

## EFFECTS OF 6- BEZYLAMINOPURINE ON MULTIPLICATION OF PROTOCORM LIKE BODIES (PLBs) OF FOUR *CYMBIDIUM* HYBRIDS CULTURED *IN VITRO*

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### Abstract

The protocorm like bodies (PLBs) of four *Cymbidium* cultivars *i.e.* Levis Duke 'Bella Vista', Vivacious 'Super White', Pine Clash 'Moon Venus' and Margaret Thatcher 'Perfection' were cultured on MS media invariably supplemented with 0.1 mg l<sup>-1</sup> NAA (1-Napthalene acetic acid) and various concentrations of BAP (6 – benzylaminopurine) in order to optimize the BAP requirement for the proliferation of PLBs. The PLB number, PLB diameter and fresh and dry weight of PLBs were influenced by genotypes and concentration of BAP. The BAP concentration 2.0 mg l<sup>-1</sup> for Levis Duke 'Bella Vista', 1.5 mg l<sup>-1</sup> for Margaret Thatcher 'Perfection' and 0.5 mg l<sup>-1</sup> to 1.0 mg l<sup>-1</sup> for Pine Clash 'Moon Venus' and Vivacious 'Super White' were found optimum for proliferation of PLBs. In all the cultivars, the BAP concentration beyond optimal level inhibited the proliferation of PLBs.

### Introduction

THE GENUS *Cymbidium* (Orchidaceae) comprises 44 species distributed from North Western India to China, Japan, South through Malaya Archipelago to North and East Australia (Du Puy and Cribb, 1988). *Cymbidiums* have been known for over hundred years, till now it remains one of the most important orchid in present day commerce. These are primarily grown for long, attractive, delicately coloured, long lasting waxy flowers used for indoor decorations and for corsages. Nowadays, they are also gaining popularity as decorative pot plants with smaller but equally high quality flowers. In Northeastern Himalayas, areas lying between 1500 – 2000 meters from the sea level are ideal for *Cymbidium* cultivation (Pradhan *et al.*, 1995). Apart from availability of new cultivars for cultivation, scarcity of quality planting material and its cost are major bottlenecks in expanding *Cymbidium* cultivation in the country. *Cymbidium* were the first horticultural plant species successfully mass propagated using shoot tip culture (Morel, 1960). Since Morel's work in 1960, shoot tip remained most preferred explants (Steward and Mapes, 1971; Wilfret, 1996; Wimber, 1963) to increase number of plantlets per explants various technique like protocorm segment culture (Fuji *et al.*, 1999) thin section culture (Nayak *et al.*, 2002) and callus culture (Le Van *et al.*, 2004) have been proposed. However, most of orchids of commercial importance have been propagated using tissue culture through the formation of protocorm like bodies (PLBs) except for some recalcitrant species such as *Paphiopedilum* (Tokuhara and Mii, 2001). The nutritional requirements for optimum growth of plant tissues *in vitro* vary with the species (Morel, 1952). The modern hybrids are very

complex and involve several species in their ancestry and requirements for plant growth regulators for PLB multiplication may likely vary. Hence, the present study was undertaken to optimize the BAP requirements for multiplication PLBs of selected cultivars of *cymbidiums*.

### Material and Methods

#### Plant Material

The protocorm like bodies (PLBs) of four *Cymbidium* cultivars *i.e.* Levis Duke Bella Vista, Vivacious Super White, Pine Clash 'Moon Venus', and Margaret Thatcher 'Perfection' were derived from lateral shoot culture. The PLBs were sub cultured on MS media (Murashige and Skoog, 1962) at 40 days interval and thus PLBs were multiplied and equalized physiologically and morphologically. The uniform PLBs were taken for study.

#### Media and Culture Conditions

MS medium supplemented with 20 g l<sup>-1</sup> sucrose and 8 g l<sup>-1</sup> agar was used as basal medium. The pH of the medium was adjusted to 5.6 with 0.1 N KOH or HCl before adding agar. The medium was autoclaved for 15 mins at 121°C at 1.06 kg cm<sup>-2</sup>. The 10 PLBs were cultured aseptically in each jam bottle (400 g) containing 50 ml culture media. The cultures incubated at 25 ± 2°C under 16 hr photoperiod of 3500 lux light intensity (fluorescent tubes 40 W, Phillips India Ltd, Mumbai) for 40 days. To examine the effects of BAP explants were cultured on MS medium supplemented with 0, 0.5, 1.0, 1.5, 2.0 mg l<sup>-1</sup> BAP.

