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REGENERATION COMPETENCE OF *ARUNDINA GRAMINIFOLIA* (D. DON.) HOCHR. THROUGH STEM DISC CULTURE: A STUDY *IN VITRO*

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Abstract

Regeneration competence of stem discs of *Arundina graminifolia*, procured from *in vitro* grown cultures, was assessed on Murashige and Skoog (1962, MS) medium and its different combinations of growth regulators, auxin [IAA, IBA, NAA, 2,4-D (1-5 mgl^{-1}), NAA (1-2 mgl^{-1})]; cytokinin [KN (1-5 mgl^{-1})]; and gibberellin [GA_3(1mgl^{-1})]. In the basal medium, explants turned brown and perished within 50 days. IAA was effective when used at 2-4mgl^{-1}. At 2 mgl^{-1}, it invoked regeneration via Protocorm like bodies PLBs and at 3-4 mgl^{-1}, it favoured the development of rhizomatous bodies. KN effectively induced regeneration of PLBs in 37.5-66.5% explants when used at concentration ranging from 2-5 mgl^{-1}. KN and IAA, when used together, in the ratio of 1:1 (1mgl^{-1} each) acted synergistically to induce shoot bud differentiation in 50% explants. Plantlets were transferred to clay pots containing potting mixture (brick-pieces, pine bark, charcoal, moss) in ratio of 1:1:1:1. Nearly 60% plantlet survival was recorded.

Introduction

ARUNDINA GRAMINIFOLIA, an evergreen and ground growing species of bamboo orchids, is well known for its colourful and attractive flowers. It is widely distributed in East Himalayas and adjacent hills in tropical to sub tropical climates (upto an altitude of 1000m). In Darjeeling and Sikkim hills, it dwells in open grassy and /or gravely slopes. It is also distributed in the Nilgiris and Anamoli ranges in the peninsular India. The orchid blooms in summer and autumn, showing rather open clusters of open terminal flowers. The genus, considered to possess activities of detoxification, anti-arthritis and abirritation. *Arundina graminifolia* contains benzyldihydrophenanthrene, arundinaol, stilbenoid, arundian and phenanthrene (Liu *et al.*, 2004). *Arundina graminifolia* is an endangered orchid (Jain and Sastry 1980).

In the present study, an attempt has been made to assess the regeneration competence of *Arundina graminifolia* stem disc explants with a view to formulating an effective protocol for its mass propagation and hence conserving this endangered species.

Materials and Methods

Culture Media and Incubation Conditions

The regenerative competence of stem discs (3-5 mm) procured from 23 wk old *in vitro* raised seedlings were assessed on Murashige and Skoog (1962, MS) medium and its different combinations of growth regulators, auxin [IAA, IBA, NAA, 2,4-D (1-5 mgl^{-1}), NAA (1-2 mgl^{-1})]; cytokinin [KN (1-5 mgl^{-1})]; and gibberellin [GA_3, (1mgl^{-1})]. The cultures were maintained at 25 + 2°C temperature and exposed to 12 hr illumination of 3500 lux intensity. These were subcultured at regular intervals.

Acclimatization

Healthy plantlets with 2-3 well grown leaves and 1-2 roots were gradually hardended *in vitro*, by sequential elimination of growth additives, vitamins, sucrose and minor salts from the nutrient matrix at 15 days interval. The hardended seedlings were washed thoroughly with lukewarm water to remove agar and potted in clay pots, using charcoal, moss, brick-pieces and pine bark (1:1:1:1) as the potting mixture.

Statistical Analysis

One way analysis of variance was performed with respect to each response (averge ± standard error against each additive is mentioned in Table 1). 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 and Discussion

