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An *in vitro* study on micropropagation of *Cymbidium* orchids

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Micropropagation of plants through tissue culture has become a significant and informative technique to reproduce and make the availability of crops, orchids and ornamental plants that are otherwise difficult to propagate traditionally by seed and /or vegetative means. Matured and specified explants were manipulated to give rise the multiple numbers of the original or parental plant under specialized physicochemical or environmental conditions and allow them to regulate the growth of the explants. The applications of tissue culture has the few limitations over the propagation techniques such as cost of production, choice of crops restricted to species with acceptable micropropagation and reproducibility protocols. *In vitro* micropropagation technique has many unique advantages than the conventional propagation methods such as rapid multiplication of valuable genotypes, expedition's release of improved varieties, fast production, germplasm conservation and facilitating their easy international exchange. *In vitro* techniques are persuasive tools for plant breeders in all the fields of promoting the performance of agriculture, horticulture and floriculture plant yield. Attention to the tissue culture micropropagation of *Cymbidium* orchid has evolved due to its importance throughout the world. The plants produced through this technology provide much export potential as they are shipped internationally with limited quarantine restrictions and it has the prospective for developing new cultivars of *Cymbidium* species (Hyndman, 1987).

Cymbidium is the first orchid propagated through tissue culture using shoot tips; root etc., as an explant. The shoot tip of cymbidium developed green protocorm-like bodies (PLB) which subsequently produced new PLB or plantlets. In *cymbidium*, sections 6 to 8 mm away from the apex also develop the same outgrowth. It was found that the

peripheral area of a protocorm proliferates actively through morphogenesis. This growth occurs only on the epidermal region. But the central parenchyma core remains entirely inert.

Cymbidium orchid plants were obtained from local dealers of commercial orchid supplier by ensuring that they were obviously healthy and signs free from any stress or surface blemishes. The physical condition selection was further carried out by growing the plants under environmentally controlled fresh water at 28 ± 2°C with 12-h period. For surface sterilization, plants were first scrubbed gently under running tap water for 0.5 h to remove coating layer of microorganisms ubiquitously found on them. Upper portions of the shoot twigs containing apical meristem through fourth axillary bud were isolated and submerged for 20 min in 30% commercial bleach (Axion) with 1-2 drops of Tween 20 and continuous stirring. Stirring dispersed the air bubbles adhering to explants and facilitated even distribution of the disinfectant. After discarding bleach, the explants were washed 3 times with sterile water. Apical meristems, first, second and third-fourth axillary buds from shoot tips were excised and cultured on to shoot regeneration media in Magenta GA7 vessels for 4 weeks. Explants were then cultured to subculture medium for 4 weeks. The number of explants producing shoots and the number of shoots per explant were scored after 8 weeks of culture. Well developed shoots were isolated and rooted in rooting medium. Rooted plantlets were transferred to fine sand with 4 cm spacing at 28 ± 2°C water (pH 6.0) temperature with 12 h period for 3 months.

The shoot regeneration media consisted of MS mineral salts and vitamins (Murashige and Skoog, 1962), 3% sucrose, 0.7% agar (Sigma agar type A), 0.1-0.3 mg dm-3 6- benzylaminopurine (BAP), 0.1 mg dm-3 α-naphthalene acetic acid (NAA) or 0.05-0.15 mg dm-3 thidiazuron (TDZ) and 0.1 mg dm-3 NAA. Subculture and rooting medium consisted of half-strength MS medium, 3% sucrose and 0.7% agar. The pH of the medium was adjusted to 5.6 with 1N NaOH or 1N HCl before autoclaving at 1.4 kg/cm2 and 121°C for 20 min.

Propagation of orchids is a complex process, which involves the environmental (structural & functional) and physiological changes, and may get influenced by the internal and external signaling factors. Role of cytokinins play a major role in interaction between the plant growth substances and also involves in inhibition of the plant growth substances. The cytokines, like benzyl adenine, kinetin, thidiazuron, and zeatin; adenine sulphate; auxins like indole acetic acid and naphthalene acetic acid (NAA) are involved in the process. (Ramanayake et al., 2001; Lin et al., 2003; Taylor et al., 2005; Saritha and Naidu 2007). Culture conditions like light, temperature and moisture content are important in the propagation of *Cymbidium* orchid (Dickens and Van Staden, 1988). The addition of some antibiotic mixtures like cefotaxim, tetracycline, rifampicin, streptomycin, and ampicillin to the culture medium allowed the recovery of a high percentage of contaminated explants.

