**In vitro studies on regeneration of Chrysanthemum cv. SNOW CEM**

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**SUMMARY**
A successful procedure was established for in vitro regeneration from callus derived from shoot tip, leaf, auxillary bud and internodal segment explants of Chrysanthemum c.v. SNOW CEM cultured on MS (Murashige and Skoog, 1962) medium supplemented with various combination of auxins (IAA and NAA) and cytokinins (BAP and kinetin). The biotechnological interventions were required to modify the plant traits as desired by consumer. The present investigation dealt with micropropagation of Chrysanthemum c.v. SNOW CEM and the highest regeneration frequency (45.3%) was recorded in SNOW CEM with auxillary bud explant culture when MS medium supplemented with IAA (1.5 mg/l) and BAP (2.5 mg/l).

Key words : In vitro, Regeneration, Chrysanthemum, IAA, NAA, BAP.

Chrysanthemum (Dendranthema grandiflora Tzvelev) is one of the world's most leading and popular flower crop of commercial importance. The commercial cultivars are usually propagated vegetatively through cuttings and suckers. Breeding programmes have focused on improving various characteristics to enhance the ornamental value, including the colour, size and form of the flower, production quality and reaction to the environment (Broertjes *et al.*, 1980).

Biotechnology involving modern tissue culture, cell biology and molecular biology offers the opportunity to develop new germplasm that are better adopted to changing demands. In this way, extensive studies have been carried out with Chrysanthemum on various aspects of its biotechnology, such as micropropagation, adventitious shoot bud regeneration from various explants and somatic embryogenesis. Dendranthema grandiflora has been micropropagated to meet domestic and external market. The advantages are many including propagating large number of plants in a short period with genetically true to type nature. In addition, the rapid protocols also help in fundamental studies such as understanding of plant biology and applied areas like genetic engineering and development of pathotoxin resistant cultivars and stable mutants in vitro.

**MATERIALS AND METHODS**
The present study was carried out at Tissue Culture Laboratory, Department of Genetics and Plant Breeding, College of Agriculture, Rajendranagar, Hyderabad during December, 2004 to August, 2005. From the Agricultural Research Institute, Rajendranagar, Hyderabad, the explants viz., shoot tip, leaf, auxillary bud and internodal segment of Chrysanthemum cv. SNOW CEM were collected from their mother plants. Shoot tips of one cm length, leaf bits of one cm² size, axillary bud of 0.5 cm size and internodal segment of 0.5 cm length were excised and thoroughly washed with tap water followed by rinsing with distilled water and subsequently treated with 0.1 per cent mercuric chloride (HgCl₂) for four minutes and rinsed with sterile double distilled water to remove any traces of mercuric chloride. They were placed on sterile filter paper to remove any excess moisture.

RESULTS AND DISCUSSION
Four explants of SNOW CEM cultivar viz., shoot tip, leaf, auxillary bud and internodal segment have been studied for regeneration frequency on MS medium supplemented with growth regulators in varied concentrations of IAA (1.0, 1.5 and 2.0 mg/l) and BAP (2.0, 2.5 and 3.0 mg/l). IAA (1.5 mg/l) and BAP (2.5 mg/l) recorded high frequency of regeneration (45.3%) with auxillary bud followed by IAA (1.0 mg/l) and BAP (2.0 mg/l) with 41.9 per cent when shoot tip was used as explant (Table 1). The frequency was relatively reduced.

MS medium supplemented with growth regulators of IAA (1.0, 1.5 and 3.0 mg/l) and kinetin (2.0, 2.5 and 3.0 mg/l) was used for regeneration studies in SNOW CEM cultivar explants viz., shoot tip, leaf, auxillary bud and internodal segment.