SUMMARY OF THE THESIS

MICROPROPAGATION STUDIES WITH SOME ORNAMENTAL PLANTS

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Ornamental plants are those which are appreciated for their own aesthetic qualities or which are used to beautify the appearance of a primary object. In many of the cases, aspects of practicality and utility are also important factors in the choice of ornamental plants.

Humans have planted gardens since the dawn of civilization; however it is the growth of modern cities that has made horticulture the multibillion-dollar business of today. India has a long tradition of Floriculture. We have been growing and using flowers for many centuries. Flowers are used at most of our social and religious functions. Ornamental annuals with wide array of flower colours are mainly used for garden display for aesthetic effect. However, some of the annuals produce attractive flowers and have the potentiality for being used as cut-flowers, viz., Annual *Chrysanthemum*, *Antirrhinum*, *Arctotis*, *Calendula*, Carnation, China Aster, Clarkia, *Coreopsis*, Cornflower, Cosmos, Larkspur, *Gypsophila*, *Rudbeckia*, *Salpiglossis*, Sunflower, etc. they stand well in the interior decoration as cut-flowers, floral arrangements and bouquets (Roy and Sharma, 1999).

Propagation of plants through tissue culture has become an important and popular technique to reproduce plants that are otherwise difficult to propagate conventionally by seed or vegetative means. Tissue culture is used in its broadest sense to include the aseptic culture of plant parts – explants of widely different organizational complexities, including organs, tissue, isolated cells and protoplasts under controlled conditions (Gamborg & Phillips, 1996).

Commercial application of plant tissue culture started in USA with micropropagation of orchids in 1970's. It has seen tremendous expansion globally. The floriculture trade statistics of 2000 show an excellent year of percentage increase in sales. The total exports of cut flower and flower buds, foliage and other vegetation and live plants have shown an increasing trend from year 1995 to 1999. The Indian micropropagation industry, though a late starter by almost a decade, has expanded exponentially from 5 million annual capacities in 1988 to 190 million in 1996 (Govil and Gupta, 1997). Plant tissue culture has been promoted by Government of India since last 2 decades to enhance the production and availability of disease free, true-to-type, quality-planting material.

Government of India has launched 30 functional units within the country according to BCIL (Delhi) report, 1996. Total 40 million plants annually are produced against the installed capacity of 110 million plants.

One of the major applications of tissue culture techniques is in the area of micropropagation (Berbee and Hilderb'randt, 1972; Evans, 1990). Micropropagation involves culture of small piece of meristem, shoot tip or axillary bud which results in development of large number of buds from the explants. Proliferation of shoots in the initial stage is rather slow, but gradually goes on increasing during the first few subcultures and reaches a steady plateau during subsequent subcultures. Plants developed from axillary buds, shoot tips etc are generally phenotypically homogeneous thereby indicating genetic stability (Mc Cown and Amos, 1979). Many of the herbaceous species are being successfully propagated through *in vitro* axillary bud proliferation as in the case of *Anthurium* (Kuniseki, 1961), *Gerbera* (Murashige, 1974), *Chrysanthemum* and *Dianthus* (See Hu and Wang, 1983). Micropropagation of woody plants have comparatively lagged far behind, due to the great difficulty in establishment of primary cultures. The present studies were undertaken to formulate protocols for large scale *in vitro* multiplication of ornamentals. The plants selected for the study are as follows:

1. *Bougainvillea spectablis* Willd.
2. *Syngonium podophyllum* Schott.
3. *Mussaenda luteola* Delile.
4. *Aglaonema commutatum* Schott.
5. *Schefflera arboricola*

To standardize the protocol for clonal propogation the above ornamental plants were selected because of their following advantages:

1. *Bougainvillea spectablis* Willd: The small and inconspicunes flowers are associated with large and showy magenta-purple or red bracts that constitute the decorative value of the plants. The true flowers of *Bougainvillea* are relatively

insignificant and it is the numerous striking colourful petals like bracts, which afford the glowing curtain for which these plants are famed. *Bougainvillea* can be grown as shrubs, climbers, hedges and lately as pot plants (Pal and Krishnamurthi, 1967).

2. *Syngonium podophyllum* Schott: An herbaceous ornamental pot plant which, has decorative foliage. This plant belongs to the family Araceae, has high ornamental value as indoor as well as hanging plants.

3. *Mussaenda luteola* Delile.: It is a pretty shrub belonging to family Rubiaceae, with dull green foliage, yellow flowers and foliaceous sepals. One of the calyx lobes develops into a large, yellow coloured leaf like structure, which makes the plant conspicuous and attractive. It is a native of Africa commonly grown in Indian gardens, also suitable as tall hedges for cover.

4. *Aglaonema commutatum* Schott.: A genus of ornamental herbs, belonging to the Araceae family, distributed in the Indo-Malaysian region. *Aglaonema sp* are succulent or shrubby perennials valuable as pot plants. *Aglaonema commutatum* Schott - is a plant with spotted and greyish, oblong lanceolate leaves, pale green or white flowers, and capitate berries and is of special merit to horticulturists. The leaves are used to reduce swellings as they contain α and ß carotenes. Small amounts of carotenoids are present in the floral buds too. The fruits contain the following carotenoids - Lycopene, Lycoxanthain, Violaxanthin, 4-keto-3-hydroxylycopene and α - β - γ- and δ- carotenes. The presence of lutein is also reported in the seeds (Anonymous, 1994; Burkil, 1976).

