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Research review paper

Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects

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Abstract

Recent modern techniques of propagation have been developed which could help growers to meet the demand of the horticultural industry in the next century. An overview on the *in vitro* propagation via thin cell layer, meristem culture, regeneration via organogenesis and somatic embryogenesis is presented. Available methods for the transfer of genes could significantly simplify the breeding procedures and overcome some of the agronomic and environmental problems, which other wise would not be achievable through conventional propagation methods. The development and remarkable achievements with biotechnology in ornamental pot plants made during the three decades have been reviewed. The usefulness of the pot plants in commercial industry as well as propagation techniques, screening for various useful characteristics and selection of somaclonal variation is also discussed.

Keywords: Biotechnology; Genetic transformation; *In vitro* culture; Ornamental plants; Plant propagation

Contents

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Nomenclature

BA	6-benzylaminopurine
2ip	6-(γ,γ-dimethylallylamine)purine
2,4-D	2,4-dichlophenoxyacetic acid
Kn	kinetin
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
NAA	1-naphthaleneacetic acid
MS medium	Murashige and Skoog (1962)medium
PVP	polyvinylpyrrolidone
TDZ	thidiazuron
TCL	thin cell layer

1. Introduction

The commercial production of ornamental plants is growing worldwide. Its monetary value has significantly increased over the last two decades and there is a great potential for continued further growth in both domestic and international markets (Jain, 2002). Major pot plants such as *Begonia*, *Ficus*, *Anthurium*, *Chrysanthemum*, *Rosa*, *Saintpaulia*, and *Spathiphyllum* are being produced in the developed countries (Anonymous, 2003). About 212.5 million plants including 157 million ornamental plants amounting to 78% of the total production were reported (Pierik, 1991a,b). The Netherlands dominates export of ornamental plants including pot plants like *Begonia*, *Ficus*, *Cyclamen*, *Philodendron*, *Saintpaulia*, *Spathiphyllum* and *Rhododendron* (O'Riordain, 1999; Anonymous, 2003). About 156 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide. The shares of major producers are The Netherlands (33%), Japan (24%), Italy (11%), USA (12%), Thailand (10%) and others (14%). The major exporting countries are The Netherlands (59%), Colombia (10%), Italy (16%), Israel (4%), Spain (2%), Kenya (1%) and others (18%). The four leading exporters (The Netherlands, Colombia, Italy and Israel) constitute about 80% of the world market. The share of the developing countries of Africa, Asia and Latin America is less than 20% (Rajagopalan, 2000; Schiva, 2000) Planting material of ornamental plants is in great demand for commercial production as well as for domestic gardens and landscaping. The better quality planting material is a basic need of growers for boosting productivity. Chebet et al. (2003) reported the use of biotechnological approaches to improve horticultural crop production. The present review emphasizes the application of biotechnology on *in vitro* manipulation and propagation of ornamental pot plants.

2. *In vitro* propagation

In vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plant cells, a concept proposed by Haberlandt (1902) and unequivocally demonstrated, for the first time, by Steward et al. (1958). Tissue culture is alternatively called cell, tissue and organ culture through *in vitro* condition (Debergh and Read, 1991). It can be employed for large-scale propagation of disease free clones and gene pool conservation. Ornamental industry has applied immensely *in vitro* propagation approach for large-scale plant multiplication of elite superior varieties. As a result, hundreds of plant tissue culture laboratories have come up worldwide, especially in the developing countries due to cheap labour costs. However, micropropagation technology is more costly than conventional propagation methods, and unit cost per plant becomes unaffordable compelling to adopt strategies to cut down the production cost for lowering the cost per plant (IAEA-TECDOC-1384, 2004).

2.1. Micropropagation via meristem culture or axillary bud/shoot tip culture

In vitro propagation through meristem culture is the best possible means of virus elimination and produces a large numbers of plants in a short span of time. It is a powerful tool for large-scale propagation of horticultural crops including pot plants. The term 'meristem culture' specifically means that a meristem with no leaf primordia or at most 1–2 leaf primordial which are excised and cultured. The pathway of regeneration undergoes several steps. Starting with an isolated explant, with de-differentiation followed by re-differentiation and organization into meristematic centres. Upon further induction the cells can form unipolar structures i.e. organogenesis, or bipolar structures called somatic embryogenesis. The organization into morphogenetic patterns can take place directly on the isolated explant or can be expressed only after callus formation, which is called indirect morphogenesis. When shoots are developed directly from leaf or stem explants it refers to direct morphogenesis. Micropropagation is an alternative method of vegetative propagation, which is well suited for the multiplication of elite clones. It is accomplished by several means, i.e., multiplication of shoots from different explants such as shoot tips or axillary buds or direct formation of adventitious shoots or somatic embryos from tissues, organs or zygotic embryos. The first significant use of plant tissue culture in ornamental was made during 1920s when orchid seeds were germinated under laboratory conditions (Knudson, 1922). Micropropagation generally involves four distinct stages: initiation of cultures, shoot multiplication, rooting of *in vitro* grown shoots, and acclimatization. The first stage: culture initiation depends on explant type or the physiological stage of the donor plant at the time of excision. Explants from actively growing shoots are generally used for mass scale multiplication. The second stage: shoot multiplication is crucial and achieved by using Plant Growth Regulators i.e. auxin and cytokinin. The third stage: the elongated shoots, derived from the multiplication stage, are subsequently rooted either *ex vitro* or *in vitro*. In some cases, the highest root induction occurs from excised shoots in the liquid medium when compared with semi-solid medium. The fourth stage: acclimatization of *in vitro* grown plants is an important step in micropropagation.

In vitro plants are exposed to invariably controlled growth conditions such as high amount of organic and inorganic nutrients, Plant Growth Regulators, carbon source, high humidity, low light and poor gaseous exchange. Although they may support rapid growth and multiplication, the controlled conditions induce structural and physiological changes in plants rendering them unfit to survive when transferred directly to the field. Thus, a gradual acclimatization from laboratory to field condition is necessary. The plants are gradually shifted from high humidity/low irradiance conditions to low humidity/high irradiance conditions, enabling them to survive under 'adverse' climatic conditions. Carbon dioxide enrichment in the greenhouse for the cultivation of ornamental plants has a positive impact on production. Increased CO_2 concentration also lessens water stress of microcuttings by closing the stomata as reported by Matysiak and Nowak (1995). In CO_2 enriched atmosphere (1200 μl/l) of the greenhouse and the highest level of electrical conductivity (EC = 2.8 mS cm) of the medium produced the best growth of gerbera microcuttings taken from *in vitro* plants (Matysiak and Nowak, 2001). Photoautotropic micropropagation of ornamental plants have been reviewed (Kozai et al., 1988; Kozai, 1990a,b), and is suggested to use for reducing production costs, and automation to use robots for micropropagation process (Kozai et al., 1988; Kozai, 1991a,b).

Many commercial ornamental plants are being propagated by *in vitro* culture on the culture medium containing auxins and cytokinins (Preil, 2003; Rout and Jain, 2004). Several different explants have been used for direct shoot formation. Mayer (1956) succeeded first time regeneration of *Cyclamen* shoots from tuber segments on MS medium supplemented with 10.7 μM NAA.

Furthermore, plants have been regenerated from leaf tissues and petiole segments of *Cyclamen* (Geier, 1977; Geier et al., 1983; Schwenkel, 1991; Dillen et al., 1996), *Heuchera sanguinea* (Hosoki and Kajino, 2003), and *Begonia* (Takayama, 1983). *In vitro* clonal propagation of *Dracaena deremensis* has been reported by several groups (Debergh, 1975, 1976; Miller and Murashige, 1976; Chua et al., 1981). The first report on shoot multiplication and rooting of rose (*Rosa multiflora*) was made by Elliott (1970) by using shoot tip explants and later on followed by others (Hasegawa, 1979; Skirvin and Chu, 1979; Rout et al., 1989). AboEl-Nil (1983) reviewed on the large-scale production of *Pelargonium* by using different explants. Atta-Alla et al. (1998) reported the shoot bud regeneration from leaf and petiole explants of *Anthurium parvispathum* and subsequently establishment in soil. Martin et al. (2003) succeeded in direct shoot bud regeneration from lamina explants of *Anthurium andraeanum* on MS medium fortified with 1.11 μM BA, 1.14 μM IAA and 0.46 μM Kn. Furthermore, the regenerated shoots were rooted on half-strength MS medium supplemented with 0.54 μM NAA and 0.93 μM Kn. Nearly 300 plantlets of each cultivar were transferred to soil with 95% survival rate (Joseph et al., 2003). Thao et al. (2003) achieved shoots regenerated from petiole-derived callus of *Alocasia micholitziana* "Green velvel" on MS medium fortified with 0.5 μM Kn and 0.5 μM 2,4-D. The regenerated shoots were rooted on hormone free MS medium and subsequently established in the field. Skirvin et al. (1990) reported rapid method of shoot multiplication and rooting of *Rosa hybrida* cultivars. Now shoot tip explant is being routinely used for the micropropagation of ornamental plants including *Rhododendron* (Ettinger and Preece, 1985; McCown and Lloyd, 1983; Brand and Kiyamoto, 1994a,b), *Zantedeschia albomaculata* (Chang et al., 2003) and *Ebenus cretica* (Hatzilazarou et al., 2001). Later on Brand and Kiyomoto (1997) accomplished shoot multiplication of *Rhododendron* "Montego" on woody plant medium (Lloyd and McCown, 1980) supplemented with 10–50 μM 2ip. The number of shoots increased with subsequent subcultures on the fresh culture medium. Micro-shoots were rooted in moist *sphagnum* moss and vermiculite (3 : 1 ratio), and 90% microshoots survived and grown in the greenhouse. Several researchers have reported on clonal propagation of *Spathiphyllum* (Fonnesbech and Fonnesbech, 1979; Orlikowska et al., 1995; Wated et al., 1997).

Cytokinin alone in the culture medium induces shoot formation in many plants. MS medium supplemented with 3.0 mg/l BA was suitable for micropropagation of *Ficus benjamina* vars. Natasja and Starlight (Rzepka-Plevnes and Kurek, 2001). Jain (1997) micropropagated *Saintpaulia ionantha* by culturing leaf disks on MS medium containing 0.22–0.50 μM BA. Addition of auxins with cytokinins becomes essential for shoot induction and multiplication depending on the plant type. In *Petunia hybrida*, mass shoot multiplication was achieved on MS medium amended with 2.2 μM BA and 5.7 μM IAA within 4 weeks of culture (Sharma and Mitra, 1976). High concentration of cytokinins is unsuitable for shoot formation from leaf or petiole explants in some ornamental pot plants. Takayama and Misawa (1981, 1982) used 1.3 μM BA or 4.6 μM Kn in combination with 5.4 μM NAA for shoot bud regeneration from leaf, petiole or inflorescence segments of *Begonia* species. Further, low concentration of cytokinins also influences high rate of shoot bud regeneration (Reuter and Bhandari, 1981; Bigot, 1981a,b; Roest et al., 1981; Mikkelson and Sink, 1978a,b; Welander, 1977, 1979, 1981; Simmonds, 1984; Appelgren, 1976, 1985). The addition of 1–2 g/l activated charcoal in the culture medium increased the rooting efficiency from excised shoots of *Begonia* × *hiemalis* (Bigot, 1981a,b). Activated charcoal seems to adsorb Plant Growth Regulators, prompting to better response for rooting and even for shoot formation. It seems *Begonia* has high endogenous cytokinin and auxins, and by adding activated charcoal in the medium certainly promotes organogenesis. Jain (1997) used two cytokinins (Kn and zeatin) for regeneration of plantlets of *Begonia* × *elatior*. He suggested that two cytokinins did not affect the basic plant characteristics including flower colour. Similarly, many reports indicated mass multiplication of *Ficus* species by adding cytokinins in the culture medium (Gabryszewska and Rudnicki, 1997; Debergh and DeWael, 1997; Nobre and Romano, 1998). Demiralay et al. (1998) achieved shoot multiplication of *Ficus carica* var. Bursa Siyaki on MS medium containing 1.0 mg/l BA and 89 mg/l phloroglucinol; shoot multiplication rate was 4.43 shoots/explant; and 68.33% rooting rate of the micropropagated shoots on rooting medium containing 1 mg/l IBA. The rooting efficiency enhanced by addition of 0.05% PVP in the culture medium containing 2.5 μM IBA (Nobre and Romano, 1998). The addition of PVP helps in oxidising polyphenols leached in the medium, and promotes high rate of organogenesis.

