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REGENERATION COMPETENCE OF *AERIDES MULTIFLORA* ROOT SEGMENTS: A STUDY *IN VITRO*

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Abstract

The paper reports the possibility of using root segments for micropropagating *Aerides multiflora*. Mitra *et al.* (1976) medium supplemented with a combination of BA (2 mgl^{-1}) and NAA (0.5 mgl^{-1}) favoured a callus and/or direct PLB generation in nearly 50% explants within 6 wks. The neoformations organogenated subsequently. Nearly 19 plantlets each with 3-4 leaves and 1-2 roots were produced within 30 wks from each responding explant. The plantlets were subjected to a two step (*in vitro* and *ex vitro*) hardening procedure and established with 80% survival frequency.

Introduction

AERIDES MULTIFLORA Roxb., a species of foxtail orchids with long-lasting, fragrant, and pinkish-white flowers in lateral racemes (Fig. 1a), has progenated a variety of inter-generic hybrids (Bose *et al.*, 1999). Its natural populations are on decline under the duress of unregulated habitat destruction and commercial collection pressures. An appropriate propagation system needs to be developed for the species so as to ensure its sustainable development. The application of shoot-tip culture technique has added new dimensions to orchid propagation *in vitro*. The utility of this technique is, however limited in monopodial taxa like *A. multiflora*, since it requires the excision of the only growing point. Hence, the use of root segments whose excision is not detrimental to the survival of mother plant has often been stressed (Arditti and Ernst, 1984; Vij *et al.*, 1987; Vij and Pathak, 2006). We have assessed the regenerative competence *in vitro* of root explants in *A. multiflora*. Some of the salient features of the study are reported in this paper.

Material and Methods

Roots (0.5-0.7 cm long) from 30 wks old *in vitro* cultures were segmented into apical and basal halves and cultured on M (Mitra *et al.*, 1976) medium and its combinations with PGRs [6-benzyl amino purine (BAP), 1-Napthalene acetic acid (NAA) see Table 1]. The cultures were incubated in the ambience of 25±2°C and a 12 hrs photoperiod of 3,500 Lux light intensity. These were subcultured regularly at 4-6 wks intervals depending upon the growth of the cultures. Results were analyzed using Completely Random Design (CRD) by subjecting to One Way Analysis of Variance (ANOVA)

Table 1. Regeneration response *in vitro* of root segments of *Aerides multiflora* on M medium*

Additives (mgl^{-1})	Regeneration Response (%)	Number of Meristematic Loci evoked	Time Taken in wks for Initiation of Response	Regeneration Pathway	Number of Plantlets obtained after 30 wks
—	00.0 ^{a}	—	—	—	—
$BA_{0.5}+NAA_{0.5}$	00.0 ^{a}	—	—	—	—
$BA_{0.5}+NAA_{1}$	00.0 ^{a}	—	—	—	—
$BA_{0.5}+NAA_{2}$	00.0 ^{a}	—	—	—	—
$BA_{1}+NAA_{0.5}$	15.0 ^{b}	1.0 ^{a}	8.2 ^{c}	Callus-PL	14.8 ^{a}
$BA_{1}+NAA_{1}$	00.0 ^{a}	—	—	—	—
$BA_{1}+NAA_{2}$	00.0 ^{a}	—	—	—	—
$BA_{2}+NAA_{0.5}$	50.0 ^{c}	1.8 ^{b}	6.0 ^{a}	Callus/PLB-PL	19.2 ^{a}
$BA_{2}+NAA_{1}$	45.0 ^{c}	1.6 ^{ab}	6.3 ^{b}	Callus-PL	18.2 ^{a}
$BA_{2}+NAA_{2}$	00.0 ^{a}	—	—	—	—

*Values in a column with same superscripts are not significantly different at $p \leq 0.05$

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Fig. 1 a-g. Micropropagation of *Aerides multiflora* Roxb. using root segments: a, An Inflorescence; b-d, Pale yellow, nodular, and highly proliferative callus along the cut ends and further differentiation on M+BA (1 mgl^{-1}) + NAA (0.5 mgl^{-1}); e-f, Different stages of PLB mediated morphogenetic development on M+BA (2 mgl^{-1}) +NAA (0.5 mgl^{-1}); g, Healthy plantlet growing on AC supplemented medium.

to detect the significant differences ($p \leq 0.05$) among the treatments.