5. *Schefflera arboricola*: This plant is grown for their showy foliage. The glossy, variegated digitate, pamately compound leaves have high ornamental value. Their shrubby appearance, shade preference for their growth, make them perfect indoor plants.

The basic protocol followed for the standardization of large-scale production of these ornamental plants through axillary bud or shoot tip culture are as follows –

Establishment of cultures: The contamination of free cultures was established via axillary nodal and shoot tip explants using routine procedure.

Bud induction and multiplication: Bud initiation and multiplication of shoots was achieved on MS basal medium supplemented with cytokinins (BA / KN) alone or in combination with auxins (NAA / IAA).

Elongation of shoots: The multiple shoots were allowed to elongate and grow on the MS medium supplemented with growth regulators - cytokinins.

Rooting: The well-grown shoots were allowed to root on MS liquid medium by pulse treatment to the shoots with auxin in *Bougainvillea* and *Mussaneda* or on MS basal medium supplemented with auxin (NAA / IAA) in other plants.

Hardening of plantlets: Hardening of plants was achieved in the culture room by normal routine process.

Transfer to the field: Once the plantlets were hardened in the culture room, they were transferred to the field

RESULTS

1. *Bougainvillea spectablis* Willd.: June to September was the best period found for bud proliferation. The axillary bud sprouting was achieved on MS medium containing BA (6.65 μM) with IAA (2.85 μM). For shoot elongation and multiple shoot induction BA alone was very effective in terms of shoot length and number of nodes than other cytokinins, whereas low levels of auxin (IAA) with high level of cytokinin (BA) significantly increased the multiple shoot formation. Root induction was achieved by pulse treating the shoots with IBA (2 min). Liquid

medium favored the initiation of roots compared to the poor growth of the roots in the solid medium. Hardening of the plants was achieved by transferring them to plastic vermiculite and perlite (5:2) and then transferring them to earthen pots (8 cm diameter) containing garden soil, compost and sand (2:3:1) before transferring to botanical garden.

2. *Syngonium podophyllum* Schott.: For controlling the contamination in the cultures, among the two surface sterilants tried viz $HgCl_2$ and NaOCl; $HgCl_2$ (0.1%) for 6 minutes proved much better than NaOCl. The prriod from July to September proved to be the best for bud initiation. Cytokinin - BA was found to be best for bud initiation (2.21 μM) and for multiple shoot induction (8.87 μM). On comparing solid and liquid medium for bud initiation and for multiplication of shoot; solid medium gave much better response as compared to the liquid medium. Incisions given to the bud did not give good number of multiples. BA (33.9μM) and IAA (1.42μM) was most effective for multiple shoot induction from shoot apices; prior to this buds were incubated on MS medium containing BA (44.39μM). After 5 subcultures in approximately 4 months, maximum 123 shoots were produced in the same medium. Root induction was achieved *in vitro* from the axils in the shoot multiplication medium only. The hardened plantlets were transferred to the Botanical garden and their morphological characters were found to be same with parent plants indicating regenerated plantlets were true-to-type to the initial stock.

3. *Mussaenda luteola* Delile.: BA (0.44μM) and IAA (0.571μM) at very low concentrations gave best results for bud sprouting. BA in combination with IAA elicited a better response for multiple shoot induction than KN with IAA. Callus was produced at the base of the multiple shoots when tried on the medium containing KN with IAA; this resulted in poor root formation and field survival of plants. GA_3 in very low concentration (0.26μM) with IAA and BA resulted in elongated but weak shoots. For rhizogenesis, the shoots, pulse treated with IBA (49.20 mM) for 10 min and then transferred to ½ strength liquid basal medium

gave best response. Though roots were also produced in the multiplication medium. The plants were hardened similarly as the other above plants and after 1 month transferred to field in earthen pots

4. *Aglaonema commutatum* Schott.: BA (0.88μM) was found effective for axillary sprouting. Multiple shoots were induced on BA (2.21μM) and auxin IAA (0.57μM) supplemented medium. *In vitro* and *ex vitro* rooting was achieved on the shoots, in the shoot multiplication medium and during the hardening of plantlets respectively. The apical meristem culture tried failed to give any positive response.

5. *Schefflera arboricola*: The bud initiation was induced directly on the MS medium supplemented with BA (2.21μM). Higher concentration of BA (8.87μM) favoured multiple shoot induction. Roots were induced on MS solid medium containing NAA (2.63μM), whereas the other auxins tried IBA and IAA could not show significant results. These rooted shoots were then hardened subsequently in plastic pots containing vermiculite and perlite (1:1) covered with plastic sheet in the culture room and followed by transfer of plantlets in earthen pots containing garden soil and compost (1:1 v/v) and then transferred to the field.