The quality of light also influences shoot induction. Gabryszewska and Rudnicki (1997) developed a micropropagation protocol for *F. benjamina* by using shoot meristems; shoot numbers increased on MS medium supplemented with 15 mg/l 2ip by red light treatment; and root initiation occurred in all light treatments (white, blue, green and red). However, the

rooting and number of roots/shoot were highest in red light on the medium having 0.5 mg/l IAA.

Liquid medium seems to be more effective for shoot regeneration and root induction, which is due to better aeration. Simmonds and Werry (1987) used liquid medium for enhancing the micropropagation profile of *Begonia*×*hiemalis*. Wated et al. (1997) compared performance of agar-solidified medium and interfacial membrane rafts floating on liquid medium for shoot multiplication and root induction. The results showed shoot multiplication was highest on membrane rafts floating on the liquid medium, and also plants rooted much better. Similarly, Osternack et al. (1999) succeeded in inducing somatic embryogenesis and adventitious shoots and roots from hypocotyl tissues of *Euphorbia pulcherrima* on cytokinin containing medium. Subsequently, Preil (2003) noted that the regeneration potential of isolated cells, tissue or organs and the callus cultures is highly variable. Furthermore, petiole cross sections cultivated on auxin and cytokinin containing medium give rise to adventitious shoots from epidermal cells and subepidermal cortex cells, never from pith cells of the central regions of the petiole.

The direct shoot bud formation without any callus phase from appropriate explants is of great success for large-scale clonal multiplication of desired clone all round the year to boost the commercial floriculture. The micropropagation of major ornamental pot plants are presented in Table 1.

2.2. Micropropagation via somatic embryogenesis

Somatic embryos, which are bipolar structures, arise from individual cells and have no vascular connection with the maternal tissue of the explant (Haccius, 1978). Embryos may develop directly from somatic cells (direct embryogenesis) or development of recognizable embryogenic structures is preceded by numerous, organized, non-embryogenic mitotic cycles (indirect embryogenesis). Somatic embryogenesis has a great potential for clonal multiplication. Under controlled environmental conditions, somatic embryos germinate readily, similar to their seedling counterpart. The commercial application of somatic embryogenesis will be accomplished only when the germination rate of somatic embryos is high up to 80–85%.

Considerable success has been achieved in inducing somatic embryogenesis in ornamental pot plants like chrysanthemum (*Dendrathema grandiflorum*) (May and Trigiano, 1991; Tanaka et al., 2000), *Cyclamen persicum* (Wicart et al., 1984; Pueschel et al., 2003), rose (*R. hybrida*) (Rout et al., 1991, Kim et al., 2003a), *Begonia gracilis* (Castillo and Smith, 1997), *S. ionantha* cv. Benjamin (Murch et al., 2003), and *E. pulcherrima* (Osternack et al., 1999). In chrysanthemum, somatic embryos were produced from leaf mid-rib explants on modified MS medium supplemented with 1.0 mg/l 2,4-D and 0.2 mg/l BA (May and Trigiano, 1991). Highest somatic embryos were produced on the medium containing 6–8% sucrose and kept in the darkness for first 28 days, followed by 10 days in the light. Twelve cultivars produced somatic embryos, but complete plantlets were recovered from only five cultivars. Castillo and Smith (1997) induced direct somatic embryogenesis from petiole and leaf blade explants of *B. gracilis* on MS medium supplemented with 0.5 mg/l kinetin and 2% (v/v) coconut water. Somatic embryos were obtained with greater frequency from petiole explants than from leaf blade sections. Osternack et al. (1999) succeeded in achieving somatic embryos from hypocotyl tissues of *E. pulcherrima* on MS medium supplemented with 2.0 mg/l IAA (Fig. 1). About 1400 embryos were developed from 320 calli derived from outer regions of the hypocotyls. However, only 8% developed normal plantlets. In most cases, shoots were rooted in hormone free medium. Both orientation of the petiole explants and auxin transport system are crucial factors for the induction of somatic embryogenesis of *Saintpaulia* (Murch et al., 2003), and TDZ helped in the development of somatic embryos. Winkelmann et al. (1998) used cell suspension culture of *Cyclamen* for rapid development of somatic embryos, and later on followed by Hohe et al. (2001), who developed a large-scale propagation system of *Cyclamen* from embryogenic cell suspension cultures. Bouman et al. (2001) reported that the efficiency of embryogenic callus of *Cyclamen* seems to be stable for more than 5 years; however, suspension cultures can lose embryogenic potential after a number of subcultures. Therefore, it is necessary to determine the number of subcultures before embryogenic cell suspensions lose their potential of embryogenic nature. Pueschel et al. (2003) succeeded in plant regeneration via somatic embryogenesis of *C. persicum* and maintained the regeneration ability for prolonged period.

There are advantages and disadvantages of somatic embryogenesis in large-scale plant multiplication (Jain, 2002). The major advantages are large-scale somatic embryo production in bioreactors, encapsulation, cryopreservation, genetic transformation and clonal propagation. The major limitations are genotypic dependence of somatic embryo production and poor germination rate. Somatic embryogenesis in major ornamental pot plants is presented in Table 2.

Table 1
Micropropagation of major ornamental pot plants

Species/Cultivars	Response	References
Alocasia micholitziana 'Green Velvet'	sbr, r	Thao et al. (2003)
Anthurium andraeanum	sbr, r, pt	Pierik et al., 1974; Pierik, 1976
Anthurium patulum	ads, r	Eapen and Rao (1985)
Anthurium scherzerianum (flamingo flower)	ms, r, pt	Liu and Xu (1992)
Anthurium spp.	ms, r, pt	Matsumoto and Kuehnle (1997)
Anthurium parvispathum	ms, r, pt	Atta-Alla et al. (1998)
Anthurium andraeanum cvs. Tinora Red, Senator	ads, r, pt	Martin et al. (2003)
Begonia × *elatior* cvs. Aphrodite Rose, Aphrodite Rose Pale, Nixe, Schwabenland Orange, Tacora	ads, r, pt	Bigot, 1981a,b
Begonia × *elatior* cvs. Aphrodite Rosa, Claudis Mayer, Mayers Rote, Mayers Rosa	ms, r, pt	Reuter and Bhandari (1981)
Begonia × *elatior* cvs. Krefeld Orange, Schwabenland Orange, Schwabenland Pink, Schwabenland Red	sbr, r, pt	Takayama and Misawa (1982)
Begonia × *hiemalis* cv. Schwabenland Red	sbr, r, pt	Simmonds (1984)
Begonia tuberhybrida	sbr, r	Peak and Cumming (1984)
Begonia × *elatior*	ms, r, pt	Jain (1997)
Dendranthema grandiflora cvs. Blue Bird, Montana, Meladion, Delaware	ms, r, pt	Wang and Ma (1978)
Dendranthema grandiflora cv. Super Yellow	ads, r, pt	Lazar and Cachita (1983)
Dendranthema hortorum cvs. Pink Camino, Super Yellow, Spider	sbr, r, pt	Gertsson and Andersson (1985)
Dendranthema grandiflora cvs. Winter westland, Yellow westland, Dark westland, Snowdon, Yellow Snowdon, Altis, Blanche	ms, r, pt	Ahmed (1986)
Dendranthema grandiflora	ms, r, pt	Kaul et al. (1990)
Chrysanthemum coccineum	sbr, r, pt	Fujii and Shimzu (1990)
Dendranthema grandiflora cv. Royal Purple	sbr, r, pt	Lu et al. (1990)
Dendranthema grandiflora	ads, r, pt	Bhattacharya et al. (1990)
Dendranthema maximum	ads, r, pt	Kumar and Kumar (1995)
Dendranthema grandiflora cv. Deep Pink	sbr, r, pt	Rout et al. (1996)
Dendranthema grandiflora	ms, r, pt	Mandal et al. (2000)
Dendranthema grandiflora	sbr, r, pt	Teixeira de Silva and Fukai (2003b)
Cyclamen persicum	ads, r, pt	Geier, 1977, 1978
Cyclamen persicum	ads, r, pt	Ando and Murasaki (1983)
Cyclamen persicum	ads, r, pt	Wainwright and Harwood (1985)
Cyclamen persicum	ads, r, pt	Hawkes and Wainwright (1987)
Dracaena deremensis cv. Warneckii	ms, r	Debergh (1975)
Dracaena marginata Tricolour	ms, r, pt	Chua et al. (1981)
Euphorbia pulcherrima	ads, r, pt	Langhe et al. (1974)
Euphorbia fulgans	ms, r, pt	Zhang et al. (1987)
Euphorbia pulcherrima cv. Angelika	ads, r, pt	Osternack et al. (1999)
Ficus lyrata	ms, r	Debergh and DeWael (1997)
Ficus religiosa	sbr, r	Narayan and Jaiswal (1986)
Ficus benjamina cv. Golden King	ms, r	Gabryszewska and Rudnicki (1997)
Ficus carica var. Bursa siyahi	ms, r, pt	Demiralay et al. (1998)
Ficus carica cvs. Berbera, Lampa	ms, r, pt	Nobre and Romano (1998)
Ficus religiosa	ms, r, pt	Deshpande et al. (1998)
Ficus religiosa	ms, r, pt	Nagaraju et al. (1998)
Ficus carica cv. Gular	ms, r, pt	Kumar et al. (1998)
Ficus benjamina cvs. Natasja, Starlight	ms, r, pt	Rzepka-Plevnes and Kurek (2001)
Petunia hybrida, *Petunia inflata*	sbr, r, pt	Rao et al. (1973)
Petunia hybrida	ms, r, pt	Sharma and Mitra (1976)
Pelargonium × *hortorum*	ms, r	Horst et al. (1976)
Pelargonium spp.	ms, r, pt	Debergh and Maene (1977)
Pelargonium spp.	ms, r, pt	Theiler (1977)
Pelargonium zonale hybrid	ms, r, pt	Jelaska and Jelencic (1980)
Rhododendron spp.	ms, r	Economou and Read (1984)
Rhododendron spp.	ms, r, pt	Anderson (1984)
Rhododendron spp.	ms, r, pt	Norton and Norton (1985)
Rhododendron P.J.M. hybrid	ms, r	Ettinger and Preece (1985)
Rhododendron '*Montego*'	ms, r, pt	Brand and Kiyomoto (1997)
Rosa hybrida cvs. Crimson Glory, Glenfiditch	ms, r, pt	Barve et al. (1984)
Rosa hybrida cv. Amanda	ms, r	DeVries and Dubois (1988)