#### Statistical Analysis

The experiment was laid down in complete randomized



block design and each treatment was replicated thrice. Each treatment consisted of 5 bottles and in each bottle, 10 explants were cultured. For PLB multiplication 5 numbers of PLBs were taken out from each replicate and studied for number of PLBs/explant, PLB diameter (mm), fresh weight of PLBs/explants and dry weight/ g fresh weight was recorded. The experiment was repeated twice and the data was pooled and analyzed statistically.

## Results

### PLB Number

The number of PLBs were found to influence by cultivar and treatment (Fig.1). The maximum number of PLBs were produced in Margaret Thatcher 'Perfection' followed by Vivacious 'Super White'. However, no significant variation with PLB number was noticed in Levis Duke Bella Vista and Pine Clash 'Moon Venus'. As the concentration of BAP was increased, a significant increase in PLB number was observed in all the cultivars. However, the optimal requirement varied with the cultivar. Increasing concentration beyond optimal level decreased the number of PLBs per explant. Cultivar Margaret Thatcher 'Perfection' recorded maximum number of PLBs on the medium supplemented with 1.5 mg l<sup>-1</sup> BAP whereas no significant difference was observed in Vivacious 'Super White' when the BAP concentration was increased from 0.5 mg l<sup>-1</sup> to 1.5 mg l<sup>-1</sup>. Levis Duke 'Bella Vista' and Pine Clash 'Moon Venus' recorded maximum number of PLBs on medium supplemented with 2.0 mg l<sup>-1</sup> and 1.5 mg l<sup>-1</sup> BAP respectively.

### PLB Diameter

The PLB diameter was also influenced by cultivar and BAP concentration (Fig. 2). The largest diameter was observed in PLBs of Pine Clash 'Moon Venus' Margaret Thatcher 'Perfection' followed by whereas the smallest diameter was found PLBs of Levis Duke 'Bella Vista'. The PLBs were largest in control and was reduced when the growth regulator was applied. The maximum diameter was observed under control in all the cultivars. Levis Duke 'Bella Vista' had smallest PLBs on medium supplemented with 2.0 mg BAP mg l<sup>-1</sup>. Whereas Vivacious 'Super White' and Margaret Thatcher 'Perfection' on medium supplemented with 0.5 and 2.0 mg l<sup>-1</sup> respectively.

### PLB Fresh Weight

The fresh weight of PLBs was significantly influenced by cultivars and concentration of BAP in the medium (Fig. 3). Cultivar Vivacious 'Super White' recorded

highest fresh weight of PLBs whereas it was lowest in Levis Duke 'Bella Vista'. No significant variation in fresh weight of PLBs/clump was observed in Vivacious 'Super White' and Margaret Thatcher 'Perfection'. Addition of BAP in the medium increased fresh weight of PLBs and maximum fresh weight in cultivar Vivacious 'Super White' was recorded on medium supplemented 1.0 or 1.5 mg l<sup>-1</sup> BAP. However, it was not significantly different from the fresh weight of PLBs of Margaret Thatcher 'Perfection' cultured on medium supplemented with 1.5 or 2.0 mg l<sup>-1</sup> BAP.

### Dry Weight

Dry weight of the PLBs varied significantly among the cultivars and BAP concentrations. The maximum dry weight recorded in Pine Clash 'Moon Venus' and Margaret Thatcher 'Perfection' followed by Levis Duke 'Bella Vista' when cultured on medium supplemented with 0.5 mg l<sup>-1</sup> BAP. However, in cultivar Vivacious 'Super White' the maximum dry weight was obtained when the medium was supplemented with 1.0 mg l<sup>-1</sup> BAP.