Presently, the stem explants (3-5mm) acquired

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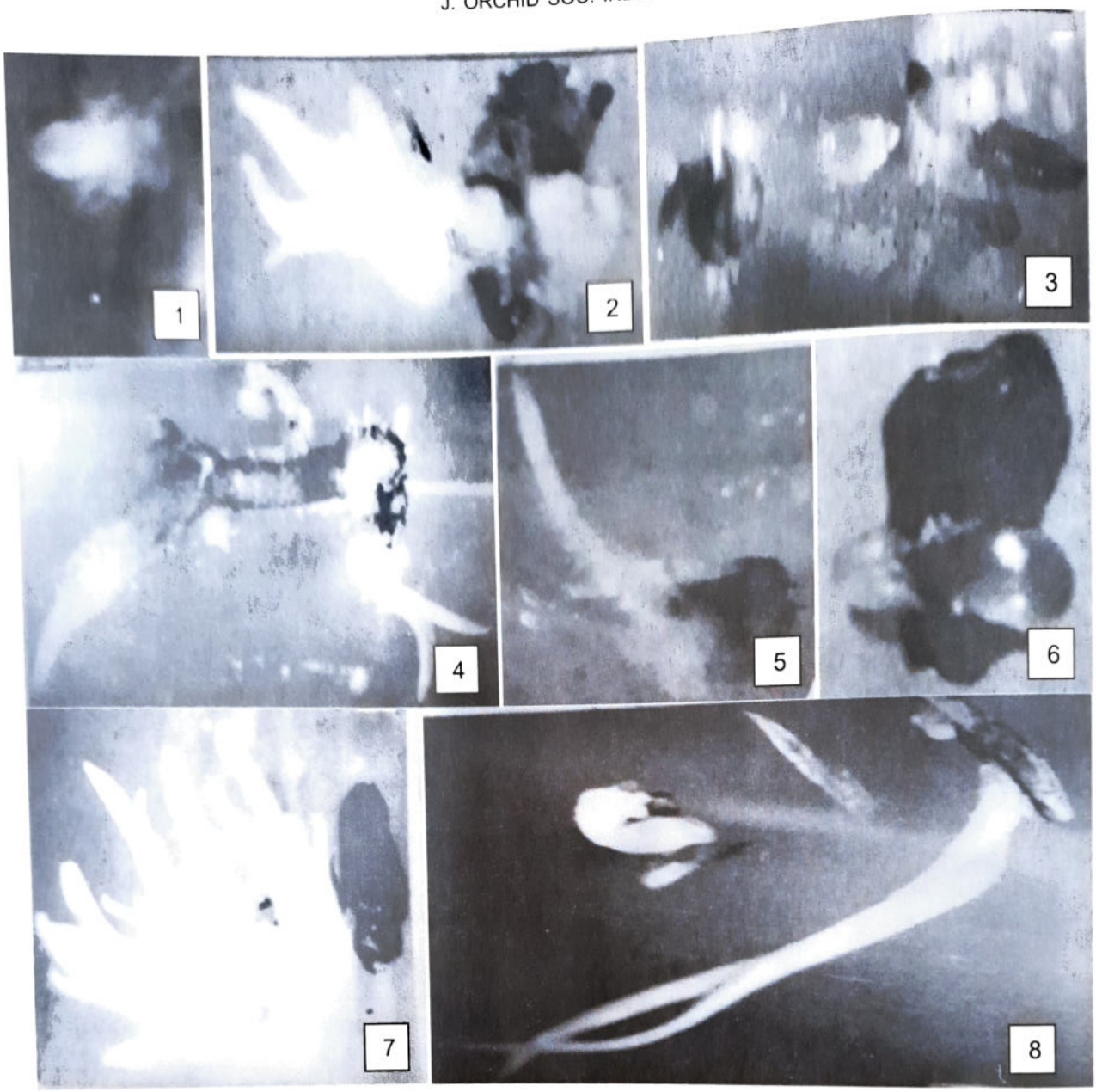

Figs. 1-8. Regeneration competence of stem discs of *Arundina graminifolia:* 1, Initiation of PLBs at the cut end [MS + IAA (2mgl^{-1})]; 2, PLBs differentiating leaves [MS + IAA (2mgl^{-1})];3 Root differentiation from rhizomatous bodies [MS + IAA(1 mgl^{-1})]; 4, Differentiation of leafy shoots and roots at the nodes of rhizomatous bodies [MS + IAA(3 mgl^{-1})]; 5, Root differentiation [MS + IBA (4 mgl^{-1})]; 6, Ring of PLBs formation at the cut end [MS + GA_3(1 mgl^{-1})]; 7, Enhanced PLB generation [MS + KN (5mgl^{-1})]; 8, Shoot bud differentiated into a leafy shoot[MS + IAA (1mgl^{-1}) + KN(1mgl^{-1})].

