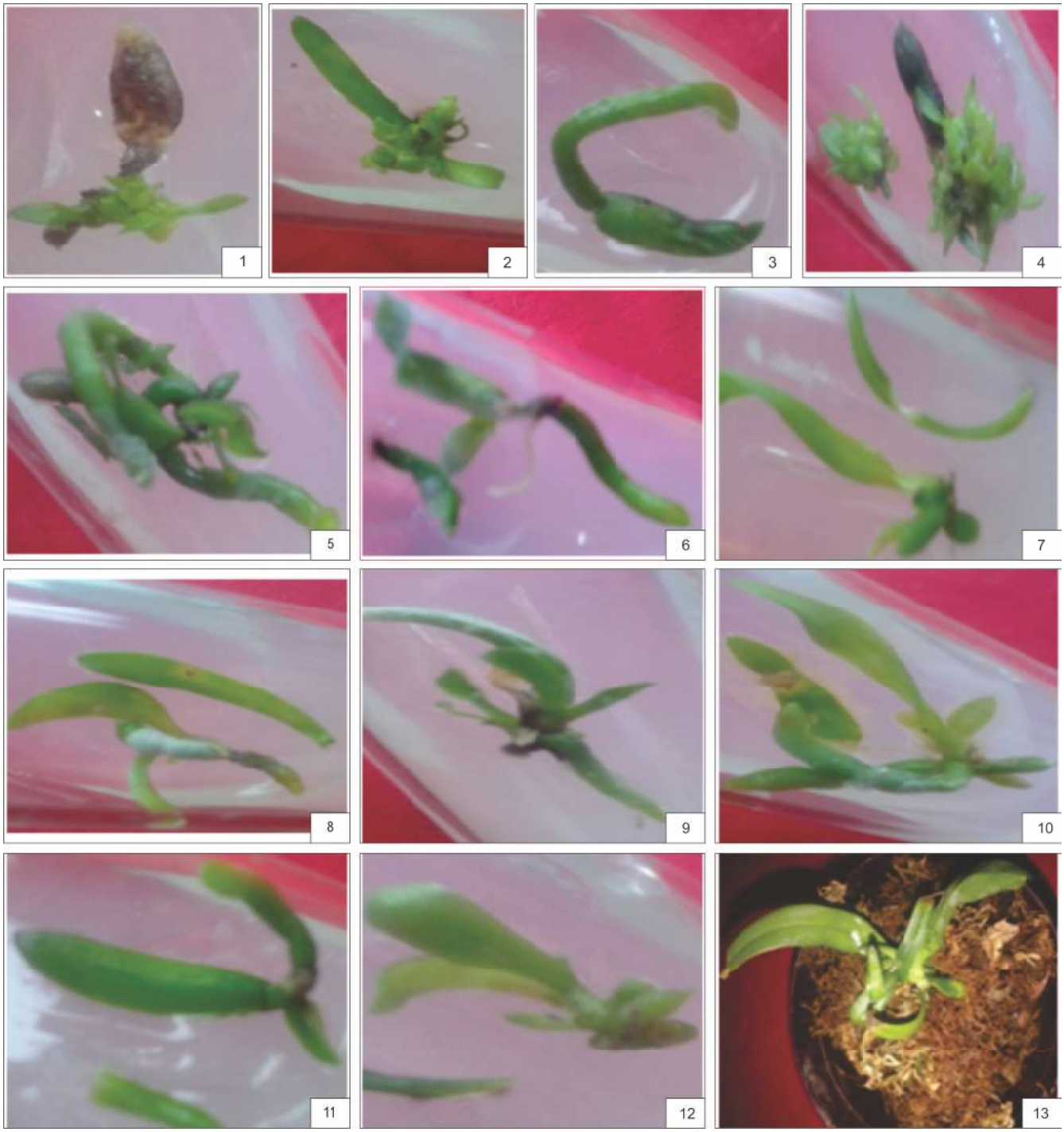
Table1. Regeneration potential of *in vitro* sourced leaf explants (entire) in *Rhynchostylis gigantea* on Mitra *et al.* (1976, M) medium.