Results

The explants failed to respond in the basal medium unless subjected to a combined treatment with BA and NAA in the nutrient mix. The treatment favoured cell proliferations along the cut-ends. A combination containing BA (1 mgl^{-1}) and NAA (0.5 mgl^{-1}) favoured cell proliferations along the cut ends in 15% explants within 8.2 wks. The callus was pale yellow in colour, granular, and highly proliferative (Fig. 1b, c). It differentiated several growing points, each of which differentiated, leaf (Fig. 1d) and root primordia. On an average, 14.8 plantlets could be obtained within 30 wks. Increased doses of NAA (1-2 mgl^{-1}) in the above combination proved inhibitory to regeneration.

When BA was used at 2 mgl^{-1} and NAA at 0.5 mgl^{-1} in the medium, nearly 50% explants callused and/or generated PLBs (Fig. 1e) within 6 wks prior to entering organogenetic phase. More than 19 plantlets each with 3-4 leaves and 1-2 roots (Fig. 1f) were produced within 30 wks from each of the responding explant. Increased level of NAA (1 mgl^{-1}) in the above combination suppressed PLB phase of development; the explant, instead followed a callus mediated pathway of regeneration. Further increase in the level of auxin proved detrimental; the explants invariably failed to respond.

The plantlets were grown on AC supplemented medium and those with 4-5 leaves and a number of roots (Fig. 1g) were subjected to a two-step hardening procedure by subjecting them to *in vitro* and *ex vitro* hardening. These survived with 80% frequency.

Discussion

The utility of orchid roots for regeneration purposes was first suggested by Beechey (1970), and their reported ability to spontaneously develop callus/shoot primordia in some naturally growing orchids hinting at their inherent regeneration potential (Champagnat, 1971; Churchill *et al.*, 1972), further supported such a suggestion. Root explants have been successfully used to micropropagate a number of orchid species (Champagnat, 1971; Chen and Chang, 2000; Park *et al.*, 2001; Philip and Nainar, 1986; Sanchez, 1988; Tanaka *et al.*, 1976; Vaz *et al.*, 1998; Vij, 1993; Vij and Pathak, 2006; Vij *et al.*, 1987, 2004; Wu *et al.*, 2004; Yam and Weatherhead, 1991) suggesting thereby that regeneration from root explants can be triggered *in vitro*. According to Vij (1993), the roots due to their easy availability and amenability, can be used as an effective alternative to meristem culture and their regenerative potential is markedly influenced by their genotype, physiological status, and the chemical stimulus *in vitro*.

In the present studies, regeneration competence of excised juvenile roots (0.5-0.7 cm long) with poorly developed root caps was positively tested. The explants selectively proliferated depending upon the medium formulation. Vij (1993) reported that the regeneration is mostly confined to the sub apical portions of the root explants, thereby substantiating the role of root cap in root proliferation. In this connection, it may not be out of place to mention that the earlier contention of Peterson (1975) that root meristem consists of highly determinate cells which have limited capacity for bud formation has failed to find favour with the subsequent investigators who have demonstrated the initiation of regeneration in the root apices of a number of orchid species (Champagnat, 1971; Philip and Nainar, 1986; Sanchez, 1988; Tanaka *et al.*, 1976; Vaz *et al.*, 1998).

A treatment with a combination of cytokinin (BA) and auxin (NAA) proved useful; the PLBs followed a callus mediated multiplication in accord with similar reports on *Oncidium* (Chen and Chang, 2000; Wu *et al.*, 2004). According to Sanchez (1988), the morphogenetic response of *Cyrtopodium* root explants is significantly effected by the level of NAA and BA in the nutrient pool; lower doses stimulates PLB development whereas increased level of auxin favoured a callus mediated fasciated root development. Similar synergistic effects of auxins and cytokinins have been documented by Vij (1993) in a number of orchid taxa. In *Doritaenopsis*, root tips responded favourably to a sucrose less medium containing TDZ (0.5 mgl^{-1}) and CW (20%) to produce PLBs; substitution of CW with sucrose proved detrimental as the explants became necrotic (Park *et al.*, 2001). The inhibitory effects of BA on the longitudinal growth of explants have also been reported (Colli and Kerbauy, 1993; Kerbauy, 1991).

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