## Discussion

The growth regulating effects of BAP has been reported on various plant species. It reinforces regenerative response like somatic embryogenesis (Cheng, *et al.*, 2002; Kim and Kako, 1982; Swamy *et al.*, 2004), adventitious shoot formation (Chang and Chang, 1998; Sinha and Roy, 2004), and callus shoot proliferation (Chang and Chang, 1998; Le Van *et al.*, 2004; Roy and Banerjee, 2003) depending upon source of explants and the genotype. In this experiment promotive as well as inhibitory effects of BAP on multiplication of *Cymbidium* PLBs (Fig. 1) were noticed. The optimum concentration of BAP for PLB multiplication was cultivar dependent and it was lowest (0.5 mg l<sup>-1</sup> BAP + 0.1 NAA mg l<sup>-1</sup>) for Margaret Thatcher 'Perfection' whereas highest for (2.0 mg l<sup>-1</sup> + 0.1 mg l<sup>-1</sup>) for Levis Duke 'Bella Vista'. The results agree with those of Fujii *et al.*, 1999 who reported that BAP and NAA have synergistic effect on number of PLBs derived from outers tissues of *Cymbidium* cv. Thanksgiving 'Nativity' and *Cymbidium* cv. Lucky Rainbow 'Lapine Dancer'. Fuji *et al.*, (1999) did not find any difference in number of PLBs produced in two *Cymbidium* cultivars. These might be very closely related to each other. However, we observed a significant difference among the cultivars in production of PLBs and their requirement for NAA and BAP. Increasing BAP concentration beyond optimal level caused significant reduction in PLB diameter of all the cultivar. Fuji *et al.* (1999) also made similar observation and concluded that higher concentration of BAP reduces PLB diameter. A significant increase in fresh weight of

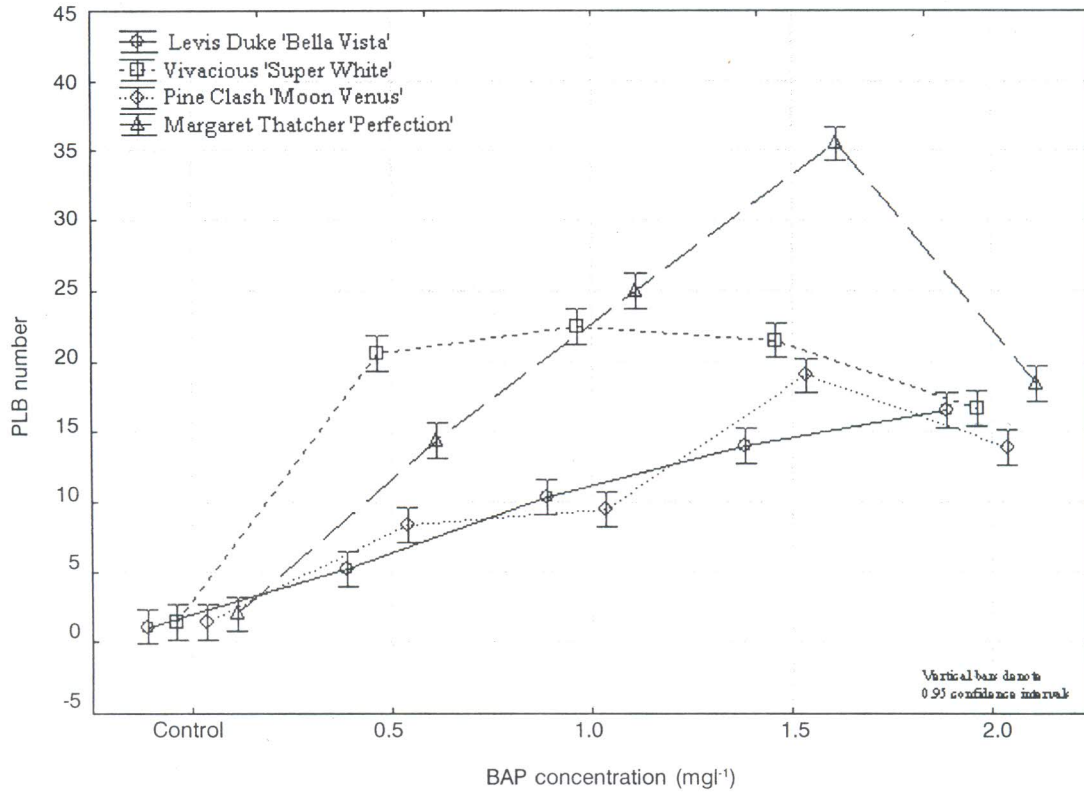


Fig.1. Effect of various concentrations of BAP on number of PLBs of four cultivars of *Cymbidium*.