Additives	Explants responded (%)	Time taken in days for initiation of response	Number of proliferative loci/explant	Regeneration pathway	Number of plantlets obtained/explant	Time taken in days for development of complete plantlets	Remarks
-	-	-	-	-	-	-	-
IAA (1.5 mgl^{-1})	50.00 ± 0.81^{b}	30.00 ± 0.81^{b}	1	Sb-PLBs	10	124.00 ± 0.81^{d}	Formation of PLBs at base of shoot
IAA (2 mgl^{-1})	100.00 ± 0.81^{c}	32.00 ± 0.81^{b}	1	-	-	-	Direct rooting
NAA (1.5mgl^{-1})	50.00 ± 0.81^{b}	48.75 ± 0.95^{ef}	2	Sb	2	60.00 ± 0.81^{b}	Plantlets with long, thick and healthy roots
NAA (2 mgl^{-1})	25.00 ± 0.81^{a}	50.00 ± 0.81^{f}	1	Sb	1	60.00 ± 0.81^{b}	-
BAP (1.5mgl^{-1})	-	-	-	-	-	-	-
BAP (2 mgl^{-1})	100.00 ± 0.81^{c}	42.00 ± 0.81^{c}	6	Sb	6	80.75 ± 0.95^{f}	-
KN (1.5mgl^{-1})	100.00 ± 0.81^{c}	20.00 ± 0.81^{a}	5	PLBs	25	45.75 ± 1.70^{a}	Rapid multiplication of PLBs and early plantlet formation
KN (2 mgl^{-1})	50.00 ± 0.81^{b}	22.00 ± 0.81^{a}	1	Sb	1	46.50 ± 3.10^{a}	-
IAA (1 mgl^{-1}) +KN (2mgl^{-1})	100.00 ± 0.00^{c}	46.00 ± 0.81^{d}	2	Sb	2	62.50 ± 0.81^{c}	Complete plantlet formation
IAA (2 mgl^{-1}) +KN (2mgl^{-1})	50.00 ± 0.81^{b}	47.00 ± 0.81^{de}	2	Sb	2	121.75 ± 0.95^{d}	Delayed plantlet formation
NAA (1 mgl^{-1}) +KN (2mgl^{-1})	100.00 ± 0.00^{c}	49.50 ± 1.29^{f}	1	Sb	4	65.00 ± 0.81^{e}	Additional shoot buds at the base of shoot

Entries in column nos. 2, 3 and 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. 1-13. *In vitro* leaf explant (entire) culture of *Rhynchostylis gigantea*: 1, Formation of PLBs at the base of shoot [M+IAA (1.5 mgl^{-1})]; 2, Direct rooting at the base of leaf [M+IAA (2 mgl^{-1})]; 3, Formation of multiple leaves [M+BAP (2mgl^{-1})]; 4-5, Multiplication of PLBs and early complete plantlet [M+KN (1.5 mgl^{-1})]; 6-10, Formation of complete plantlet [M+KN (2mgl^{-1}), M+IAA (1 mgl^{-1})+KN (2 mgl^{-1})], [M+IAA (1 mgl^{-1})+KN (2 mgl^{-1})], [M+ IAA (2 mgl^{-1})+KN (1 mgl^{-1})]; 11, Plantlets with long, thick and healthy roots and complete plantlet formation [M+NAA (1.5 mgl^{-1}), NAA(2 mgl^{-1})]; 12, Additional shoot buds at the base of shoot [M+NAA (1mgl^{-1}) +BAP (2mgl^{-1})]; 13, Seedlings transferred to plastic pots.

combinations and concentrations of PGRs (IAA, NAA, BAP, KN, IAA+KN, IAA+KN, NAA+BAP; Figs. 1-13). The leaf segments failed to respond in the basal medium; these turned brown and subsequently perished within 5 wks of inoculation. In the present study, initiation of morphogenetic response was restricted to the basal region of the leaf. The morphogenetic potential of leaf base has also been reported in several other species

such as *Arachnis labrosa* (Deb and Temjensangba, 2007) and *Vanda coerulea* (Vij and Aggarwal, 2003). Mathews and Rao (1985) considered the leaf base to be the decisive factor for culture initiation from foliar plants.