Table 1 (*continued*)

Species/Cultivars	Response	References
Rosa hybrida cv. Bridal Pink	ads, r	Burger et al. (1990)
Rosa damascena	ads, r, pt	Ishiooka and Tanimoto (1990)
Rosa hybrida cvs. Landora, Virgo, Happiness, Sea Pearl, Super Star, Queen-Elizabeth	ms, r, pt	Rout et al. (1990)
Rosa hybrida cv. Landora	ads, r, pt	Rout et al. (1992)
Rosa chinensis var. minima cvs. Debut, Ginny	ms, r, pt	Rogers and Smith (1992)
Rosa chinensis var. minima (cvs. Baby Katie, Lavender Jewel, Red Sunblaze, Royal Sunblaze)	ms, r, pt	Chu et al. (1993)
Hybrid tea 'Dr. Verhage'	ms, r, pt	Voyiatzi et al. (1995)
Rosa multiflora	ads, r, pt	Rosu et al. (1995)
Rosa hybrida	ads, r, pt	Van der Salm et al. (1996)
Hybrid tea rose cv. Peace	ms, r, pt	Ara et al. (1997)
Saintpaulia ionantha	sbr, r, pt	Starts and Cummings, 1976; Grunewaldt, 1977; Vazquez et al., 1977
Saintpaulia ionantha	ms, r, pt	Molgaard et al. (1991)
Saintpaulia ionantha	sbr, r, pt	Hoshino et al. (1995)
Saintpaulia ionantha 2 confusa hybrids	sbr, r, pt	Lo et al. (1997)
Saintpaulia ionantha	ms, r, pt	Jain (1997)
Saintpaulia ionantha cvs. Benjamin, William	sbr, r, pt	Mithila et al. (2003)
Spathiphyllum cv. Clevelandii	ms, r, pt	Fonnesbech and Fonnesbech (1979)
Spathiphyllum	sbr, r, pt	Orlikowska et al. (1995)
Spathiphyllum floribundum cv. Petite	Sbr, r	Werbrouck and Debergh (1995)
Spathiphyllum 'Petite'	ms, r, pt	Wated et al. (1997)
Yucca aloifolia	ms, r, pt	Atta-Alla and Van Staden (1997)

Abbreviation: ads=adventitious shoot bud development, ms=multiple shoot, pt=plantlet formation, r=rooting, sbr=shoot bud regeneration.

2.3. Micropropagation via thin cell layer

Thin cell layer (TCL) is a simple but effective system that relies on a small size explant derived from a limited cell number of homogenous tissue. They are excised longitudinally or transversely from different organs ranging from floral parts to root/rhizome of plants. Longitudinal TCL (lTCL) (0.5–1 mm wide and 5–10 mm long) is used when a definite cell type (epidermal, sub-epidermal, cortical, cambial or medullar cell) is to be analysed. TCLs can be excised from stem, leaf, vein, floral stalk, petiole, pedicel, bulb-scale, etc. As for the transverse TCL (tTCL) (0.1–5 mm), other organs (leaf blade, root/rhizome, floral organs, meristems, stem node, etc.) can be used. The reduced cell number in TCL is important for the developmental process or the morphogenetic programme, which can be altered by making changes in organ/tissue and size to be uniformly exposed to the medium (Tran Thanh Van, 1980).

Thin cell layer is the model systems and find applications in higher plant tissue and organ culture and genetic transformation (Teixeira da Silva, 2003a, 2005). Moreover, thin cell layer technology is a solution to many of the issues currently hindering the efficient progress of ornamental and floricultural crop improvement, since it solves the initial step i.e. plant regeneration problem. This technology has also been effectively used in the micropropagation of various crops including floricultural crops (Tran Thanh Van and Bui, 2000; Fiore et al., 2002; Nhut et al., 2003a,b; Teixeira de Silva and Nhut, 2003a). Recently, Teixeira da Silva (2003a) published a detailed review on the use of thin cell layer technology in ornamental plant micropropagation and biotechnology, which highlights organogenesis and somatic embryogenesis for plant regeneration and genetic improvement via transformation. Mulin and Tran Thanh Van (1989) indicated that *in vitro* shoots and flowers were formed from thin epidermal cells excised from the first five internodes of basal flowering branches in *P. hybrida*. Explants (1×10 mm^2) consisting of 3–6 layers of subepidermal and epidermal cells produced vegetative buds within 2 weeks of culture. Ohki (1994) reported that 100–200 shoots per tTCL (transverse thin cell layer) explants were obtained from 0.3 to 0.5 mm petiole or 3×3 mm^2 lamina sections, respectively of *S. ionantha* within 4 weeks of culture. Over 70,000 plants were produced from a single leaf within 3–4 months. Gill et al. (1992) used tTCL hypocotyl explants (10 mm) of 1-week-old geranium (*Pelargonium*×*hortorum*) hybrid seedlings for induction of somatic embryogenesis. They observed that the development of somatic embryos was rapid and the number of embryos was about 8-fold higher than the culture of whole hypocotyl explants. Hsia and Korban (1996) achieved organogenic and embryogenic callus and subsequent regeneration from lTCL (longitudinally thin

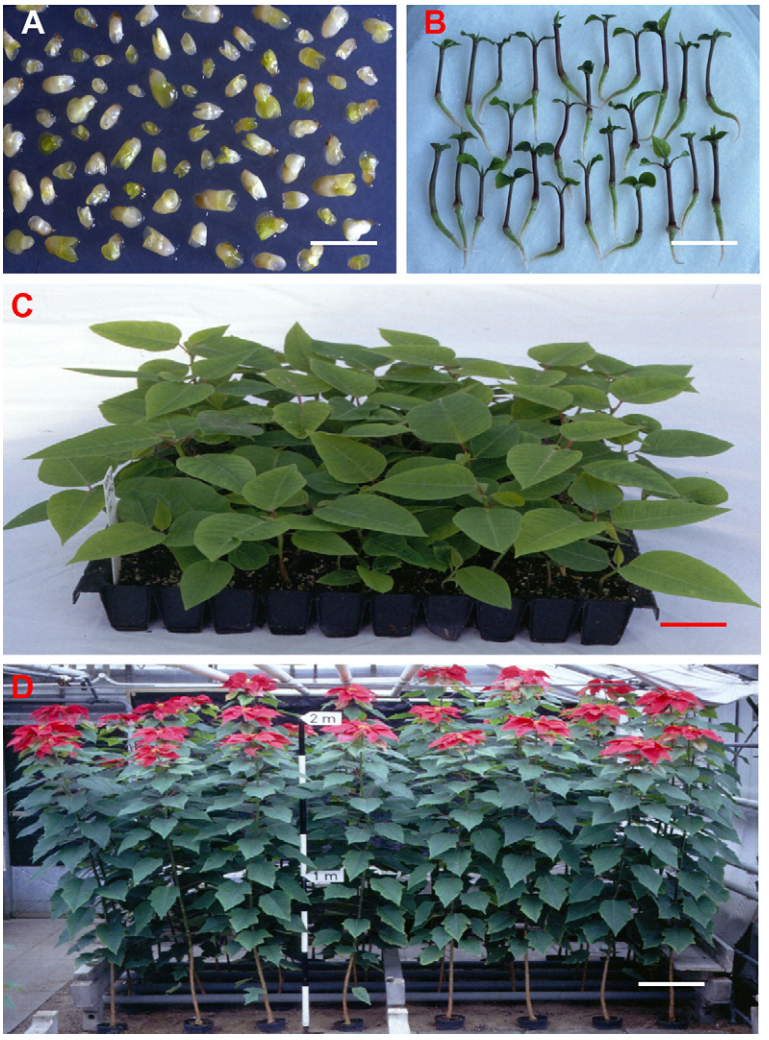

Fig. 1. *In vitro* somatic embryogenesis of *Euphorbia pulcherrima*. (A) Isolated somatic embryos of *E. pulcherrima* (bar=0.1 cm). (B) Germination of somatic embryos (bar=0.25 cm). (C) Somatic embryos derived plantlets acclimatised in the greenhouse (bar=0.5 cm). (D) Flowering of somatic embryo-derived plants (bar=25 cm).

cell layer) explants derived from dormant bud floral stalks of *R. hybrida* cv. Baccara.

Thin cell layer systems could be used as a tool for *in vitro* regeneration and micropropagation. The efficiency is very high compared to the conventional technique of tissue culture. The TCL method is also very useful in virus elimination in combination with antiviral compounds. Recent progress in thin cell layer technology has opened new possibilities for improvement of ornamental and floricultural crops.

2.4. Mechanization of in vitro plant propagation

The exploitation of *in vitro* methods for profitable plant micropropagation requires automation and scaling-up, which depend on the use of liquid cultures (Takayama and Misawa, 1981). The use of bioreactors is a step forward for commercial propagation of ornamental plants. Bioreactors with computer control systems offer various advantages over conventionally produced culture due to possibilities of automation, saving labour and production cost (Aitkens-Christie, 1991; Preil, 1991; Ziv, 1991, 1995; Paek et al., 2001; Eide et al., 2003). Since microbial fermentation techniques were first used in studies on growth kinetics of higher plant cell suspensions (Tulecke and Nickell, 1959), major progress has occurred in the area of large-scale liquid culture and in the development of bioreactor process control system. Since then bioreactor system was applied for meristem, embryogenic and organogenic

Table 2
In vitro somatic embryogenesis of major ornamental pot plants

Species/Cultivars	Culture response	References
Begonia gracilis	emc, gse, pt	Castillo and Smith (1997)
Dendranthema grandiflora cv. Yellow Spider	emc, gse, pt	Sauvadet et al. (1990)
Dendranthema grandiflora	emc, gse, pt	May and Trigiano (1991)
Dendranthema grandiflora cv. Yellow Spider	emc, gse, pt	Pavingerova et al. (1994)
Dendranthema grandiflora	emc, gse, pt	Tanaka et al. (2000)
Cyclamen persicum	ecs, gse, pt	Hohe et al., 2001; Schwenkel (2001)
Cyclamen persicum	emc, gse, pt	Pueschel et al. (2003)
Euphorbia pulcherrima cv. Angelika	emc, gse, pt	Osternack et al. (1999)
Rosa hybrida cvs. Domingo, Vickey Brown, Tanja, Azteca	emc, gse	de Wit et al. (1990)
Rosa hybrida cv. Landora	emc, gse	Rout et al. (1991)
Rosa rugosa	emc, gse, pt	Kunitake et al. (1993)
Rosa sp. cvs. Baccara, Mercedes, Ronto, Soray	emc, gse, pt	Kintzios et al. (1999)
Rosa hybrida, *Rosa chinensis minima*	emc, gse, pt	Li et al. (2002a)
R. hybrida cv. Sumpath	emc, gse, pt	Kim et al. (2003a)
Saintpaulia ionantha cvs. Benjamin, William	emc, gse, pt	Mithila et al. (2003)
Saintpaulia ionantha cv. Benjamin	emc, gse, pt	Murch et al. (2003)

