Chapter I

GENERAL INTRODUCTION

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In contrast to plants that yield a tangible product, 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. Criteria of what is beautiful are in large part culturally determined and differ between countries, within parts of a country, between individuals and from generation to generation. There are however, a few basic elements of beauty common to art forms in general which can be applied to ornamental plants as well. 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 and others. They stand well in the interior decoration as cut-flowers, floral arrangements and bouquets (Roy and Sharma, 1999).

The business and marketing of flowers provides livelihood to scores of people. Annuals constitute a major share of loose flower production in India. According to an earlier estimate by APEDA (Agriculture and Processed Food Export Development Authority), out of total floricultural trade of India, which is estimated to be Rs 205 crores, the traditional flowers, including annuals, contribute Rs 105 crores while modern cut-flowers (flowers with stem) fetch Rs 100 crores. Recently, the trade is around Rs 250 crores, shared equally by traditional and modern cut-flowers. By proper planning and management, one can earn a net profit of Rs 30,000-50,000 per acre by the sale of flowers and seeds / annuals (Roy and Sharma, 1999). In our export of flowers significant growth has been observed from Rs 8.6 crores in 1990-91 to 30.60 crores during 1994-95 (Dadlani, 1996) and recently has exceeded the target of Rs 200 crores worth.

In our increasingly urban environment, ornamentals often constitute our only link with the natural growing plant world. Ornamentals can be divided into three major groups:

1. Nursery plants
2. Florist plants
3. House plants.

Nursery plants are used for outdoor plantings, often to complement a structure or to beautify an area. Florist crops are those, which yield flowers or varied foliage and used for cut arrangements. House plants are confined to pots and used for interior decoration. Different qualities are sought in plants belonging to each of the groups, because they serve different purposes (Anonymous, 1979) but one quality, the aesthetic appeal, is common to all (Simpson and Gauzily, 1986).

Floriculture:

Flowering ornamentals play an important role in the garden by their tremendous capacity of adding colours to the landscape. Annuals have a dazzling array of flower colours. Some are very brilliant while others are soft and pleasing to the eye. They render tremendous colour effect to the landscape, within a very short period, as compared to other plants. Seasonal flowers, in dehydrated form, can be preserved for a considerable long period and are used in various ways for decoration purposes. Dried flowers encapsulated in glass casings as decorative items are in heavy demand in commercial market. Annuals suitable for dehydration and making value added products are: *Acroclinum*, *Antirrhinum*, Aster, *Brachycome*, *Calendula*, Candytuft, *Helichrysum*, Marigold, Pansy, Sunflower etc.(Roy and Sharma, 1999).

Recent floriculture trade statistics shows increase in sales. The total exports of cut-flower and flower buds, foliage and other vegetation and live plants have maintained almost same trend from the year 1995 to 1999 (Table-1.1).

The trade for cut foliage and potted plants was $ 7,885 million in 1999, whereas live plant trade around the world was $ 3,185 million, which is little under half the importance of cut flower market. Whereas cut foliage was up by 3 % in export market

worth US $ 620 million in the year 1999. Floral and ornamental plants are also the fast growing business due to high added value and profit (Nancy, 2001).

Table-1.1: Export values in Floriculture trade across the world (Nancy, 2001)

EXPORT MATERIAL	YEARLY SALES IN US $ X 1000				
	1995	1996	1997	1998	1999
Cut flowers, flower buds	3,830,984	3,869,510	3,615,735	3,686,417	3,880,886
Foliage, other vegetation	487,528	522,762	521,703	549,250	568,696
Other live plants, and cuttings	3,143,336	3,230,606	3,036,175	3,119,985	3,336,738
Total	7,461,848	7,622,878	7,173,613	7,355,652	7,786,320

Propagation of ornamentals:

Most of natural and conventional propagation of ornamentals is by seeds. However, this method poses intrinsic problems like:

(1) Low multiplication rate

(2) Dependent upon the season

(3) The progenies produced are highly heterogeneous

(4) There is no surety of quality of propagules in advance

(5) Sometimes cuttings are difficult to root

(6) They may produce plagiotrophic plants except, when taken from apical shoots which give orthotrophic plants.

So to avoid these, problems, vegetative propagation methods are being employed. The most widely used methods of vegetative propagation include cuttings of vegetative parts, layering, grafting and budding. Some of the widely used methods of conventional vegetative propagation of plants are:

Plantlets: In certain species, the miniature replicas of the plant itself is produced on stems or leaves e.g. *Kalanchoe*. Gardeners use these little plants for propagation.

Offsets: Sometime plants produce small branches, that spring directly from the main stem, if carefully cut and potted up after reaching a certain size, they rapidly grow into a mature plants. This method of propagation is especially appropriate for bromeliads, a number of cacti, and bulbous plants.

Division: Plants that grow in clumps are generally propagated by being divided into two or more smaller clumps. Each segment of a divided clump should carry a cluster of leaves and well-formed roots.