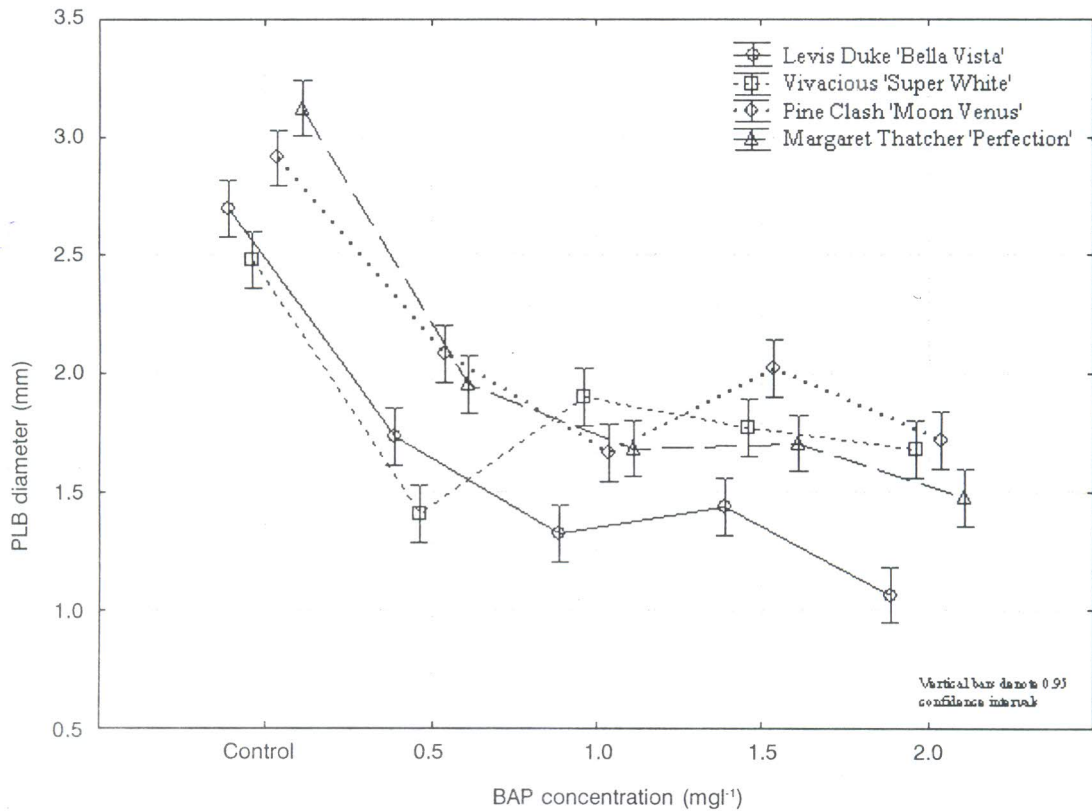


Fig. 2. Effect of various concentrations of BAP on PLB diameter of four cultivars of *Cymbidium*.