Abbreviation: emc = embryogenic callus, ecs = embryogenic cell suspension, gse = germination of somatic embryos, pt = plantlet development.

cultures of several plant species (Levin et al., 1988; Preil et al., 1988; Takayama and Akita, 1994, 1998; Takayama, 2002; Eide et al., 2003). The various propagation aspects of several plant species in bioreactors, applications, and some of the problems associated with the operation of bioreactors have recently been reviewed (Takayama and Akita, 1998; Ziv, 2000; Paek et al., 2001). Liquid media have been used for plant cells, somatic embryos and cell suspension cells in either agitated flasks or various types of bioreactors (Smart and Fowler, 1984; Tautorus and Dunstan, 1995; Takayama, 2000; Ziv, 2000; Paek et al., 2001; Eide et al., 2003). Considerable attention has been given to automation of the repeated cutting, separation, subculture, and transfer of buds, shoots, or plantlets during the multiplication and transplanting phases (Levin et al., 1988; Aitkens-Christie, 1991; Vasil, 1994; Aitkens-Christie et al., 1995). Automation of tissue culture will depend on the use of liquid cultures in bioreactors, allow fast proliferation, mechanized cutting, separation, and automated dispensing (Sakamoto et al., 1995). These techniques were used in some plants, which involve minimal hand manipulation and thus reduce *in vitro* plant production costs (Levin et al., 1988; Ziv, 1991, 1992, 1995; Vasil, 1994; Aitkens-Christie et al., 1995; Curtis, 2002). Eide et al. (2003) reported two liquid culture systems for plant propagation i.e. temporary immersion systems and permanent submersion of the plant cells/tissue that requires oxygen supply through rotary shakers or bioreactors. Temporary immersion system, e.g. RITA bioreactor, seems to be better than the permanent submersion system for shoot proliferation.

However, Takayama et al. (1986) demonstrated vigorous growth of organogenic cultures of *Begonia* in a bioreactor. The oxygen partial pressure in bioreactors helps cell proliferation and subsequent differentiation of somatic embryos from suspension cultures of *C. persicum* (Hvoslof-Eide and Munster, 1998, 2001). A significant high number of germinating embryos were obtained from the cultures grown at 40% pO_2 than from those grown in flasks or in bioreactors at 5%, 10% and 20% pO_2 (Hohe et al., 1999). Kim et al. (2003b) established a large-scale propagation of chrysanthemum through bioreactor system, and obtained 5000 plantlets after 12 weeks of culture in 10 l column type bioreactor. They also found that the bioreactors maintained at 25 °C, 100 μmol/m^2/s PPF and 0.1 vvm air volume as optimal conditions for this propagation. Weber et al. (1994) reported the propagation efficiency of *Clematis tangutica* in a bioreactor. Preil (2003) established successfully eleven hybrid cultivars and a wild type of *C. tangutica* in a bioreactor (Fig. 2). This method resulted rapidly increased pro-embryogenic clusters up to 4500/ml. Later, some 200 globular embryos, 300 heart and torpedo-shaped embryos per ml were determined after 4 weeks of culture in auxin-free medium. About 500,000 cotyledonary embryos were obtained from 1 l cell suspension culture. Further, the clusters of embryos developed into plantlets differing in length. The plantlets were transferred to the greenhouse.

Somatic embryos and shoot cultures could be grown in both liquid systems, embryogenesis possibly being the most suited for full automation through a synthetic seed scheme. Adapting bioreactors with liquid media for

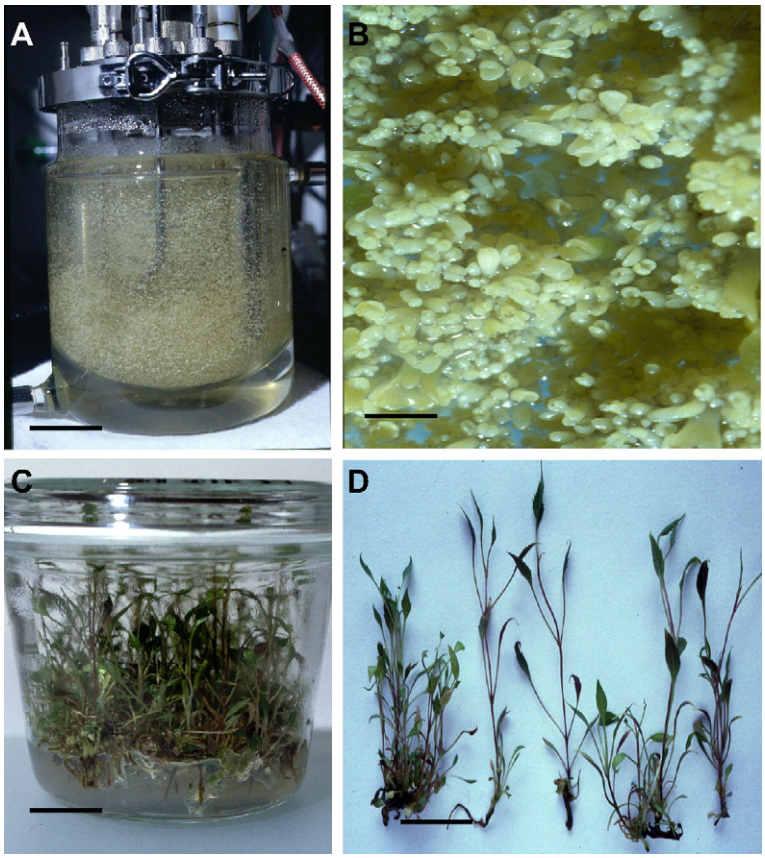

Fig. 2. Development of somatic embryos in liquid culture. (A) Somatic embryos of *Clematis tangutica* were developed in a liquid culture (bar=0.5 cm). (B) Somatic embryos of *C. tangutica* cultured in agar-gelled medium (bar=0.25 cm). (C) Germination of somatic embryos of *C. tangutica* in agal-gelled medium (bar=0.5 cm). (D) Cluster of somatic embryo derived plantlets developed in agar medium (bar=5 cm).

micropropagation is highly suitable due to the ease of scaling-up (Preil, 1991; Preil and Beck, 1991) and the ability to prevent the physiological disorders of shoot and leaf hyperhydricity (Ziv, 1999) and, thereby, lowering production costs. The major risk in using bioreactors for large-scale plant production is contamination.

3. Propagation of important pot plants

3.1. Begonias

Begonias are important perennial ornamental plants and distributed throughout tropical and subtropical regions of the world. It is used as potted as well as garden plants. Begonias are propagated by vegetative means i.e. stem cuttings and leaf cuttings. About 200 species have been introduced by commercial growers, and among them *Begonia tuberhybrida*, *Begonia*×*hiemalizs*, *Begonia*×*elatior*, *Begonia*×*cheimantha* and *Begonia*×*socotrana* are important species (Takayama, 1983). The conventional methods of propagation are problematic due to rapid occurrence of diseases. The production of large numbers of genetically homogenous plants is also very difficult. Plant cell culture technique is an alternative method for mass cloning of *Begonia* plants and also to overcome the problems occurring in the conventional propagation. Most of the researchers used petiole, leaf or inflorescence segments for mass propagation of *Begonia* species (Takayama and Misawa, 1981, 1982; Roest et al., 1981). Takayama and Misawa (1982) reported that the medium containing 1.3 μM BA or 4.6 μM Kn along with 5.4 μM NAA showed rapid regeneration of shoot buds from leaf and petiole segments. Reuter and Bhandari (1981) indicated that the combination of low concentration of cytokinin and auxin initiated rapid propagation of *Begonia* species. The better aeration of cultures in the liquid medium is beneficial for shoot formation while shaking on a shaker when compared with cultures growing on the solid

medium. Takayama and Misawa (1982) developed a liquid culture system with shaking which helped the buds to develop efficiently and quickly into plantlets. Also, reduction of growth hormones in the culture medium benefits shoot culture, which is done by adding activated charcoal in the culture medium. Bigot (1981a, b) reported that the addition of 1–2 g/l activated charcoal in the culture medium showed vigorous rooting from excised shoots of Begonias. Plant regeneration from leaf disk callus of *Begonia*×*elatior* was achieved on MS medium supplemented with 5.0 μM Kn and 0.5 μM zeatin (Jain, 1997). He also reported that about 84% of callus cultures showed shoot bud regeneration and rooting *in vitro*. Castillo and Smith (1997) reported the direct somatic embryogenesis in *B. gracilis* by using micro-cultured laminar segments and petioles. The rate of somatic embryogenesis induction was greater from petiole explants than from leaf blade sections on MS medium supplemented with 0.5 mg/l Kn and 2% (v/v) coconut water. The production of somatic embryos was significantly higher on responding laminar explants (60–70 embryos/leaf section) than on petioles (40–50 embryos/petioles). Subsequently, somatic embryos were germinated into plantlets (Castillo and Smith, 1997) and transferred to the field.

3.2. Chrysanthemum

Chrysanthemum (*Dendranthema grandiflora* syn. *Chrysanthemum morifolium* Ramat.) is extensively grown as a pot plant as well as a cut flower worldwide. It is vegetatively propagated with cuttings and suckers. Breeding programmes have focussed on improving various characteristics to enhance ornamental values, including flower colour, size and form, and production quality. Although desirable traits have been introduced by classical breeding, there are limitations to this technique. Firstly, there is a limited gene pool. Secondly, distant crosses may be limited by incompatibility or differences in ploidy level between mutant parents. Thirdly, characteristics such as uniform growth and synchronous flowering are polygenic. Hence sexual crossing may alter the delicate balance of factors determining plant growth and development. Plant biotechnology offers an opportunity to develop new germplasms and conservation. The techniques of stimulating axillary branching or culturing nodal sections *in vitro* are probably most commonly used in micropropagation (Lawrence, 1981). A number of factors have influenced the induction of morphogenesis in chrysanthemum. Rout and Das (1997) have reviewed at length the recent developments of chrysanthemum biotechnology. They emphasized on the application of *in vitro* culture for mass-scale propagation and also discussed the various possibilities for improvement of chrysanthemum by using modern biotechnological tools. Recently, Teixeira da Silva (2003b) published a detailed review on tissue culture of chrysanthemum, which highlights organogenesis, thin cell layer, and somatic embryogenesis for plant regeneration.