Stem cuttings: A common way to multiply plants is by rooting pieces of the stem. These stem cuttings are often taken up as tip cuttings, which are trimmed a few inches below a growing point (Anonymous, 1979).

Leaf cuttings: In some plants it is possible to root leaves by inserting the leaf stalk or base of the leaf into rooting mixture and new plants will develop. In few cases the leaf can be cut into segments, each of which will produce a young plant, often from a vein e.g. *Begonia* (Anonymous, 1992).

Layering: It is also an easy method of propagating plants; simple layering consists of pegging down a branch into the soil, where it will form roots. Later on, these rooted branches are detached from the parent plant and planted elsewhere. There are different types of layering used for different plants, these are:

1. Serpentine layering: This is a minor modification of simple layering, and is done with plants possessing long stem particularly climbers like *Clematis, Jasmine, Lonicera (honey suckle), and Passiflora.* Here, the long stems are wounded and pegged down at intervals along the ground, so that we get number of new plants from one stem.

2. Tip layering: In this method of propagation, the extreme tip of a young stem 7 to 8 cm is buried deep in the soil in the late summer or early autumn, e.g. *Strawberries* are propagated by such layering (Anonymous, 1992).

3. **Air layering:** It is a useful method where stems cannot be pulled down to the ground i.e. in trees or shrubs. The young stem is cut diagonally halfway through it, making the cut about 5 cm in length. Make this wound about 30 cm away from the tip and keep the cut fully covered by packing it with moist *Sphagnum* moss. It may be treated with rooting hormone or liquid to speed up root production. Then this prepared part of the stem bandaged with moist sphagnum moss can be held in place by wrapping with a piece of clear polythene sheet with both ends tied with twine or the polythene 'sleeve' tightly sealed with self adhesive water proof tape.

Budding and grafting: Grafting and budding are horticultural techniques used to join parts from two or more plants so that they appear to grow as a single plant. In grafting, the upper part (scion) of one plant grows on the root system (root stock) of another plant. In the budding process, a bud is taken from one plant and grown on another. Although budding is considered a modern art and science, grafting is not new. The practice of grafting can be traced back 4,000 years to ancient China and Mesopotamia. There are different types of budding and grafting technique:

1. T- budding
2. Chip budding
3. Whip and tongue grafting
4. Saddle grafting
5. Splice grafting
6. Bridge grafting
7. Approach grafting.

Propagation from seed: Large numbers of new ornamental plants can be raised from the seed. This is the only way to propagate most of the annuals / seasonal plants. Therefore, quality and purity of seeds are very important. As a common practice by gardeners, seeds are collected from present year crop and utilized in the next year. Healthy, mature and disease free seeds should be collected. Immature seeds lead to poor germination due to loss of viability during

storage. Collected seeds are to be air dried and stored in airtight containers like glass jars, desiccators with calcium chloride, or in tin containers with silica gel to protect them from moisture. Seed production for commercial purposes require scientific and technical knowledge on plant breeding, isolation distance, bagging etc.

Problems occurring among ornamentals:

The demand for ornamentals has increased with increasing urbanization in the industrialized countries. A continuous breeding of new cultivars, domestication and experimentation with new species is the only way to satisfy the customer demand for variation (Koenig, 1980). Annuals are often affected by a number of diseases and infested by several insects at seedling as well as mature stage. As they are herbaceous in nature, the extent of damage will be more, if not cared properly and timely (Roy and Sharma, 1999). There are many problems occurring among ornamentals:

1. The major drawback for raising perennial foliage plants from seeds is that it can take a long time to bring such plants to maturity (Anonymous, 1979).

2. Many ornamentals are virus infected and such pathogens spread by different means e.g. mechanical contact, through horticultural equipments; insects, especially aphids; from soil through nematodes and fungi or even by pollen etc. Once a plant becomes infected by a virus it normally cannot be cured by spraying chemicals. Since most ornamentals are propagated vegetatively, all the progeny of a diseased plant will be infected too (Koenig, 1980).

3. The lack of identification of suitable planting material of a particular species and the arrangement for their large-scale import.

4. For flowering ornamentals in India we lack adequate knowledge on harvesting, post-harvest handling of flowers, and on packing according to the international standards (Pandey et al, 1994).

5. Standardization of production and post-harvest technology is available for only few and local species.

6. In-adequate arrangement of financial assistance for the infra-structural development and the import of necessary equipments required for growing high valued ornamentals (Pandey et al., 1994).

7. Irrigation procedure is also important with certain crops as certain bacterial pathogens spread with overhead-applied water splashing off the foliage of infected plants (Henley, 1991).

8. There are certain physiological problems occurring among foliage plants like water soaked leaves, long thin internodes and rot of single-node cuttings in the propagation beds etc. may be due to the lack of sufficient sunlight, decrease in air temperatures etc. (Spinazzola, 1990).