IAA when used at 1.5 mgl^{1} in the medium induced initiation of regeneration response in 50.00±0.81% segments in 30.00±0.81 days via shoot bud formation; 5 additional PLBs were activated at the base of shoot (Fig. 1) and plantlets were developed within 124.00±0.81 days. Interestingly, direct rooting was induced at the base of the whole leaf when IAA was used at higher concentration (2 mgl^{-1}) in the nutrient pool (Fig. 2). NAA when used at lower concentration (1.5mgl^{-1}), 50.00±0.81% segments responded in 48.75±0.95 days, 2 meristematic loci were activated and plantlets with bright green leaves and long, healthy and thick roots were obtained in 60.00±0.81 days (Fig. 10). NAA when used at higher concentration (2 mgl^{1}); the segments responded via shoot bud formation and complete plantlets were obtained in 60.00±0.81 days (Fig. 11). BAP at lower concentration (1.5 mgl^{-1}) proved inhibitory whereas its higher concentration (2 mgl^{1}) proved beneficial for inducing regeneration response, cent per cent segments responded via shoot bud formation; 6 meristematic loci were activated (Fig. 3) and plantlets were obtained in 80.75±0.95 days. KN at lower concentration (1.5 mgl^{-1}) proved beneficial as cent per cent explants responded within 20.00±0.81 days; nearly 5 meristematic loci were activated which developed into PLBs. These PLBs rapidly multiplied (Figs. 4-5) and a rich crop of nearly 25 plantlets was obtained (45.75±1.70 days); in this combination. KN at higher concentration (2 mgl^{-1}) when used in the nutrient pool, induced regeneration response via shoot bud formation within 22.00±0.81 days; shoot buds developed into complete plantlets in 46.50±3.10 days (Fig. 6).

When IAA (1 mgl^{-1}) was used with KN (2 mgl^{-1}), cent per cent explants responded in 46.00±0.81 days; 2 meristematic loci were activated (Fig. 7-8) and shoot buds formed, developed into complete plantlets in 62.50±0.81 days. When the concentration of this auxin was increased to 2 mgl^{-1} and that of KN was decreased to 1 mgl^{-1}, though the explants responded via shoot bud formation (Fig. 9) in 47.00±0.81 days, plantlet formation was delayed (121.75±0.95 days). NAA (1 mgl^{-1}) when used in combination with BAP (2 mgl^{-1}) in the medium; cent per cent explants responded via shoot bud formation and interestingly, 4-5 additional shoot buds were generated at the base of the shoot (Fig. 12) and complete plantlets were obtained in 65.00±0.81 days.

The role of growth hormones in stimulating meristematic activity and promoting proliferation in leaf explants is well documented in orchids (Arditti and Ernst, 1993; Deb and Sungkumlong, 2010; Deb and Temjensangba, 2007; Li and Xu, 2009; Temjensangba and Deb, 2005; Vij and Pathak, 1990; Yam and Weatherhead, 1991). Murashige (1974) opined that *in vitro* plant regeneration occurs frequently through adventitious shoot formation and rarely through somatic embryogenesis (Deb and Pongener, 2013). Presently, regeneration also occurred via PLBs formation, in accord with similar earlier observations in case of *Dendrobium* and *Epidendrum* (Churchill *et al*., 1970, 1971), where shoot regeneration from leaf explants has been reported to occur through the formation of PLBs.

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