Prasad et al. (1983) reported that the rate of shoot multiplication is genotypic dependent in *D. grandiflora*. Datta et al. (2001) established a protocol using direct shoot regeneration system from ray florets of 28 genotypes. The regeneration frequency and average number of shoots per explant varied among the cultivars. Shoot tip size also plays an important role in shoot regeneration efficiency. Wang and Ma (1978) reported that shoot tip between 0.2 and 0.5 mm and shoot meristems between 0.1 and 0.2 mm diameter produced only a single shoot. Larger explant (0.5–1.55 mm diameter) formed multiple shoots. Mandal et al. (2000) used various explants for regeneration of *D. grandiflora* and regenerated new plants from mutated tissues. Liquid medium has also proven beneficial in root induction in several plants, especially in some recalcitrant plants for rooting, due to better aeration of cultures. Roest and Bokelmann (1975) successfully induced roots in the adventitious shoots of chrysanthemum in the liquid MS medium containing 1.0 mg/l IAA. In general, shoots and roots developed on a single medium containing 4.4 μM BA and 5.7 μM IAA. Rooting was achieved in 90% cultures of 'Deep Pink' rooted with about 2.0 klx (kilolux) of light, whereas higher light intensities (3.0 klx) gave a lower rooting percentage (Roberts et al., 1992; Rout et al., 1996). The rooted plants were successfully established in the soil (Rout et al., 1996; Roberts and Smith, 1990). Kim et al. (2003b) reported the propagation system and reduction of transplant production period. They reported that 5000 cuttings were obtained after 12 weeks of culture in 10-l column type bioreactor and subsequently transferred to the greenhouse with 100% survival. Belarmino and Gabon (1999) induced rapid multiplication of *D. grandiflora* on MS medium supplemented with 1.0 mg/l BA, 2.0 mg/l NAA and 10 mg/l gibberellic acid. Kumari et al. (2001) used cytokinins and auxins to scale up the multiplication efficiency of chrysanthemum. Hosokawa et al. (2004) developed a new method to regenerate chrysanthemum plants from leaf primordia-free shoot apical meristem domes (LP-free SAMs) by establishing the meristem dome on the cut surface of root tips of chrysanthemum or different plant species from the Compositae (cabbage). The highest shoot regeneration rate was observed with cabbage root tips.

Induction of somatic embryogenesis in chrysanthemum has been achieved by using leaf mid-rib explants (May and Trigiano, 1991), which depended on the photoperiod and sucrose concentration. The highest number of somatic embryos was produced on the medium containing 9–18% sucrose, in the darkness for first 28 days of culture, followed by 10 days in the light. Twelve of the 23 cultivars evaluated produced somatic embryos, but complete plantlets were recovered only from five cultivars. The regenerated plants were phenotypically similar to parent plants in growth habit, leaf morphology and flower colour. Pavingerova et al. (1994) reported somatic embryogenesis and plant regeneration from transform calli of *D. grandiflora*. Tanaka et al. (2000) achieved the induction of somatic embryogenesis and plant regeneration in chrysanthemum from ray-floret explants by using IAA and kinetin. The somatic embryo derived plantlets were established in the greenhouse. The genotypic dependence remains the major limitation on the use of somatic embryogenesis in chrysanthemum.

3.3. Cyclamen

Cyclamen belongs to the family Primulaceae, and is grown as a pot plant. It is widely growing in Europe and very popular in Germany. In addition to pot plants, tubers are produced as planting material and have commercial importance. It is distributed in the Mediterranean region and areas adjoining to the North and to the East. *Cyclamen* is propagated exclusively through seeds. It is cross-pollinated, and many cultivars are autotetraploid. Since repeated self-fertilization leads to inbreeding depression, the traditional cultivars are maintained by crossing selected plants of similar appearance. As a result, uniformity is poor. Hence, *in vitro* clonal propagation of *Cyclamen* has been widely studied as an alternate method for mass scale production of high quality planting material. *In vitro* clonal propagation of *Cyclamen* has been very well worked out. Mayer (1956) first used tuber segments on MS medium supplemented with 1.1 μM NAA for shoot formation. Subsequently, Okumoto and Takabayashi (1969) and Pierik (1975) achieved shoot bud regeneration from tuber explants. Geier (1977) obtained shoot and root formation on the medium containing 14.3–28.6 μM IAA and 0.9–2.3 μM Kn. He also compared types of explant on plant regeneration and observed less morphogenetic potential in other plant parts as compared to tuber tissue. *In vitro* cloning of *C. persicum* through organogenesis has been reported by different researchers (Geier, 1978; Geier et al., 1983; Schwenkel, 1991; Dillen et al., 1996).

Hoffmann and Preil (1987) established shoot bud regeneration protocol in 13 genotypes of *Cyclamen* and subsequently rooting. Similar genotypic-specific differences in shoot bud regeneration from peduncle explants were observed by Schwenkel and Grunewaldt (1988). Winkelmann et al. (1998) produced 90,000 plantlets from 1 l of embryogenic cell suspension culture. Subsequently, Hohe et al. (2001) and Schwenkel (2001) also reported clonal propagation of *C. persicum* by using embryogenic cell suspension culture. Pueschel et al. (2003) highlighted the mass-scale propagation of *C. persicum* via somatic embryogenesis.

3.4. Ficus spp.

Genus *Ficus* has more than 800 species, and are used as foliage plants including *Ficus altissima*, *Ficus benjamina*, *Ficus binnedijkii*, *Ficus elastica*, *Ficus microcarpa*, *Ficus pumila*, *Ficus retusa* and *Ficus rubiginosa*. *Ficus* is one of the most popular indoor plants. It is native to India, Southeast Asia and Northern Australia. It is propagated either by air layering or rooting by stem cutting. Some varieties have appealing aesthetic appearances, and their performance is of high quality under interior low light conditions (Chen et al., 2001). The propagation however, is slow and limited. Hence, *in vitro* micropropagation of *Ficus* species has been widely studied as an alternate method for mass-scale production of high quality planting material. Debergh and DeWael (1997) reported micropropagation of *Ficus lyrata*. Subsequently, Dijkshoorn-Dekker (1996) studied the influence of light and temperature on propagation profile of *F. benjamina*. Propagation of different *Ficus* species by using shoot tips or axillary bud explants has been reported (Deshpande et al., 1998; Kumar et al., 1998; Demiralay et al., 1998; Nobre and Romano, 1998; Nagaraju et al., 1998). Deshpande et al. (1998) induced multiple shoots from nodal explants of 35-year-old tree of *Ficus religiosa* on MS medium supplemented with 5.0 mg/l BA and 0.2 mg/l IBA, and obtained multiple shoots as well as rooting on MS medium containing 1.5 mg/l BA and 1.5 mg/l Ads and 1/2 MS plus 2.0 mg/l IBA and 1.0 mg/l NAA, respectively. Kumar et al. (1998) established micropropagation protocol for *F. carica* cv. Gular by using apical buds from 8-year-old trees, and succeeded in getting multiple shoots and rooting in the liquid half-strength MS medium supplemented with 2.0 mg/l IAA and 0.2% activated charcoal. The micropropagated plantlets were successfully established (68%) in soil. Rzepka-Plevnes and Kurek (2001) regenerated multiple shoots from nodal explants of *F. benjamina* on MS

medium supplemented with 3.0 mg/l BA. The plantlets grown in the medium with cytokinins were generally shorter and developed shorter leaves as compared to the growth medium without cytokinin.

3.5. Rose

Rose is the most important cut flower as well as pot plant. Roses attribute to great variation in flower and plant characteristics and to their wide adaptability to varied agro-ecological conditions. The genetic resources of roses can be grouped into four categories: exotic varieties, indigenously evolved varieties, native rose species and exotic species. Being an important commercial flower plant, systematic investigations have been carried out for its propagation and improvement in production both in quality and in quantity during the last three decades. Budding or grafting is done for the propagation of roses. The breeding programmes are focused on the improvement of various characteristics to enhance the ornamental value, including the flower colour, size and keeping quality of the bloom and the response to various diseases. Although desirable traits were introduced by conventional breeding, there were limitations to this technique; firstly, because of the limited gene pool, secondly, distant crosses were limited by incompatibility or differences in ploidy level between putative parents and thirdly, characteristics such as uniform growth and synchronous flowering were polygenic. Plant tissue culture offers an opportunity to propagate roses in large scale.

In vitro mass multiplication of rose is successful by micropropagation (Skirvin and Chu, 1979; Hasegawa, 1979; Rout et al., 1989, 1990; Bressan et al., 1982; Arnold et al., 1995) and several reviews have been written (Skirvin et al., 1990; Short and Roberts, 1991; Horn, 1992; Rout et al., 1999; Pati et al., 2006). They have highlighted the role of growth regulators and physical factors on shoot multiplication and rooting of the different cultivars of hybrid roses and also illustrated the application of modern technology on improvement, conservation and documentation of roses. Skirvin and Chu (1979) and Hasegawa (1979) reported a rapid method for shoot multiplication and rooting of hybrid rose cultivars. Khosh-Khui and Sink (1982a,b) observed the rate of shoot multiplication of *R. hybrida*, *Rosa damascena* and *Rosa canina* varied significantly during different subculture periods. By reducing the sucrose concentration in the culture medium, the number of multiple shoots increased (Langford and Wainwright, 1987). Similarly, size of the meristem (both shoot tip and nodal explant) of floribunda and miniature roses had significant effect on shoot multiplication; on an average 2.5–5.0 shoots were obtained per culture cycle, dependent on cultivars (Douglas et al., 1989). The growth and multiplication of shoots increased by extending the culture period from 3 to 6 weeks (Chu et al., 1993). There are several factors affecting rose micropropagation, which are: agar concentration (Ghashghaie et al., 1991), ethylene concentration (Kevers et al., 1992), growth room and vessel humidity (Sallanon and Maziere, 1992) and different types of gelling agents (Podwyszynska and Olszewski, 1995). Kumar et al. (2001) developed an efficient protocol for micropropagation of *R. damascena* on MS medium supplemented with 1.0–2.5 μM TDZ. Pre-culture soaking in thidiazuron improved the axillary shoot proliferation in rose (Singh and Syamal, 2000). Carelli and Echeverrigaray (2002) developed an efficient protocol for propagation of hybrid roses by using MS medium amended with 3.0 mg/l BA and 0.5 mg/l NAA. The multiplication rate was 30.3 plantlets per explant after 180 days. The addition of silver nitrate along with BA and IAA promoted the growth of the axillary shoots (Chakrabarty et al., 2000). The microshoots were rooted on growth medium supplemented with low concentrations of auxins (0.1 to 0.5 mg/l) and reduced concentrations of sucrose (2–2.5%) (Khosh-Khui and Sink, 1982b,c; Rout et al., 1990; Arnold et al., 1995).

Somatic embryogenesis in rose has been successfully accomplished by using leaf, internode, filament of stamen, root and zygotic embryo (Rout et al., 1991; Roberts et al., 1995; de Wit et al., 1990; Kunitake et al., 1993). Rout et al. (1991) induced embryogenic calli and later on developed somatic embryos from 8-week-old callus, derived from immature leaf and stem segments of *R. hybrida* acv. Landora. Medium amended with 2,4-D helped in the long-term maintenance of embryogenic callus (Roberts et al., 1990; Matthews et al., 1991; Noriega and Sondahl, 1991). Kunitake et al. (1993) observed zygotic embryo-derived calli of *Rosa rugosa* has ability to develop somatic embryos on media without exogenous growth regulators although embryogenic potential did not persist after 6 months. Hsia and Korban (1996) reported low frequency rate of somatic embryogenesis from the rhizogenic callus of the cut rose. Noriega and Sondahl (1991) and Roberts et al. (1990) added ABA (abscisic acid) and GA_3 in the culture medium for the germination of somatic embryos. By adding L-proline in the primary culture medium followed by its removal from the regeneration medium, stimulated embryo development and reduced abnormalities (Rout et al., 1991). Furthermore, low temperature exposure (8 °C) exposure to embryogenic calli for

4 days enhanced the germination rates. Somatic embryo-derived plantlets were successfully established in the soil (Fig. 3). Roberts et al. (1995) gave chilling treatment at 4 °C for 2 weeks, which improved the germination rates from 12% to 24%. Kunitake et al. (1993) succeeded in germination of somatic embryos of *R. rugosa* into plantlets without any growth regulator. Kintzios et al. (1999) reported that the somatic embryos derived from mature leaf explants were germinated on a MS medium supplemented with 5.2 μM BA and 5.7 μM IAA. Somatic seedlings or embryo-derived plantlets were established in the field. Sarasan et al. (2001) used 44 μM methyllaurate (Mela) to germinate somatic embryos into plantlets. Li et al. (2002a) induced somatic embryogenesis from leaf tissues of *R. hybrida* and *Rosa chinensis minima* and also germinated secondary somatic embryos. Plants were regenerated from protoplast derived embryogenic calli of *R. hybrida* on MS medium supplemented with 60 g/l *myo*-inositol, 4.4 μM BA and 1.4 μM 2,4-D (Kim et al., 2003a), and the germination rate of somatic embryos increased up to 30.9% and subsequently plantlets were established in the soil.