9. Pronounced bacterial problems occur among the ornamentals plants e.g. Bacterial leaf spots where the leaves are infected with *Erwinia*; cutting rot caused by *Erwinia sp* which causes decay of the plant cuttings (Chase, 1990).

10. Insect and mite problems are also common in the ornamental plants. The major arthropod pest of ornamental species includes aphids, moths, worms, mealy bugs, mites, scales, thrips and white flies (Price et al, 1989). All these causes distortion of new growth, stunted plants and eventual senescence or death of plant parts (Short et al, 1989).

Plant tissue culture methods:

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 organ, tissue, isolated cells and protoplasts under controlled conditions (Gamborg and Phillips, 1996). *In-vitro* techniques for the culture of protoplast, anther,

microspore, and ovule have also been used to create new genetic variations in the breeding lines. Cell-culture methods also helped to produce somaclonal and gametoclonal variants with crop improvement potential .The culture of single cells and meristems can be effectively used to eradicate the pathogens from planting material and thereby dramatically improve the yield of established cultivars (Brown and Thrope, 1995). However, one of the major applications of tissue culture techniques is in the area of clonal propagation (Berbee and Hilderbrandt, 1972; Evans, 1990). The clonal propagation is an asexual method of reproduction for genetically uniform plants that originated from a single individual, through explants.

Callus culture: Callus is an unorganized mass of cells. Induction of callus from the primary explants is necessary as a first step in many tissue culture experiments as the callus is convenient for initiating and maintaining cells required for organogenesis, selection of cell lines (cellular selections), conservation of germplasm, protoplast isolation and culture, somatic embryogenesis and secondary product formation (Gamborg and Wetter, 1975). Callus formation can be controlled by the level of plant growth regulators, mainly auxin and cytokinin in the medium. Nutritional requirement and environmental conditions (temperature, light and humidity) can vary for each plant species and even depend on the origin of the explants or individual plant species for the growth, development and morphogenesis under *in vitro* conditions (Mascarenhas, 1991).

Somatic embryogenesis: Somatic embryogenesis is a process, by which somatic (non-gametic) cells undergo differentiation to form bipolar structure containing both root and shoot axes in tissue culture. This asexual mode of embryogenesis can be induced to differentiate directly or indirectly from cells, tissues, organs or intermediate callus of a plant. Somatic embryos may proliferate further to produce secondary embryos. These embryos are similar to zygotic embryos and can mature and germinate on suitable culture medium. Lately, new advances in somatic embryogenesis has been viewed as most potential and efficient micropropagation method as large number of plants could be regenerated in a small amount of cultured cells or tissues in a short period and limited space. The somatic embryos can act as

artificial seeds via coating or encapsulation of somatic embryos with gel matrix. According to Sharp et al (1982), somatic embryogenesis can be initiated, either by **pre-embryonic determined cells** (PEDC) or by **Induced embryogenic determined cells** (IEDC). Sometimes, individual cell or cells from the group may escape and give rise to either embryoids or nodular embryogenic callus consisting of proembryoids (Williams, 1987).

Meristem culture: Meristem culture technique involves the dissection of shoot apices containing the apical dome with few primordial leaves and culturing them on nutrient medium. Broadly, this method is also known as apical-tip culture (Bhojwani et al, 1983). Meristem culture technology has been applied to many crops, especially vegetatively propagated crops such as Potato, over the last 4 to 5 decades to eliminate viruses from the important cultivars (Slack, 1980; Bhojwani et al, 1983; Hartmann et al, 1990). The meristem culture technique have been adopted for virus elimination and for maintenance and propagation of nuclear seed stocks, respectively (Bryan, 1988; Jones, 1988). The protocols for different species are set in laboratory by varying the culture media as required and by adopting these techniques to the growth habit and meristem locations in the species (Slack et al, 1995).

Single node culture: The most obvious, simple and secure method of cloning plants *in vitro* is the single node culture method, resulting in the production of the shoots and the regeneration of roots on these shoots. The technique exploits the normal ontogenic root of branch development by lateral (axillary) meristems. As an apical bud or shoot tip grows out into an elongated stem, single nodes with dormant buds are subcultured to form elongated shoots and the apical shoot tip can be rooted directly. The rate of shoot multiplication in such cases depends on the number of nodal cuttings that can be excised from the shoot at the end of each passage. This method of shoot multiplication may be initially slower than the other methods but with each passage the number of shoots increases logarithmically and within an year astronomical figure can be achieved. It is the only method of *in vitro* propagation particularly in some of the ornamental species, when it is not possible to induce the release of axillary buds

from apical dominance by manipulating the hormonal composition of the medium (Bhojwani and Razdan, 1983; Phillips and Hubstenberger, 1995; Ignacimuthu, 1996).

