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High frequency of protocorm like bodies (PLBs) induction and plant regeneration from protocorm and leaf sections of *Aerides crispum*

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Abstract

An efficient and reproducible method for the large-scale propagation of *Aerides crispum* L. using protocorm and leaf sections has been developed. Protocorm and leaf sections were cultured on Murashige and Skoog (MS) medium supplemented with cytokinins [N^6-benzyl adenine (BA), thidiazuron (TDZ), and kinetin (KN), 0.5, 1.0, 2.0 and 5.0 μM], auxins [α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), 0.5, 1.0, 2.0 and 5.0 μM] and coconut liquid endosperm (CW: 5, 10 and 15%). The explants developed protocorm like bodies (PLBs) within 5–8 weeks on the growth medium. BA supplemented medium was found best for the induction of PLBs and an optimum of 49.1 and 22.0 PLBs developed from protocorm and leaf sections on medium supplemented with 1 and 2 μM BA, respectively. Upon subculture on basal MS medium, the PLBs differentiated plantlets within 6–8 weeks. The resulting plantlets were successfully transferred to potting mixture and 85% of plantlets survived after green house transplantation. This simple protocol will be useful for large-scale propagation of *A. crispum* L.

Keywords: *Aerides crispum*; Miropropagation; Orchid; Protocorm like bodies (PLBs)

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1. Introduction

Aerides is a genus of strong-growing monopodial epiphytic orchids with some 20 species that are native to south and south-east Asia (Teob, 1989). They are popularly included in ''foxtail orchids'' along with *Rhyncostylis*. These orchids are popular for their freely produced pendent spikes of closely set, fragrant blooms. A few of the species are especially fascinating for their complex lip, which can obscure the column. *Aerides* is beginning to be used more and more in hybridizing with related species and hybrids, especially vandas and ascocendas.

Tissue culture has played an important role as a means of orchid propagation for many years and in vitro methods have been developed for large number of species using shoot tips, axillary buds, leaves, roots and inflorescences (Arditti and Ernst, 1993). *Aerides* is a monopodial orchid, which is difficult to propagate vegetatively. The characteristics of seedlings are not uniform, and propagation through tissue culture has been desired. The aim of our research was to develop an appropriate in vitro method for propagation of *Aerides crispum*, which has considerable economic potential. We report in this paper efficient in vitro methodology for mass propagation of *A. crispum* by using protocorm and leaf sections.

2. Materials and methods

2.1. Explant source

The 4 weeks old green protocorms like bodies (PLBs)/protocorms and young leaves from in vitro grown 4 weeks old plantlets were taken as explants. The protocorms were segmented into two halves and each half was considered as an explant; the leaves were cut into 3–5 mm sections and were cultured on the medium.

2.2. Culture medium and culture conditions

Murashige and Skoog (MS) (1962) basal medium and MS medium supplemented with growth regulators like indole-3-acetic acid (IAA), α-naphthaleneacectic acid (NAA), N^6-benzyladenine (BA), kinetin (KN), thidiazuron (TDZ) (0.5, 1.0, 2.0 and 5.0 μM) were added to the medium singly and in several combinations. Coconut liquid endosperm (CW: 5, 10 and 15%, v/v) was also tested with basal medium. Sucrose (2%, w/v) was the carbon source, pH was adjusted to 5.6 and 1% agar (Hi-media, Mumbai, India) was used as solidifier.

All cultures were maintained at 25 ± 2 °C under 16 h photoperiod of 40 μmol m^{-2} s^{-1} and 60% relative humidity. The cultures maintained by sub culturing at an interval every 4 weeks.

2.3. Experimental design and data analysis

Experiments were performed in a randomized design and repeated twice. Each treatment had 12 explants/replicates. Morphogenic response (PLB formation) from