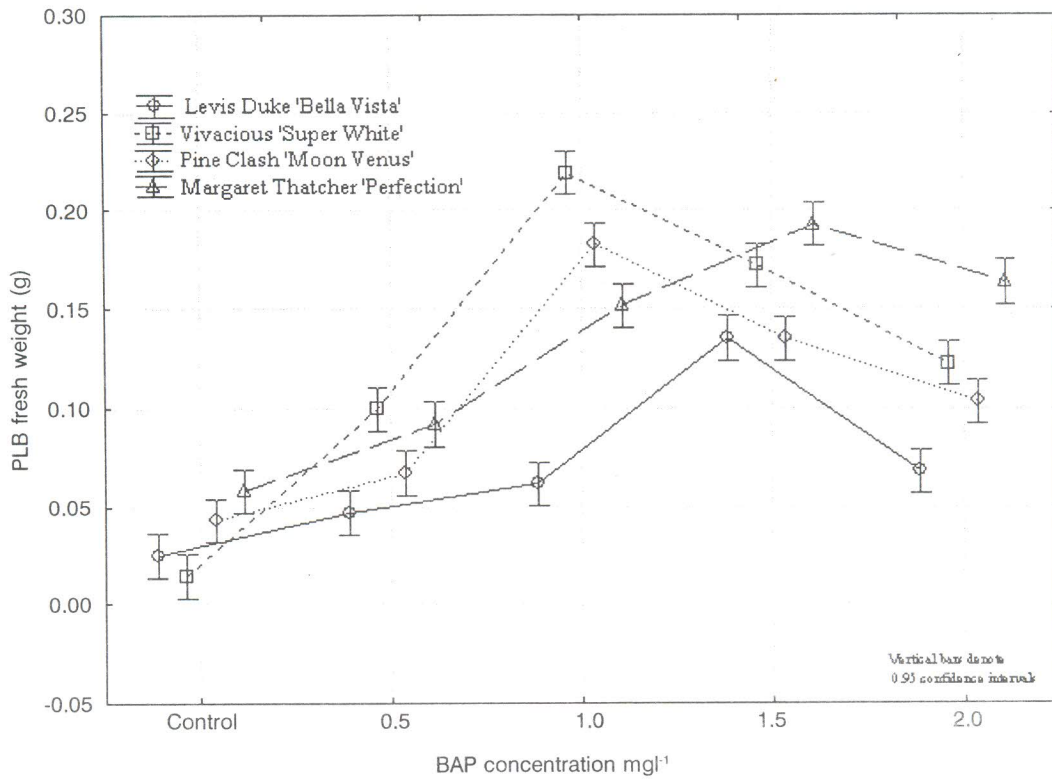


Fig.3. Effect of various concentrations of BAP on fresh weight of PLBs of four cultivars of *Cymbidium*.

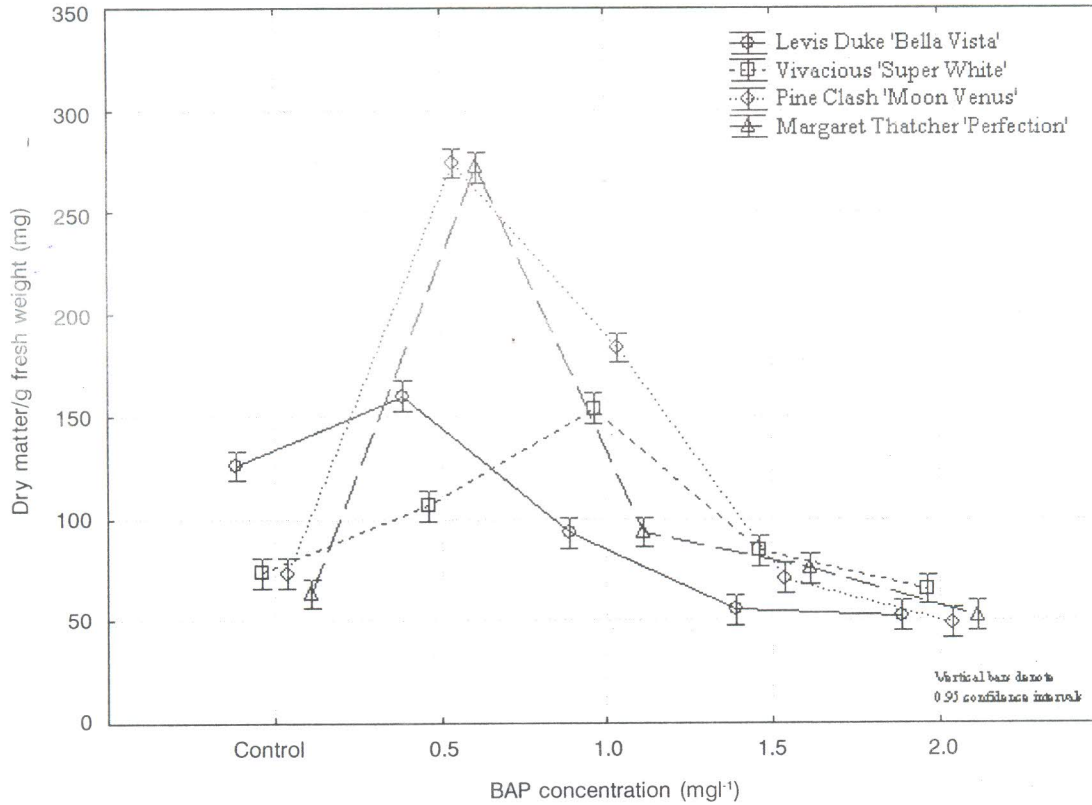


Fig.4. Effect of various concentrations of BAP on dry matter content of four cultivars of *Cymbidium*.



PLB clump was observed in all cultivars. The increase in fresh weight of PLB clump might be due to regenerative capacity of BAP and NAA. The synergistic effect of cytokinins and auxins has been reported by several workers however their concentration varied with the genotype. Meesawat and Kanchanapoom (2002) concluded that 1.0 mg l<sup>-1</sup> NAA and 2.0 mg l<sup>-1</sup> BAP is required for regeneration of callus in pigeon orchid (*Dendrobium crumenatum*)

### Conclusions

The above findings indicate that cytokinins and auxins are necessary for proliferation of *Cymbidium* PLBs and optimum requirement varies with the genotype. The excessive use of cytokinins not only reduce number of PLBs/explant but also likely to cause somaclonal variations. Hence, optimum requirement of cytokinins and auxins need to be worked out for the cultivar to be propagated *in vitro*.