Enhanced axillary branching: Another slightly artificial method is the induction of the axillary branching in isolated shoot tips. In nature, apical dominance can be broken by removing the apical bud resulting in the release of dormancy of the axillary buds.As soon as the apical bud is removed, the basipetal flow of auxin, which normally keeps the axillary buds dormant, stops. Removal or senescence of the apical bud also breaks apical dominance *in vitro*, resulting in axillary branching, but in most cases this is released by the application of cytokinins, which are capable of antagonizing the auxin produced by the apical meristem. As soon as the axillary buds or shoots are formed, they can be separated from the original shoot tip and placed on a fresh medium again with cytokinins to repeat axillary branching. When enough shoots have been obtained, the process of axillary branching can be stopped by omitting cytokinin; subsequently, auxin is applied to promote rooting of the individual shoots *in vitro* or sometimes *in vivo*. This method is frequently applied, since it is relatively simple and quite safe with reference to appearance of variation. The rate of propagation is also relatively fast.

Multiple shoot formation: This is another form of multiplication of plants *in vitro*. When single node explants are put on a semisolid medium with appropriate combination of growth regulators, instead of elongation of the existing axillary bud, a large number of shoot buds appear from the axil and elongate. Some of the shoots produced are longer and sturdier. The number of shoots produced depends on the species, the growth regulator used in the medium, physiological status of the explant and the number of the subcultures undergone. Each of these shoots can be either put for further multiplication or can be allowed to grow in a medium with less or no cytokinins compared to the multiplication medium, and then be rooted. The rate of multiplication is very high and many of the herbaceous species are propagated by these means. Although the shoots may get formed *de novo* it is not yet conclusively proved that the shoots or plantlets formed via multiple shoot induction show variability.

Commercialization of tissue culture:

Commercial application of plant tissue culture started in USA with micropropagation of orchids in 1970's. It has seen tremendous expansion globally. Advancements in commercialization of plant tissue culture and acceptance of tissue cultured plantlets by the commercial sector have led to continued exponential growth within the industry in terms of new units as well as number of plants produced by these units. The global biotechnology business is estimated to be around 150 billion US $, of which 50-60 % is in agri-business and annual demand of tissue culture raised products constitutes about 10 % of the total 15 billion US $ with annual growth rate of about 15 % (Govil and Gupta, 1997).

Plant tissue culture has been promoted by Government of India since last two decades to enhance the production, land 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). Having realized its potential for large scale clonal propagation many groups in India for example, AV Thomas & Company-Cochin, SPIC Biotech-Chennai and Coimbatore, Ramco Biotech-Rajapalaym, Indo American Hybrid Seeds-Banglore, Godrej Biotech-Hyderabad, Zandu-Mumbai, GSFC-Vadodara, Cadila-Ahmedabad, Dabur-New-Delhi, EID Parry-Bangalore, Kothari Biotech-Bangalore, EPC Irrigation-Nasik, Mericlone-Hyderabad, Kumar Biotech- Pune, *In-vitro* International-Bangalore, Khoday Biotech-Banglore etc. have commercialized tissue culture as a new business venture.

Recent National Workshop on commercialization of plant tissue culture-**Role of Micropropagation Technology Park** was convened by DBT-New Delhi on June 14, 1999 with the major objective of creating an awareness and concept of Micropropagation Technology Parks.

The state of Maharashtra emerging in tissue culture industry alone is producing 91.85 lakh tissue cultured plants every year out of which 31.30 lakh plants of ornamental crops particularly *Gerbera*, Carnation and Orchids (Prakash et al, 1996). Most of the units are

importing planting material, which consumes very heavy foreign exchange. Indigenous production of such plants would help reducing the burden on foreign exchange and also help to reduce capital investment on large-scale floriculture units.

Recent developments in ornamentals:

The use of *in vitro* procedures for rapid clonal propagation has become routine in a large number of herbaceous and ornamental plants. Similar procedures have been used for rapid selection and screening of genotypes which exhibit tolerance to abiotic and biotic stresses. Development of cost-efficient micropropagation and selection procedures in such plants is of great commercial advantage, provided that well-acclimatized transplants can be produced. The range of options available to achieve this goal includes: axillary bud regeneration, somatic embryogenesis, production in bioreactors and efficient rooting and acclimatization. Similarly, the use of small propagules for rapid screening against environmental stress (e.g. desiccation, freezing, heavy metal toxicity), as well as for germplasm collection and conservation, is of great importance worldwide. These techniques are currently used in many herbaceous plants, but only to a small extent in other species (Govil and Gupta, 1997).

During the past decade there has been a tremendous increase in the demand of ornamentals worldwide. In the floriculture industry itself the consumption of flowers in the western European countries was from US $ 12 billion in 1991 to US $ 15 billion by the end of 1995. To meet with the needs and increasing demand of the ornamentals certain steps and efforts were taken all over the world. There have been co-ordinated efforts to develop a modern floriculture industry by various agencies of the Indian Government over the past decade. At present, most of the research on flower improvement is carried out in Agricultural Universities (Anonymous, 1989). In 2001, Florigene's founded in Melbourne, Australia (1986) has launched next generation Carnation to the world market which have unique colours ranging from lavender to purple to a colour that approaches black. Various plant tissue culture methods / techniques are being employed for large-scale production of ornamentals.