meristematic activity along their cut ends in certain selected nutritional combinations (Table 1; Figs. 1-8). Subsequent development of the meristematic loci into protocorm like bodies (PLBs)/shoot buds/rhizomatous bodies, however, varied with the nature of hormonal treatments. Nodal discs have been effectively utilized for regeneration purpose in some orchid species including *Arundina* (Mitra, 1971); *Cymbidium pendulum* (Vij *et al.*, 1994); *Dendrobium* (Kim *et al.*, 1970; Mosich *et al.*, 1974); *Esmeralda clarkei* (Paudel and Pant, 2012); *Habenaria bractescens* (Medina *et al.*, 2009); *Phalaenopsis* (Sagawa, 1961; Scully, 1965; 1966; Wang, 1989); *Vanda* Miss Joaquim (Sagawa and Sehgal, 1967); and *Vanilla* (Duan and Hong, 1989; Phillip and Nainar, 1986). The stem discs (explant) failed to proliferate in basal medium unless supplemented with suitable quality and quantity of growth hormones. Similar obseravations were made earlier in *Dendrobium* species (Yasugi and Shinto, 1994). In the present study, IAA was effective when used at 2-4mgl^{-1}. At 2 mgl^{-1}, it invoked PLB generation in 25.00 ± 0.00% explants and the PLBs differentiated into plantlets in 78 days old cultures. At higher concentration (3-4 mgl^{-1}), it favoured the development

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Table 1. Regeneration potential of *in vitro* sourced *Arundina graminifolia* stem disc explants on Murashige and Skoog (1962, MS) medium.

Additives	Regeneration response (%)	Time taken in days for the development of						Remarks
		Callus	Rhizomatous growth	Shoot bud	PLBs	Root	Plantlet	
-	-	-	-	-	-	-	-	
IAA_1	-	-	-	-	-	-	-	
IAA_2	25.00 ± 0.00^{b}	-	-	-	28.00 ± 0.82^{b}	63.50 ± 0.58^{a}	77.75 ± 0.50^{c}	
IAA_3	37.50 ± 0.00^{c}	-	22.00 ± 0.82^{b}	-	-	-	-	Leaf/root formation eluded
IAA_4	37.50 ± 0.00^{c}	-	20.50 ± 0.58^{a}	-	-	35.75 ± 0.96^{b}	-	Leafy shoot eluded
IAA_5	-	-	-	-	-	-	-	
IBA_1	-	-	-	-	-	-	-	
IBA_2	25.00 ± 0.00^{b}	-	27.75 ± 0.50^{c}	-	-	49.50 ± 1.29^{d}	-	Leafy shoot eluded
IBA_3	37.50 ± 0.00^{c}	-	28.00 ± 0.00^{c}	-	-	55.75 ± 0.96^{e}	78.25 ± 1.70^{c}	
IBA_4	12.50 ± 0.00^{a}	-	-	-	-	35.00 ± 0.82^{b}	-	Leafy shoot eluded
IBA_5	-	-	-	-	-	-	-	
GA_3	50.00 ± 0.00^{d}	-	-	-	20.75 ± 0.50^{a}	64.50 ± 0.58^{f}	84.00 ± 0.82^{d}	Ring of PLBs
$2,4\text{-}D_1$	-	-	-	-	-	-	-	
$2,4\text{-}D_2$	-	-	-	-	-	-	-	
$2,4\text{-}D_3$	25.00 ± 0.00^{b}	-	-	-	28.25 ± 0.96^{b}	64.25 ± 0.96^{f}	78.00 ± 0.82^{c}	
$2,4\text{-}D_4$	-	-		-	-	-	-	
$2,4\text{-}D_5$	-	-	-	-	-	-	-	
NAA_1	-	-	-	-	-	-	-	
NAA_2	12.50 ± 0.00^{a}	27.50 ± 0.58^{a}	-	-	-	-	-	Organogenesis eluded
NAA_3	50.00 ± 0.00^{d}	-	20.25 ± 0.96^{a}	-	-	-	-	
NAA_4	50.00 ± 0.00^{d}	-	21.00 ± 0.00^{a}	-	-	-	-	Leaf/root formation eluded
NAA_5	37.50 ± 0.00^{c}	-	28.00 ± 1.63^{c}	-	-	-	-	
KN_1	-	-	-	-	-	-	-	
KN_2	56.50 ± 0.00^{e}	-	-	-	20.50 ± 0.58^{a}	78.25 ± 1.70^{g}	92.00 ± 0.82^{e}	
KN_3	37.50 ± 0.00^{c}	-	-	-	20.50 ± 0.58^{a}	25.75 ± 0.96^{a}	76.00 ± 0.82^{c}	
KN_4	50.00 ± 0.00^{d}	-	-	-	27.75 ± 0.50^{b}	64.00 ± 0.00^{f}	78.00 ± 1.41^{c}	
KN_5	50.00 ± 0.00^{d}	-	-	-	21.00 ± 0.00^{a}	64.00 ± 0.82^{f}	77.00 ± 0.82^{c}	PLB generation enhanced

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