3.6. Saintpaulia

S. ionantha Wendl. Commonly called *Saintpaulia* or African Violet is commercially most popular ornamental species. Numerous *Saintpaulia* cultivars are available with varied flower colour, leaf colour and shape. It is

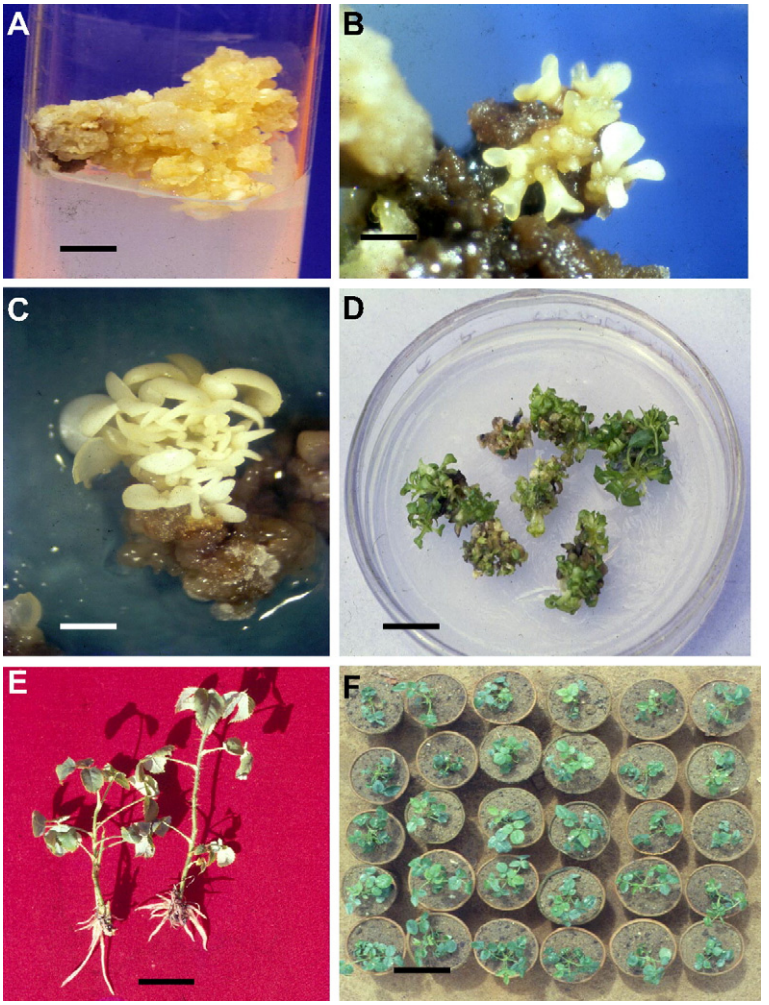

Fig. 3. Somatic embryogenesis in *Rosa hybrida* cv. Landora. (A) Embryogenic callus from leaf tissues on MS medium +2.2 μM BA, 0.05 μM NAA, 0.3 μM GA_3 (bar=0.2 cm). (B) Development of group of somatic embryos (bar=0.25 cm). (C) Development of cotyledons from group of somatic embryos (bar=0.5 cm). (D) Development of shoots from somatic embryos (bar=0.5 cm). (E) Development of roots from somatic embryo-derived shoots (bar=5 cm). (F) Plantlets grown in the pots under greenhouse condition (bar=10 cm).

propagated vegetatively, however, breeding of *Saintpaulia* has been limited to intraspecific hybridization and spot selection, and neither interspecific nor intergeneric hybridization has been incorporated (Grout, 1990). Micropropagation of *Saintpaulia* has been reported by many researchers (Starts and Cummings, 1976; Geier, 1983; Smith and Norris, 1983; Cassells and Plunkett, 1984; Molgaard et al., 1991). Vazquez et al. (1977) reported *in vitro* organogenesis from leaf callus of African violet on MS medium supplemented with 2.0 mg/l NAA and 0.2 mg/l BA. Their results showed production of precocious adventitious shoot buds directly from the surface, apparently without callusing on MS medium supplemented with 1.0 mg/l BA and 1.0 mg/l NAA. After 8 weeks of culture, each petal and leaf disc (1 cm) produced 118 and 37 number of shoot buds, respectively. The regenerated shoots were rooted on hormone free medium with 95% survival rate (Fig. 4). Lo et al. (1997) succeeded to regenerate shoots from leaf discs of *S. ionantha* on the medium having 2.0 mg/l IAA and 0.08 mg/l BA. They indicated that the cellular competence to regenerate shoots is not lost in excised leaf discs of African violet in the absence of exogenous plant hormones. Isolation and culture of protoplasts of *Saintpaulia* have been reported (Bilkey and Cocking, 1982). Hoshino et al. (1995) regenerated plants from protoplasts of *S. ionantha* on B_5 (Gamborg et al., 1968) medium containing 1 mg/l 2,4-D and 2 g/l casein hydrolysate. The regenerated shoots were rooted on half-strength MS medium and successfully transferred to the greenhouse. Subsequently, Murch et al. (2003) induced somatic embryogenesis and germinated somatic embryos from petiole explants of *Saintpaulia*. They found that the transport of calcium and sodium play an important role in cell competence and thidiazuron induced somatic embryogenesis. Mithila et al. (2003) first successfully established plant regeneration system via shoot organogenesis and somatic embryogenesis from leaf and petiole explants of greenhouse- and *in vitro*-grown African Violet plants. They observed two cultivars ('Benjamin' and 'William') had the highest regeneration potential. In 'Benjamin', higher frequencies of shoot organogenesis (twofold) and somatic

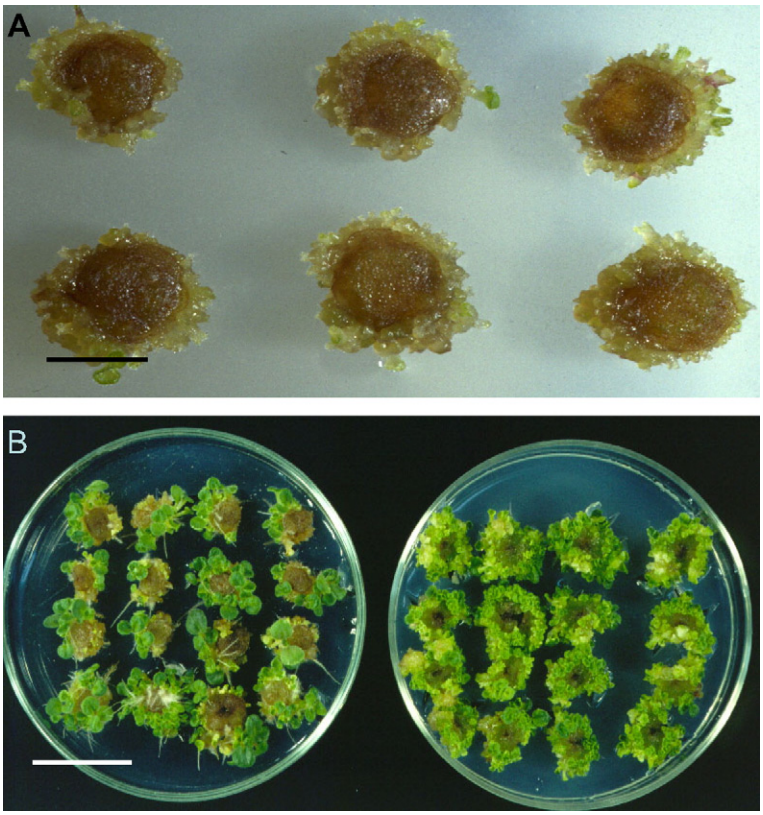

Fig. 4. Direct shoot bud regeneration from petiole segments of *Saintpaulia ionantha*. (A) Shoot buds regenerated from petiole segments of *S. ionantha* on MS medium plus 0.1 mg/l BA and 0.1 mg/l NAA (bar=0.25 cm). (B) Shoots with roots were developed from petiole segments after 3–4 weeks of culture (bar=0.5 cm).

embryogenesis (a 50% increase) were observed from *in vitro* and greenhouse-grown plants. At a lower concentration of TDZ (2.5 μM), shoots organogenesis was induced, whereas at higher doses (5–10 μM) able to induce somatic embryogenesis.

3.7. Yucca

Yucca, an important commercial ornamental pot plant, has 42 species, and is native of North America. It has also great variety of uses. Many *Yucca* species are used as raw material for synthesizing steroidal compounds, such as cortisone and sex hormones (Romo de Vivar, 1985). *Yucca* species are generally propagated by seeds, offsets and rhizome cuttings. It is a very slow growing plant and does not blossom every year. *In vitro* culture is an alternative technique to propagate on a large-scale. Stohs et al. (1974) developed callus and cell suspension from sprouts of seeds of *Yucca glauca* on MS medium, supplemented with 0.53 μM 2,4-D. Further, Meskhi et al. (1978) produced callus from flowers of *Yucca gloriosa* on MS medium supplemented with 2.6–5.3 μM 2,4-D. The callus was produced from coleoptile and leaf segments of *Yucca filifera* (Quintero et al., 1982, 1987). Khanna and Purohit (1983) developed callus tissue from *Yucca oloefolia* leaves on MS medium amended with 5.3 μM 2,4-D. Eight-week-old callus showed an index growth of 1.7. The plant regeneration from callus tissues is not yet reported. Quintero (1983) reviewed the *in vitro* culture of *Yucca* and synthesis of secondary compounds from callus and cell suspension culture. Subsequently, Atta-Alla and Van Staden (1997) succeeded to propagate *Yucca aloifolia* by using shoot tip explants and the maximum number shoot production (6.6) was obtained from a single shoot tip on MS medium supplemented with 4.5 μM TDZ and 1.1 μM NAA. The proliferated shoots readily rooted on half-strength MS medium containing 2.5–4.9 μM IBA and 1% charcoal. The rooted plants were successfully established in soil. In *Yucca*, shoot regeneration is genotypic dependent, and still requires refinement of culture medium for increasing shoot production. Temporary immersion system could be used for shoot and root production.