Indian Institute of Horticultural Research-Bangalore (IIHR) has contributed to the development of varieties of several commercially important ornamental crops, such as Rose, *Gladiolus*, Tuberose and *Bougainvillea*. It has made available, its rich collection of rose germplasm of nearly 600 accessions, to the institutes active in breeding. The Tropical Botanic Garden and Research Institute-Thiruvananthapuram (TBGRI) is working on the development of hybrid varieties of orchids. Indo-American Hybrid Seeds-Bangalore (IAHS) pioneered the production of F_1 hybrid seeds for flowers and vegetables since 1960's. The development of tissue culture facilities in India was also prompted by private sector participation. In 1984, A.V. Thomas Company, established its first tissue culture laboratory. India has an installed production capacity of around 120 million plants per year; most of it is geared to exports (Prakash and Bhandari, 1994). According to the recent initiatives the Ministry of Commerce has identified the potentials of floriculture as an **extreme focus segment.** It is expected that India's floriculture trade is to be competitive in the international market to become a major source of foreign exchange earnings, with a variety of products (Pandey and Chaturvedi, 1994). Initially, products like flower seeds were imported mainly from Europe, subsequently companies like Suttons, who started a branch in India, at Calcutta, took up indigenous production of flower seeds in the country, since then large scale flower seed production has been taken up in Punjab, Himachal Pradesh and Jammu & Kashmir in the north; Karnataka in the south and West-Bengal & Bihar in the east. Now, liberalization of import policies enables introduction of hybrids and other improved varieties of seeds in the country (Dadlani, 1996). Floral extracts with big potential has remained untapped to a large extent. There is a considerable demand in the global markets for natural extracts. The value of world production of perfumery raw materials etc. during 1987 was estimated to be over 7 billion US $. Inspite of creation of synthetic aroma chemicals and reconstituted essential oils, the demand for natural concrete of crops like jasmine, rose and tube-rose remains unlimited and matchless (Siddiquie and Seth, 1996).

Breeding for resistance: Characteristics such as flower colour, shape, longivity, plant habit and resistance to diseases and insects can be altered by the use of genetic modification techniques. Genetic engineering studies for cut flowers is done by a small number of biotechnological companies like Florigene, Calgene Pacific and

DNA Plant Technology, Zaadunie, which is now called S&G seeds, belonging to Sandoz, Switzerland. Identification, isolation and transferring of genes responsible for colours are the main focus of research at Calgene Pacific. It is expected that blue flowers command a market share close to that of red if they were freely available. The blue transgenic *Petunia* were reported in 1992, blue carnations and *Chrysanthemum* are being tested. Genetic engineering is also applied to extend vase life of flowers, by blocking the ethylene production of flowers, which triggers flower deterioration. University of California at Davis has also developed a transgenic *Chrysanthemum* with altered flower colour. DNA Plant Technology has been the first unit to report the development of a transgenic Rose. Friable embryogenic tissues of Rose have been transformed and reproduced into flowering plants (Bijman, 1994).

In Japan, three large breweries, Kirin, Sapporo and Suntory, have invested in plant biotechnology. Kirin brewery is involved in micropropagation and flower export through its Dutch subsidiary Hilico in Amsterdam. Suntory has developed a virus resistant transgenic *Petunia*. Suntory and Calgene Pacific have set up a joint venture named International Flower Development (IFD), the aim of this joint venture is to apply genetic engineering technology to produce blue flowers, particularly blue Roses. Sapporo is involved in the cultivation of *orchid* plants, using cloning techniques including tissue culture.

Micropropagation:

Most of the natural propagation takes place through seed but the progenies produced are highly heterogeneous and also there is no surety of the quality of propagules in advance . As an aternative, vegetative propagation methods are being employed. The cuttings of vegetative parts, layering, grafting and budding are the common methods of vegetative propagation. These methods have advantages over seed propagation like faster asexual multiplication and by-passing of undesirable long juvenile phase. The tissue culture technique offers an alternative means of plant propagation. The most important technique, which is used to make large number of clonal plants, is micropropagation (Phllips and Hubstenberger, 1995). The method of micropropagation basically involves regeneration of shoots from meristematic cells or tissues (Ignacimuthu, 1996).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 & Gupta, 1997).

By definition, micropropagation refers to *in-vitro* clonal propagation of plants. Through the use of various plant growth regulators the plants are made to proliferate by the induced growth of apical and axillary bud, production of adventitious shoots or through the formation of somatic embryos. However, it sometimes refers specially to axillary bud proliferation. Thus, propagation of selected elite plants through tissue culture is called micropropagation. This technology is being utilized commercially in the ornamental industry and in other plant production organizations worldwide (Chu, 1992; Huettman and Preece 1993; Mantell et al, 1985; Pierik, 1987).

There are several defined steps in a typical micropropagation system (Murashige 1974).

I) The first step is the initiation or establishment of a sterile culture of the explants (Stage 1).