### References

- Chang C. and W. C. Chang. 1998. Plant regeneration from callus culture of *Cymbidium ensifolium* var. *misericors*. *Plant Cell Reports*, **17**: 251-55
- Cheng, C. M., Y. Ye, and X. B. Ling. 2002. Seed and shoot tip tissue culture and morphogenesis of protocorms in *Cymbidium eburneum* var. *parishii* (Rchb.f.) Hook f. *Acta Hort.*, **29**: 251 - 54.
- Du Puy and P. Cribb. 1988. *The Genus Cymbidium*, Timber Press, Portland, Oregon.
- Fuji, K., M. Kawano, and S. Kako. 1999. Effects of benzyladenine and naphthaleneacetic acid on the formation of protocorm-like bodies (PLBs) from explants of outer tissue of *Cymbidium* PLBs cultured *in vitro*. *J. Japan. Soc. Hort. Sci.*, **68**: 35 - 40.
- Kim, K. W. and S. Kako. 1982. Effect of plant growth regulators on organ formation in *Cymbidium* shoot apex culture *in vitro*. *J. Japan. Soc. Hort. Sci.*, **51**: 106 -14.
- Le Van, T. H., T. Tanaka, and M. Tanaka. 2004. Callus formation and plant regeneration from callus through somatic embryo structures in *Cymbidium* orchid. *Plant Science*, **166**: 1443 - 49.
- Meesawat, U. and K. Kanchanapoom. 2002. *In Vitro* plant regeneration through embryogenesis and organogenesis from callus culture of pigeon pea orchid (*Dendrobium crumenatum* Sw.). *Tammasart International Journal of Science and Technology*, **7**: 9 - 17.
- Morel, G. 1960. Producing virus – free cymbidiums. *Orchid Soc. Bull.*, **29**: 495 - 97.
- Morel, G. and S. Martin. 1952. *Guerison de dahlia atteints d'une maladie a virus*. C. R. Acad. Sci. Paris, **235**: 1324 - 25.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.*, **15**: 473 - 97.
- Nayak N.R., S. Sahoo, S. Pathak, S. P. Rath, and S. Pathak. 2002. Establishment of thin section (TCS) method for rapid micropropagation of *Cymbidium aloifolium* (L) Sw. and *Dendrobium nobile* Lindl. (Orchidaceae). *Scientia Hort.*, **94** (1-2):107 –16.
- Pradhan, U. C., S. T. Lachungpa, and G. M. Pradhan. 1995. Scope for commercial orchid growing in Sikkim and Darjeeling hills In: *Proceedings of the Project Wesign Workshop on "Cultivation of Medicinal Plants and Orchids in Sikkim Himalaya* (eds. R. C. Sundariyal and E. Sharma) pp. 13 -18.
- Roy, J. and N. Banerjee. 2003. Induction of callus and plantlet regeneration from shoot tip explants of *Dendrobium fimbriatum* Lindl. var. *oculatum* Hk. f. *Scientia Hort.*, **97**: 333 - 40.
- Sinha, P. and S. K. Roy. 2004. Regeneration of an indigenous orchid *Vanda teres* (Roxb.) Lindl. through *in vitro* culture. *Plant Tissue Cult.*, **14**: 55 - 61.
- Steward, F. C. and M. O. Mapes. 1971. Morphogenesis in aseptic cell cultures of *Cymbidium*. *Bot. Gaz.*, **132**:65 – 70.
- Swamy, N. R., T. Ugandhar, M. Praveen, P. Venkataiah, M. Rambabu, , M. Upender, and K. Subhash. 2004. Somatic embryogenesis and plantlet regeneration from cotyledon and leaf expansts of *Solanum surattense*. *Indian J. Biotech.*, **4**: 414 - 18.
- Tokuhara K. and M. Mii. 2001. Induction of embryogenic callus and calli suspension culture from shoot tips excised from flower stalk buds of *Phalaenopsis* (Orchidaceae). *In Vitro Cell. Dev. Biol. Plant.*, **37**: 457 - 61.
- Wilfret, G.J. 1966. Formation of protocorm like bodies on excised *Cymbidium* shoot tips. *Am. Orchid Soc. Bull.*, **35**:823 – 27.
- Wimber, D. E. 1963. Clonal multiplication of cymbidiums through tissue culture of the shoot meristem. *Am. Orchid Soc. Bull.*, **24** : 380 – 92