4. Germplasm conservation

4.1. Clonal stability through in vitro culture

Clonal stability of the micropropagated plants is essential for *in vitro* germplasm conservation. Many researchers reported plants derived from meristems were more stable than the adventitious shoots derived from callus. Somaclonal variation was more common among adventitious-shoot-derived plants in many ornamental crops like *Chrysanthemum* and *Begonia* (Skirvin, 1978; Bouman et al., 1995). Skirvin and Janick (1976) were among the first to emphasize the importance of clonal variation in genotype improvement of horticultural species. Subsequently, Thorpe and Harry (1997) emphasized that *in vitro* culture techniques have played on important role in the breeding, production and improvement of horticultural crops. Various types of changes were reported in cell cultures at phenotypes, karyotypic, physiological, biochemical and molecular level. Larkin and Scowcroft (1981) reviewed extensively and reported the phenotypic variation among plants regenerated after a passage through tissue and cell culture. Hasegawa (1980) found one 'abnormal looking' plant amongst 600 tissue-culture-propagated plants of hybrid rose. Martin et al. (1981) observed no variation among 2125 rose plants raised in the field for 3 years. The lack of somaclonal variability suggests that rose is relatively stable when propagated via axillary buds. Lloyd et al. (1988) reported that callus-derived shoots of *Rosa persica*×*xanthina* exhibited considerable degree of variation in leaf shape. Malaure et al. (1991) observed shoots derived from ray florets of 16 cultivars of chrysanthemum showed more variation than plants regenerated from vegetative parts. However, *in vitro* selection and somaclonal variation are random processes and yet have to be used to achieve specific goals in chrysanthemum improvement.

Somatic hybrids resulting from protoplast fusion also show variation in morphology, cytology, fertility and others. Ploidy level of parent is very important in somatic cell fusion work. Izhar and Tabib (1980) showed that the regenerated plants derived from leaf mesophyll protoplasts of *Petunia* were diploid ($2n=2x=14$). Further, Izhar et al. (1983) observed that over 1000 fertile somatic hybrids, derived from a fusion product of *Petunia parodii* and *P. hybrida*, were tetraploids. However, when protoplasts, isolated from cell suspension cultures, were used as one of the fusion parents, the somatic hybrid plants were of a higher ploidy level ($2n=28$) (Clark et al., 1986).

The selection of explant, age of the culture, genotype, culture conditions and method of plant regeneration are very important features for genetic stability of the regenerated plants. Since most of somatic embryos originate from single cells, somaclonal variation among regenerated plants can be minimised. Of course, there is always a limit of number of subcultures before plants showing variation.

4.2. Determination of genetic fidelity

The molecular markers have facilitated research on genetic variation at the DNA level. The numerous potential applications of DNA fingerprinting have brought about their uses in plants such as in population genetics, parentage testing, and individual genotype identification and for shortening breeding programs (Ben-Meir et al., 1997). EST (Expressed Sequence Tags) database development, proteomics and expression profiling can be used to create unique database resources to identify genes that determine the quality (colour, flavour, phytonutrients) (Dandekar, 2003). He reported that ESTs represent closely related gene families could be used to define their function and to detect single nucleotide polymorphisms (SNPs). Markers such as restriction fragment length polymorphism (RFLPs) have recently been used for molecular characterization of tissue culture-derived plants. Since its development, polymerase chain reaction (PCR) has revolutionized many standard molecular techniques, with modifications of the original procedure designed to suit a number of needs. Random amplified polymorphic DNA (RAPD), arbitrarily primed PCR (AP-PCR), DNA amplification fingerprinting (DAF), inter-simple sequence repeat (ISSR), sequence-tagged sites (STSs) and amplified fragment length polymorphism (AFLP) and many others generate special classes of markers which are highly sensitive for genetic analysis of tissue culture-raised plants (Rani and Raina, 2000). RAPD markers have also been used to identify cultivars, to map important agricultural traits and to construct genetic maps (Williams et al., 1990). For varietal identification, molecular markers have been useful especially RFLP, and amplified fragment length polymorphism (AFLP) (Rajapakse et al., 1992). Bouman et al. (1992) noticed RAPD polymorphism among micropropagated plants of *Begonia* species. Debener and Mathiesch (1996) demonstrated RAPD markers for the construction of a chromosome linkage map, using crosses between *Rosa multiflora* derived genotypes that differed in a range of floral and vegetative characters. Furthermore, Debener et al. (1997) used RAPD markers for parentage analysis in interspecific crosses between different wild rose species. Vainstein et al. (1995) demonstrated that the probability of two offsprings from the crossing of similar rose genotypes having identical DNA fingerprints is very low. Ben-Meir et al. (1997) screened the hybrid rose with seven different horticultural traits through the RAPD marker. Huang et al. (2000) studied the genetic analysis of chrysanthemum hybrids with RAPD markers and classified them into seven types, i.e., markers shared bands in both parents and offspring, in male and female parents, in male parent and offspring, in female parent and offspring, in the male parent only, in the female parent only, and markers were present in offspring only. Only male parent and offspring markers were suitable for identifying the true male parent. Their results concluded that there were no definite rules as to whether markers in offspring were more similar to female or to male parents by similarity analysis. Recently, Dandekar (2003) mentioned that the rapid identification of cultivar/progeny could be detect by using micro-arrays and single nucleotide polymorphisms (SNPs).

5. Applications of *in vitro* propagation

5.1. In vitro mutagenesis

Most of the available genetic variation used in breeding programs has occurred naturally and exists in germplasm collections of new and old cultivars, land race and genotypes. This variation through crosses is recombined to produce new and desired genes combinations (Maluszynski et al., 1995). Then existing germplasm fails to provide the desired recombinants, and it is necessary to resort to other resources of variation. Since spontaneous mutations occur with extremely low frequency, mutation induction techniques provide tools for the rapid creation and increase in variability in crop species. The impact of mutation techniques on crop improvement has already been evaluated (www.iaea.org; Broertjes and Van Harten, 1978, 1988; Micke, 1999). *In vitro* culture methods has facilitated the use of mutation techniques for improvement of both seed and vegetatively propagated plants (Jain and Maluszynski, in press). In many vegetatively propagated crops mutation induction in combination with *in vitro* culture techniques may be the only effective method for plant improvement (Jain, 2002). There has been considerable work done on induced mutations in roses, using ethylmethanesulphonate (Kaicker, 1982), ionizing radiations (Broertjes and Van Harten, 1978; Smilansky et al., 1986). Benetka (1985) irradiated single bud cuttings with 0, 20, 30, 40 and 60 Gy γ-rays and subsequently observed four bud-propagated generations. He found that 40 and 50 Gy were optimum doses and that chimerism decreased with successive generations. Walther and Sauer (1986) used *in vitro* techniques to increase plant variability by irradiation with X-rays. Variability has been reported in different chrysanthemum cultivars through physical or chemical mutagenesis or low temperature tolerant mutants (Huttema et al., 1986). Nikaido and Onogawa (1989)

isolated mutants having higher levels of flavonoids and carotenoids. Mandal et al. (2000) induced sectoral somatic mutations in flower colour of chrysanthemum Root cuttings were treated with gamma rays and cultured on agar-gelled MS medium supplemented with cytokinin and auxin. Direct shoot organogenesis was achieved within 2 weeks of culture on MS medium supplemented with 0.2 mg/l NAA and 0.5 mg/l BA. Shoot regenerated from mutated ray florets were rooted and transplanted in the field. The plants flowered and exhibited true to type in two successive generations.

By induced mutations a wide range mutants can be isolated including abiotic and biotic stresses. Preil et al. (1983) developed low temperature tolerant mutants of *E. pulcherrima* and *Dendranthema* from irradiated cell suspension cultures by using X-irradiation (15 and 20 Gy). *Euphorbia* mutants adapted better at low temperature in the greenhouse as compared to the parental cultivar. The *Dendranthema* mutants flowered 7–10 days earlier than the original variety. Most of the low-temperature tolerant mutants were obtained by single step selection procedure (Huttema et al., 1989, 1991; Preil et al., 1991). During 1993, Japanese group headed by S. Nagatomi, developed six flower colour mutants of chrysanthemum by chronic irradiation (low radiation dose treatment for longer period of time) of plants (Nagatomi, 1993). Mandal et al. (2000) used various explants for plant regeneration of *D. grandiflora*, and regenerated new mutant plants from mutated tissues. Latado et al. (2004) induced mutations in immature floral pedicels of *Dendranthema* by ethylmethane sulphonate (EMS) (0.77%) for 1 h and 45 min and developed adventitious buds through *in vitro*. Forty-eight mutants were identified from 910 plants, which deviated in petal colour. Most of them were phenotypically uniform. Lamseejan et al. (2003) used chrysanthemum var. 'Taihei' for mutation induction with chronic and acute gamma irradiation treatment, and obtained mutants with different traits such as flower colour, form and size. The mutation frequency for flower colour was higher than other traits. Six mutant varieties were officially registered with Kasestart University. Misra et al. (2004) developed two *Dendranthema* mutants by γ-irradiation (0.5 Gy). Both mutants were yellow but one having flat spoon shaped ray florets similar to the original cultivar, while the other having tubular florets. Up to now, the mutation studies were helped to induce colour variants of commercialized cultivars, similar to those obtained by spontaneous mutation. The combination of micropropagation and induced mutations can develop and multiply elite mutants in a short period of time in most of the ornamental plants.

5.2. Somaclonal variation

Somaclonal variation involves all forms of variation among regenerated plants derived from tissue culture (Larkin and Scowcroft, 1981; Jain et al., 1998a; Jain and De Klerk, 1998), such as: i) physical and morphological changes in undifferentiated callus; ii) differences in the ability to organize and form organs *in vitro*; iii) changes manifested among differentiated plants; and iv) chromosomal changes. Somaclonal variation has been reviewed at length (Skirvin, 1978; Scowcroft and Larkin, 1988), and has proven useful in plant improvement (Skirvin et al., 1993; Jain et al., 1998a,b; Jain and De Klerk, 1998), and could be of much interest to the horticultural breeders. In chrysanthemum, little variation is observed in plants derived from shoot tips (Khalid et al., 1989). Most of the variation is observed in plants originating from protoplasts, which is termed as protoclonal variation (Kawata and Oono, 1997; Jain, 1997; Jain and De Klerk, 1998). Plants regenerating from unorganized callus vary more than those from organised callus, whereas no or hardly any variation occurs when plants are regenerated directly without an intermediate callus phase (Bouman and De Klerk, 1996). Malaure et al. (1991) found somaclonal variation in plants regenerated from ray-florets of *D. grandiflora*. Subsequently, Ahloowalia (1992) developed 20 new variants, which differed in height, leaf, flower shape and petal size and curvature. Increase in variability for flowering date, plant height, plant width, number of flowers, and flower morphology was reported for *Chrysanthemum* (Votruba and Kodyteck, 1988) and *Begonia*×*elatior* and *S. ionantha* (Jain, 1993a,b,c). Differential somaclonal variations were observed in *Saintpaulia* (2–10%), *Dracaena* (10%) and *Chrysanthemum* (60%) (Jain et al., 1998c). Jain (2001) reviewed the variations occurred in tissue culture raised plants and their detection through molecular markers. Exploitation of somaclonal variation through callus culture might become a source for new cultivars if this method is combined with strategic and efficient *in vitro* selection pressures (Gudin and Mouchotte, 1996). The selected somaclones should be genetically stable in seed and vegetatively propagated crops for routine induction of genetic variability through tissue culture, and this aspect should be thoroughly checked before using them in regular crop improvement programs. Somaclonal variation is unpredictable in nature and can be both heritable (genetic) and non-heritable (epigenetic) in regenerated plants. DNA methylation causes genetic instability in somaclones, which probably comes from epigenetic changes (Jain, 2001). Since somaclonal variation can

broaden the genetic variation in number of crop plants, a broader range of plant characteristics can be altered, including plant height, yield, no. of flower/plant, early flowering, resistance to diseases, insects and pests and salt. The reduction, and even the total loss of regeneration ability, is a general phenomenon observed during undifferentiated cell culture. The somaclonal variation creates problem for micropropagators by the production of offtypes in clonally propagated plants. This can be controlled by reducing the subcultures and the age of the cultures, depending on the plant species. Hirochika et al. (1996) reported that certain types of retrotransposons are activated as the tissue cultures get older and the regenerated plants show an increase in retrotransposon copy numbers leading to offtypes.