II) The second step is the multiplication of shoots or other propagules from the explants (Stage 2). Axillary bud proliferation or adventitious shoot proliferation is the most frequently used multiplication technique in micropropagation systems (Chu, 1992).

III) The third step is the development of roots on the generated shoots to produce plantlets (Stage 3).

IV) The final step is to produce self-sufficient plants (Stage 4), which usually involves a hardening process and acclimatization for growth in soil mixtures under greenhouse conditions (Phillips et al, 1995).

Micropropagation involves culture of a small piece of meristem, shoot tip or axillary bud, which results in development of large number of shoot buds from the established explants. Proliferation of shoots in the initial stage is rather slow, but gradually goes on increasing during the subsequent subcultures. Plants developed from axillary buds, shoot tips etc. are generally phenotypically homogeneous, thereby indicating genetic stability (McCown and Amoss, 1979). The major flowering plants which get micropropagated, in order of priority are *Chrysanthemum*, Carnations, Tulip, *Fressia*, Gerbera, Lily, Orchid,

Gysophila and *Iris*, where as top ten foliage plants are *Ficus, Dracaena, Begonia, Saintpaulia, Yucca, Azalea, Poinsettia, Kalanchoe* and *Dieffenbachia* (Rathnavel and Prakash, 1993). Micropropagation or clonal multiplication of plants aims at the production of plants breeding true-to-type, in larger numbers, in quicker time and disease free (Naranyanswamy, 1994). During the last 30-40 years it has become possible to regenerate plantlets directly from explants or through callus of all types of plants. As a result, laboratory-scale micropropagation protocols are available for a wide range of species (Debergs and Zimmerman, 1991). At present micropropagation is of the widest use among plant tissue culture technology. Murashige (1990) reported that there were over 300 commercial operations world wide. In Europe there were 172 firms and about 1800 different lines (species and varieties) in culture among the 501 plant tissue culture laboratories identified in 1993 (O'Riordin, 1994). The wide spread use of micro propagation has many advantages:

1. When classical methods of *in-vivo* vegetative propagation prove inadequate, *in vitro* cloning is an important tool in speeding up the propagation.

2. Adult plant material, which often cannot be cloned *in-vivo*, can be rejuvenated *in-vitro* and then propagated.

3. Growth of *in-vitro* propagated plants is often stronger, more vigorous in growth than those cloned *in-vivo* (mainly due to rejuvenation).

4. By *in-vitro* propagation, expensive and laborious methods such as grafting and budding on a rootstock can be proved absolute.

5. It also enables us to isolate and clone the spontaneous and induced mutants or selected elite material.

6. It is immune to variable environmental conditions; therefore round the year production is feasible.

In-vitro storage and propagation facilitates the creation of gene-bank (preservation of valuable plant material) and storage under pathogen free conditions in a relatively small

area. By use of low temperature storage and freezing, the time spent on cloning as well as space required can be drastically decreased. This technique allows us to do genetic manipulations, which would be impossible when no methods for regenerating protoplast, cells and tissue exist.

Factors affecting growth and morphogenesis:

The ability of the explant to survive, multiply and regenerate is a consequence of wide variety of factors such as origin and history of explants, physiological state of explants, endogenous hormone concentrations and general culture conditions (light, photo-period, room temperature). All these can be grouped under four main headings:

Effect of genotype: Response of cultured tissue or organ for *in-vitro* regeneration is influenced by the genotype used. Media and cultural environment often need to be varied for different genera or species or even closely related varieties. A major difference in the survival of shoot tips has been noted in different peach cultivars (Hammerschlag, 1982). Similarly, Roca et al, (1978) recorded that different varieties of potato and their hybrids differed in the behaviour of their shoot tip in culture.

Media components: Correct balance of inorganic and organic, growth regulator components is essential for plant growth and regeneration under *in vitro* conditions. Growth and organogenesis *in vitro* is highly dependent on the interaction between naturally occurring endogenous growth substances and growth regulators added to the medium. Auxin and cytokinins are the two types of phytohormones most often needed as growth regulators in the culture.

Environment: Physical nature of the media is also known to influence the regeneration capacity of explants. Cells can be grown in semi-solid or liquid medium. For semi-solid medium, agar, gelrite, phytagel or complex polysaccharides are the most commonly used chemicals. The environments in which plant cells are cultured often determine the type of growth and differentiation *in vitro.* These include the pH of the medium, relative humidity, type and intensity of light, temperature and

photoperiod. The pH range for growth and organized development of most species although not critically assessed, is set between 5.0 to 6.5 during media preparation. However, pH drifts do occur during autoclaving and culture (Street, 1969; Minoch, 1987).

Tissue dependent factors: The process of organized development begins with changes in individual cells in response to some inductive signals which cause quiescent cells to undergo the altered path of differentiation (Thrope, 1980), de-differentiation (Gautheret, 1966) and cellular interaction (Street, 1976) leading to the formation of meristemoid cell cluster or embryo. However, not all the cells are competent to respond to morphological signals and a major effort is still needed to understand the blocks.

