IN VITRO MULTIPLICATION AND ECOREHABILITATION OF RARE ORCHID AERIDES CRISPUM

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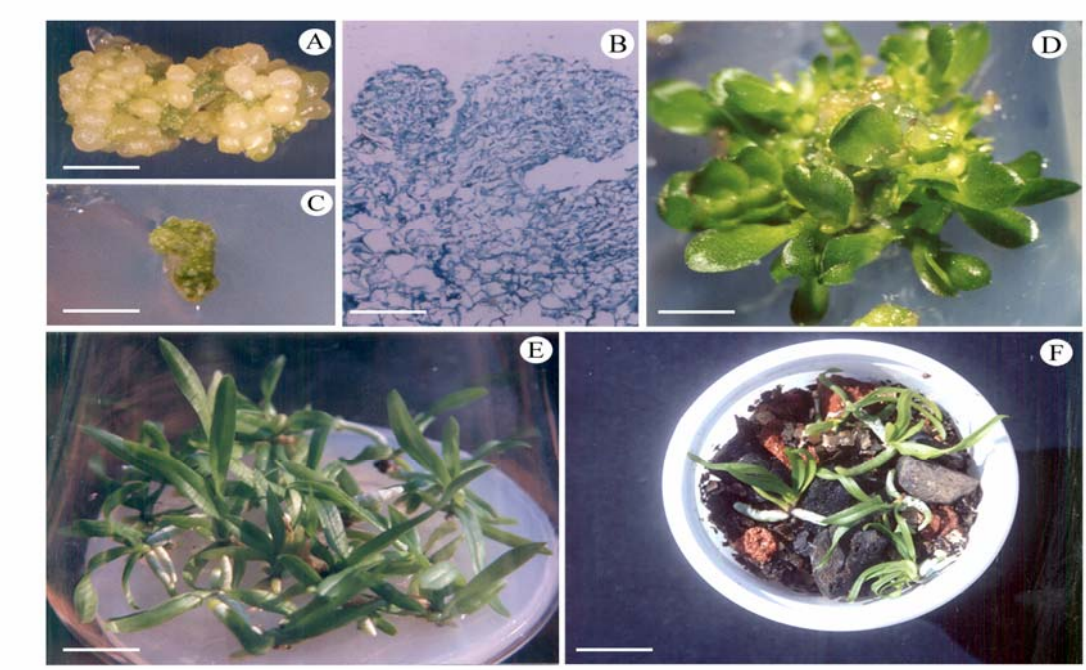
Summary
In vitro methodology has been developed for propagation of *Aerides crispum* L. using protocorm and leaf sections on MS medium supplemented with cytokinins, auxins and coconut liquid endosperm. The explants developed protocorm like bodies (PLBs) within 5-8 weeks on growth medium and upon sub culturing PLBs differentiated into plantlets. In vitro raised plants were reintroduced into alien forest habitats.

Materials & Methods, Results
Aerides crispum is one of the most important orchids, valued for its beautiful inflorescence/flowers. This species is endemic to South India and its natural populations are dwindling due to over exploitation (Rao, 1998). Multiplication of this species in nature is through seeds and only 0.3% of seeds germinated in the presence of suitable mycorhiza. Since vegetative propagation methods are not available, development of in vitro methods are essential for conservation and commercialization of this species. In the present paper, we describe rapid multiplication of *Aerides crispum* through protocorm and leaf section culture and successful establishment of clonal plants into natural habitats.
The four weeks old green protocorm like bodies (PLBs)/protocorms and young leaves from in vitro grown four week old plantlets were cultured on Murashige and Skoog (MS, 1962) medium supplemented with growth regulators like indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA), N^6-bezyladenine (BA), kinetin (KN), thidiazuraon (TDZ) (0.5, 1.0, 2.0, 5.0 μM) were added to the medium singly and in several combinations. Coconut liquid endosperm (CW, 5, 10, 15%, v/v) was also tested with basal medium. Sucrose (2% w/v) was carbon source, pH was adjusted to 5.6 and 1% agar was added as solidifier. All cultures were maintained at 25 ± 2 ^{o}C under 16 h photoperiod of 40 μmol m^{-2} s^{-1} and 60% relative humidity.
On the medium supplemented with growth regulators both protocorm and leaf sections swell in size within three weeks and developed PLBs in another two weeks. PLBs were differentiated on the explants by the end of eight weeks. Of the three cytokinins tested, BA was more effective in inducing PLBs from both protocorm and leaf sections (Fig. 1A and 1C). All the protocorm sections responded and differentiated an optimum 49.1 PLBs per explant on medium supplemented with 1.0 μM BA. Similarly, all leaf sections showed response and differentiated an optimum of 22.0 PLBs per explant on medium supplemented with 2.0 μM BA. Similarly, medium supplemented with TDZ, KN, NAA, IAA and combination of BA + NAA and TDZ + NAA have induced PLB regeneration from prtocorm sections but frequency of PLB regenerations was less compared to medium supplemented with BA. Direct PLB regeneration was observed from both protocorm and lead sections without mediation of callus. Histological observations revealed that the PLBs have originated from the subepidermal layer of the protocorm sections (Fig. 1B). The PLBs, which were regenerated on different media, were excised and transferred to MS basal medium upon which they developed shoots and roots in 6-8 weeks (Fig. 1D and 1E). In 10-12 weeks the plantlets were

fully differentiated and were ready to transplantation. The plantlets were transferred to potting medium (Fig. 1F) and reared in controlled environment in growth chamber. After two weeks pots containing plantlets were shifted to green house. Well acclimatized plants were reintroduced into alien forest habitats.

Key words: *Aerides crispum*, micropropagation, rare orchid, ex situ conservation, protocorm like bodies.

Fig. 1.



Legends to Figure: 1A. Developing PLBs from protocorm section on MS medium with 1.0 µM BA. 1B. Section through explant showing direct development of PLBs. 1C. PLBs developed from leaf explant on MS medium with 2.0 µM BA. 1D. Developing shoots from PLBs on MS basal medium. 1E. Plantlets developed on MS basal medium. 1F. Transplanted plantlets.

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