Conversely, the investigation and successful research in the field of *in vitro* micropropagation of different types of orchids are limited in production. Commercial varieties of *Vandaceoys* orchids, some *Phaleanopsis*, and some of the *Dendrobium* orchids can be grown quite easily in wooden slatted baskets, without any compost and other growing factors, but for daily watering. Some other orchids like *Cymbidium* and *Cattleya* varieties do well in a mix of bark chippings, mixed with little perlite, and charcoal to keep it sweet. In vitro propagation techniques has been tried in orchids like *Cymbidium* (Wang, 1990), *Dendrobium* (Goh, 1992; Wang et al., 1993; Rajkumar et al., 2002), *Eulophia* (Sharma and Vij, 1986), *Doriella* (Duang and Yazawa, 1994), *Phalaenopsis* (Duang and Yazawa, 1995), Spiranthes, Vanda and Zeuxine (Vij et al., 2000). Yu and Goh (2000) have shown that genes involved in transcriptional regulation, cell division, respiration are differentially expressed during flowering in orchids.

This study in cymbidium orchid also revealed a significant influence of medium components and less concentration of sugar on *in vitro* micropropagation. The figures show the significant growth of cymbidium orchid against low concentration of sucrose supplemented with the Murashige and Skoog (1962) medium. Further studies on other media components and culture conditions are in progress.

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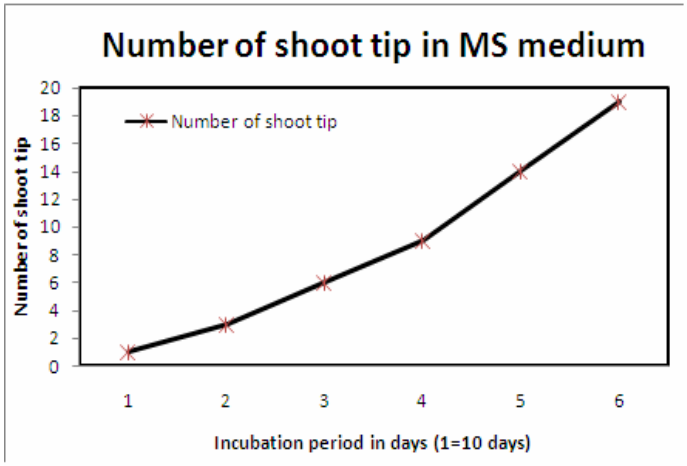

Fig.1: Number of shoot tips in MS Medium supplemented with low concentration of sucrose

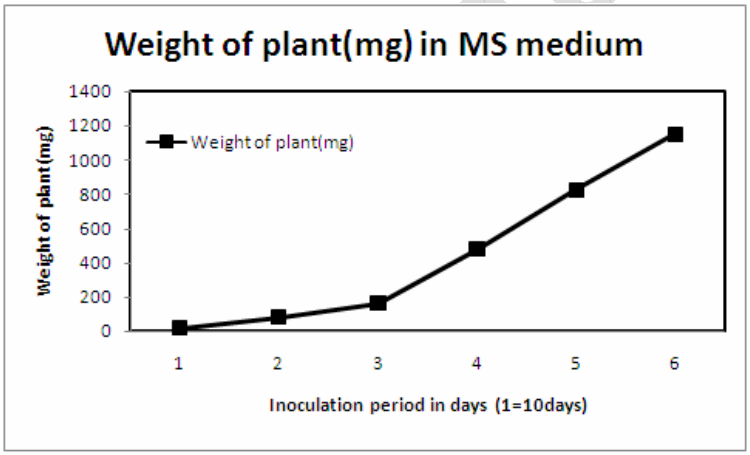

Fig.2: Weight of the plant in MS Medium supplemented with low concentration of sucrose

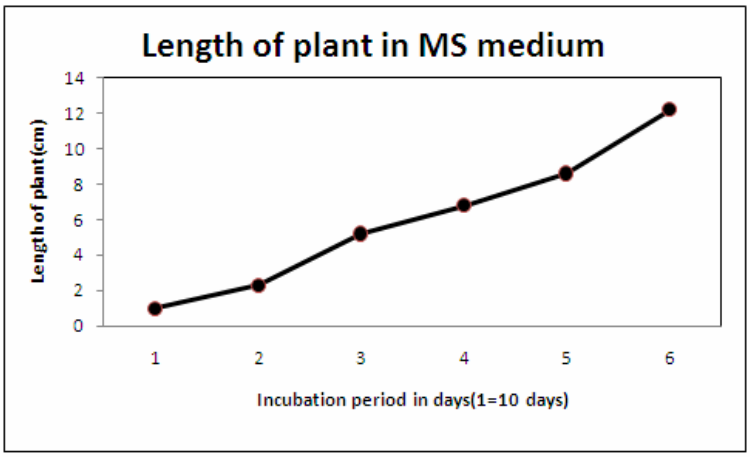

Fig.3: Length of the plant in MS Medium supplemented with low concentration of sucrose

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