Thus the proper empirical selection of the nutrient medium and the cultural conditions allow component cells to demonstrate their intrinsic capacity for organized development, which is ultimately a reflection of selected gene activity.

Advantages of micropropagated plants:

Certain morphological and physiological characteristics of tissue cultured plants make them superior to conventionally propagated materials. Tissue cultured plants particularly *Amelanchier, Rhododendron* and *Betula* tend towards a more branched habit. Micropropagated plants produce cuttings to have increased rooting, when rooted by conventional means. The most obvious advantage inherent to micropropagation is that more plants are produced quicker from a limited number of stock plants. Thus micropropagation is rapidly becoming a standard method in the nursery crops industry, much as it has in the foliage plants industry. The comprehensive list of ornamental species which are propagated by *in vitro* methods has been reviewed by Govil and Gupta (1997). Moreover, during the last few years, extensive work has been carried out on *in vitro* propagation of various ornamentals (Table-1.2 and 1.3).

Table-1.2: Various Tissue Culture Reports on Herbaceous Ornamentals

PLANT	EXPLANT AND REGENERATION	REFERENCE
Agave fourcroydes Lem.	Bulbs	Robert et al (1987); Binh et al, (1990)
Amaryllis sp.	Bulb	Prasad & Chaturvedi (1993)
Amaranthus sp.	Nodal explants	Flores & Teutonico (1986)
Anagallis arvensis L.	Adventitios buds and embryos	Bajaj & Mader (1974)
Antirrhinum majos L.	Embryos	Poirer – Hamon et al (1974)
Anthurium scherzerianum	Leaf explant	Geier (1988)
Arabidopsis thaliana(L.) Heynh	Embryos & plantlets	Corcos (1973)
Aranda wendy Scott.	Apical axillary buds	Cheah & Sagawa (1978)
Aranda "Deborah"	Stem explants	Lakshmanan (1995)
Aranthera James storei	Apical Axillary buds	Cheah & Sagawa (1978)
Asclepiias curassavica L.	Embryos	Prabhdesai & Narayanswamy (1973)
Asparagus officinalis	Anther, protoplast	Pelletier et al (1972); Raquin (1973); Hondelmann & Wilberg (1073); Mukhopadhyay & Desjardins (1994)
Begonia rex Putz.	Shoot buds & roots	Chlyah (1972)
Begonia X cheimantha	Plantlets	Fonnesbech (1974)
Blandfordia grandiflora	Shoot tips	Kristina et al (1991)
Carnation	Nodal explants	Van Altvorst (1995); Van Altvorst et al (1994)
Chrysanthemum morifolium	Petal segments & petal epidermis	Susan et al (1976); Roset & Bokelmann (1975); Bhattacharya et al (1990); Singh et al (1996)
Chrysanthemum sp.	Shoot tip	Earle & Langhans (1974); Knehra et al (1995)
Codiaeum variegatum Blume.	Shoot buds	Chikkannaiah & Gayatri (1974)
Consolida orientalis (Gay) Schrod	Shoot buds	Nataraja (1971)
Cordyline terminalis (L.) Kunth	Stem explants	Kuniąsaki (1975)
Dianthus caryophyllus L.	Stem & petal explants	Nugent et al (1991)
Dianthus sp.	Shoot buds & plantlets	Peter & Handa (1974); Hackett & Anderson (1967)

Episcia cupreata	Stem explants	Johnson (1978)
Eschscholtzia californica Champ.	Embryos	Kavathekar & Ganapathy (1973)
Euphorbia pulcherrima Willd.	Shoot buds	Nataraja et al (1973); De Langhe et al (1974); Nataraja (1975)
Gentiana trifolia	Single nodal explants	Zhang & Leung (2000)
Gerbera jamesonii Bolus.	Shoot buds & plantlets	Pierik et al (1973); Murashige et al (1974)
Gladiolus grandiflorus Hort.	Cormel tips	Remotti & Loffler (1995)
Gladiolus sp.	Cormel tips	Ziv et al (1970); Dantu et al (1987); Arora et al (1996); Stefaniak (1994)
Helianthus annuus L.	Shoot buds	Sadhu (1974)
Hydrangea quercifolia	Leaf callus	Sebastian & Heuser (1987)
Kalanchoe pinnata Pers.	Shoot buds, embryos & plantlets	Wadhi & Mohan Ram (1965)
Lachenalia sp.	Axillary Buds	Ault (1995)
Lachenalia sp.	Leaf explants	Niederwieser (1990); Niederwieser (1992)
Larix occidentalis Nutt.	Shoot buds & stems	Chesik et al (1990)
Lilium longiflorum	Anther	Sharp et al (1971)
Lily	Nodal segments, Shoot apices and Flower stalks	Grewal et al (1996)
Lunaria annua L.	Shoot buds	Pierik (1967)
Macleaya cordata R.Br.	Embryos	Kohlenbach (1965)
Mazus pumilus Loor.	Shoot buds	Raste (1971)
Mesembryanthemum floribundum Haw.	Shoot buds and Embryos	Mehra & Mehra (1972)
Montdretia crocosmaeflora Lemoine	Shoot buds	Matsuzawa & Sato (1972)
Nerine	Inflorescence	Ziv et al (1994)
Origanum vulgare L.	Cotyledons, Hypocotyle and Root segments	Kumari & Saradhi (1992)
Paronychia chartacea	Seeds	Mckently & Adams (1994)
Passiflora coerulia L.	Embryos and Shoot buds	Raman and Greyson (1974); Banerjee & Gupta (1975)
Pelargonium graveolens	Leaf segments	Rao (1994)

Pelargonium hortorum	Shoot buds and Plantlets	Chen & Galston (1967); Pillai & Hildebrandt (1967); Marsolias et al (1991)
Phalaenopsis sp. (Orchid)	Shoot tips of flower stalk buds	Tokuhara & Masahiro (1993)
Pitunia hybrida	Anther	Raquin & Pilet (1972); Daykin et al (1976)
Renanthera Imschootiana	Florets	Seeni & Latha (1992)
Rhododendron	Floral buds	Shevade & Preece (1993)
Rhododendron hybrids	Leaf explants	Preece & Imel (1991)
Rhododendron prinophyllum	Ovary	Dai et al (1987)
Rhynocostylis retusa B I.	Leaf segments	Viz et al (1984)
Saintpaulia ionantha Wandt.	Shoot buds and Plantlets	Kukulczanka & Suszinska (1972)
Sego Lily	Bulbs	Hou et al (1997)
Salpiglossis sinuate L.	Shoot buds and Plantlets	Hughes et al (1974)
Spathyphyllum	Nodal segments	Werbrouck et al, (1996)
Vanda coerulea Griff ex. Lindl.	Shoot tip and Leaf base	Seeni & Latha (2000)
Yucca aloifolia	Shoot tips	Atta-Alla & Staden (1997)
Yucca glauca	Nodal explants	Bentz et al (1988)

Table-1.3: Various Tissue Culture Reports on Shrubby Ornamentals

PLANT	EXPLANT AND REGENERATION	REFERENCES
Arbutus xalpensis H.B.K	Shoot tips	Mackay (1996)
Bougainvillea glabra Magnifica	Shoot apex	Sharma et al (1981)
Bougainvillea spectabilis	Axillary node	Misra et al (1997)
Cercis cnadensis	Cotyledonary node	Yusnita et al (1990); Distabanjong & Geneve (1997)
Eriostemon myoporoides	Axillary nodes	Ault (1994)
Frazinus angustifolia	Seeds	Perez-Parron et al(1994)
Ficus benjamina	Axillary buds	Del-Amo & Picazo (1992)
Gardenia jasminoides Ellis.	Shoot tips and Axillary buds	George et al (1993)
Garrya elliptica Doughl.	Shoot tips	Woodword & Thomson (1996)
Genista monosperma Lam.	Axillary nodes	Ruffoni et al (1999)
Hedera canariensis L.	Axillary nodes	Al-Juboory et al (1991)
Jasminum officinale L.	Nodal explants	Bhattacharya & Bhattacharya (1997)
Kalmia latifolia	Shoot tip	Lloyd & McCown (1981)
Leontochir ovallei	Seeds	Chunsheng Lu et al (1995)
Lingonberry	Shoot tip, nodal buds	Hosier et al (1985)
Lonicera sp.	Axillary buds	Karhu (1997)
Lonicera japonica	Callus	Georges et al (1993)
Mussaenda Dona Luz	Shoot tips	Cramer & Bridgen (1997)
Mussaenda erythrophylla	Sumatic embryos, callus cultures	Panda et al (1989); Das et al (1993)
Mussaenda lueteola	Axillary nodes	Jasrai et al (1999)
Photinia fraseri	Apical buds	Ramirez-Malagon et al (1997)
Prunnus mume	Axillary nodes	Harada & Murai (1996)
Rosa hybrida	Shoot tips and Lateral buds	Hasegawa (1979)
Rosa hybrida	Leaf explants	De Wit et al (1990)
Rosa hybrida	Leaf and Stem segments	Rout et al (1991)
Viburnum tinus	Apical shoot tips and single node explants	Nobre et al (2000)

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 propagation the above ornamental plants were selected because of their high ornamental and commercial value. Conventionally these plants are propagated through seeds and stem cuttings but there are certain problems, which makes the conventional method laborious and expensive. They are

- viability of seeds is short spanned
- the germination frequency of seed is very low in the field
- the seedling growth is very slow
- the seedling growth is season bound
- cuttings are not efficient under various climate conditions.

Therefore studies have been undertaken in these plants with the following objective:

To standardize protocols for micropropagation studies for large scale *in vitro* multiplication.