5.3. Cryopreservation

Cryopreservation of tissue and cells has been utilized for long-term storage of elite genetic material. In the mid 1900s, the science of cryobiology improved rapidly with the discovery of the beneficial effects of cryoprotectant substances that are added to cell freezing solutions. Prior to freezing, the cells must be treated with a cryoprotectant solution such as glycerol, dimethyl sulfoxide (DMSO) or ethylene glycol. These substances protect the cells and their membranes from damage during the freezing process. After the cells have been exposed to the freezing medium containing the cryoprotectant, they must be dehydrated so that the water inside the cells will not form ice crystals that can also damage the cell/tissues. The cells/tissues are dehydrated and cooled slowly prior to plunging them into liquid nitrogen.

Sakai (1960) first reported the survival of plant tissues after exposure to ultra low temperature −196 °C and the significance of using DMSO as a cryoprotectant (Quatrano, 1968). Preservation of cultured plant tissues in liquid nitrogen is efficient and appropriate method of germplasm conservation. Fukai et al. (1988) used cryopreservation techniques to study the survival rates of 12 species and two inter-specific hybrids of *Chrysanthemum*. Fukai and Oe (1990) established cryopreservation method for *Chrysanthemum*. Shoot tips were placed in MS medium supplemented with 0.1 mg/l BA, 1.0 mg/l NAA, 2% sucrose and 5% DMSO for 2 days, slowly cooled with a cryoprotectant solution (10% DMSO and 3% sucrose) at a rate of 0.2 °C/min, then immersed and stored in liquid nitrogen. Shoot regeneration rate of the frozen shoot tips varied from 94% to 100%, depending on the species. Shoot tips of *Chrysanthemum* showed high viability even after 8 months of storage in the liquid nitrogen. Chartier-Hollis et al. (1996) reported that the pre-freeze encapsulation and dehydration (with 0.5 M sucrose and 2 h exposure to silica gel) of shoot tips of *R. multiflora* helped in successful recovery from cryogenic storage. Recently, Hitmi et al. (2000) developed a simple method for efficient cryopreservation of *Chrysanthemum cinerariaefolium*. The shoot tip explants were treated with 0.55 M sucrose and 4 mM abscisic acid for 3 days and immersed in liquid nitrogen and subsequently rapid warming was done at 4 °C. The treated shoot tips were cultured on solid nutrient medium containing 11 μM NAA and 4.5 mM BA, about 75% shoot tips proliferated into plantlets. This technique will greatly help in the *in vitro* conservation of germplasm on long-term basis, and that will lead to the establishment of cryo-storage bank.

5.4. Genetic transformation

The study of genetic transformation of ornamental plants will considerably enhance the existing efforts of traditional and molecular breeding in generating new cultivars. Plant genetic transformation has assisted plant breeders in crop improvement as well as in better understanding of the basic mechanism involved in plant gene regulation (Wising et al., 1988). Gene transfer enables the introduction of foreign genes, or specifically designed hybrid genes, into host plant genomes, thus creating novel varieties with specifically designed characters including resistance to environmental stress, pest and disease (Ahmed and Sagi, 1983). Alternative to traditional plant breeding methods, the genetic changes can be made by using *Agrobacterium*-based gene vectors (Hutchinson et al., 1989; Hutchinson et al., 1992). In chrysanthemum, genetic transformation by using *Agrobacterium* based gene vectors have been reported (Ledger et al., 1991; Firoozabady et al., 1991a; Van Wordragen et al., 1992; Lowe et al., 1993; Kudo et al., 2002). Urban et al. (1992) transformed three cultivars of *D. grandiflora* with *Agrobacterium* EHA105 (pB1121). Subsequently, they developed an efficient, high frequency transformation protocol for cvs. Iridon, Hekla and Polaris. The transformed shoots were rooted on medium containing 50 mg/ml kanamycin. Seo et al. (2003) regenerated plants efficiently from transformed leaf explants (*Agrobacterium*-mediated) of *D. grandiflora* Teixeira da Silva and Fukai (2002a,b) tested four different methods of gene transfer for stable transgene expression in chrysanthemum. The results showed a 2- to 10-fold increase in stable transformation efficiency rate, however, genotype dependence still plays an important role; other factors such as low regeneration rate, variation in shoot regeneration capacity on selection media are also critical.

Genetic transformation of rose has been established (Firoozabady et al., 1991b; Noriega and Sondahl, 1991; Robinson and Firoozabady, 1993; Matthews et al., 1991; Dohm et al., 2002; Li et al., 2002b; Condliffe et al., 2003). Matthews et al. (1991) reported *Agrobacterium*-mediated transformation (LBA 4404 strain) of *R. persica* × *xanthina* protoplasts, derived from embryogenic cell lines, by hygromycin selection system. GUS expression of a transformed callus and subsequent regeneration of shoots were reported. Firoozabady et al. (1994) regenerated transgenic rose (*R. hybrida* cv. Royalty) plants from transformed embryogenic callus, which were confirmed by enzyme assays and polymerase chain reaction (PCR). More than 100 transgenic plants were established in the greenhouse. Subsequently, Van der Salm et al. (1996) established the regeneration protocol from stem segments of *R. hybrida* cv. Money way by co-cultivation with *Agrobacterium tumefaciens* strain GV3101 containing NPT II gene and individual *rol* genes from

Table 3
Genetic transformation study of major ornamental pot plants

Species/Cultivars	Foreign genes	References
Begonia tuberhybrida	rol A, B and C	Kiyokawa et al. (1996)
Cyclamen persicum	GUS, NPT II, HPT (hygromycin phosphotransferase)	Aida et al. (1999)
Dendranthema grandiflora	NPT II, GUS	Van Wordragen et al. (1991)
Dendranthema grandiflora cv. Yellow Spider	GUS, NPT II	Pavingerova et al. (1994)
Dendranthema grandiflora cv. Kitamura	NPT II, GUS	Seiichi et al. (1995)
Dendranthema grandiflora cvs. Polaris, Hekla, Iridon	GUS, NPT II	Sherman et al. (1998)
Dendranthema grandiflora cv. Peach Margaret	NPT II	Boase et al. (1998)
Dendranthema grandiflora	GUS	Seo et al. (2003)
Petunia hybrida var. Ultra Blue	Delta-9, fatty acid desaturase	Choudhury et al. (1994)
Petunia hybrida	Tryptophan decarboxylase [aromatic L-amino acid decarboxylase]	Thomas et al. (1999)
Petunia axillaries × (*Petunia axillaries* × *Petunia hybrida*)	*rol* C	Winefield et al. (2000)
Petunia hybrida	[naringenin-]chalcone synthase-A (CHS A)	Shao et al. (1996)
Petunia hybrida	GUS (uid A) naringenin-chalcone synthase (GTCHSI)	Kobayashi et al. (1998)
Pelargonium (*Pelargonium* × *domesticum*) 'Dubonnet'	NPT II [Neo], GUS A, als, dfr	Boase et al. (1996)
Pelargonium × *hortorum*	NPT II, hygromycin B phosphotransferase, GUS	Robichon et al. (1995)
Pelargonium cv. Frensham	Antimicrobial protein (*Ace-AMP1*)	Bi et al. (1999)
Rhododendron cultivars ('America', 'Catawbiense grandiflorum roseum', 'Madame Carvalho', 'Mars' and 'Nova Zembla')	GUS, NTP II	Pavingerova et al. (1995)
Rhododendron species	GUS (uid A), NPT II	Pavingerova et al. (1997)
Rhododendron yakushimanum cv. Percy Wiseman	NPT II, GUS	Ueno et al. (1996)
Rhododendron hybrids cvs. Hino-Crimson, Fuchsia	uid A, HPT	Hsia and Korban (1998)
Rhododendron hybrida cv. Moneyway	NPT II, *rol* A, B and C	Van der Salm et al. (1997)
Rhododendron hybrida cv. Glad Tidings	GUS (uid A), chitinase	Marchant et al., 1998a,b
Floribunda roses cvs. Heckenzauber, Pariser charme	Chitinase, GUS	Dohm et al. (2002)
Rhododendron hybrida cv. Carefree Beauty	uid A *Ace-AMP1*, NPT II	Li et al., 2002b, 2003
Saintpaulia ionantha	uid A, NPT II	Mercuri et al. (2000)

Agrobacterium rhizogenesis. Marchant et al. (1998a,b) succeeded to introduce the chitinase gene in embryogenic callus of *R. hybrida* cv. Glad Tidings through biolistic transformation. All transgenic plants were grown in the greenhouse and morphologically true-to-type, with reduced blackspot disease by 13% to 43%. Subsequently, floribunda roses were transformed with genes for antifungal proteins to reduce their susceptibility to fungal diseases (Dohm et al., 2002). Li et al. (2003) developed transgenic rose lines harbouring an antimicrobial protein gene (*Ace-AMP1*) to enhance resistance to powdery mildew. They have confirmed the stable integration of *Ace-AMP1* and NPT II genes by Southern blotting. Condliffe et al. (2003) reported the optimised protocol for transformation (*Agrobacterium*-mediated) in different rose cultivars by using GUS (uidA[2]) gene. They found stable integration of the transgene was confirmed at each stage of somatic embryogenesis and in regenerated plants.

Pavingerova et al. (1995) developed transgenic *Rhododendron* cultivars by *Agrobacterium*-mediated transformation with GUS and NPT II gene as a selectable marker gene. Hsia and Korban (1998) reported the successful transformation of two cultivars of *Rhododendron* by using a helium pressure bombardment device. Aida et al. (1999) developed transgenic *Cyclamen* and successfully transferred to greenhouse. Mercuri et al. (2000) established the *Agrobacterium*-mediated transformation system in *S. ionantha* by using *in vitro* grown leaves and petioles explants and co-cultivation with two strains (i.e., EHA105 (pKIWI105) and A281 (pKIWI105)) carrying the genes uidA and NPT II. The transient transformation was confirmed by PCR and southern hybridization. Transgenic *Petunia* plants with *rol* C gene, which reduces plant height, leaf and flower size, increased branching and decrease male and female fertility has been reported (Winefield et al., 2000). The genetic transformation studies in ornamental pot plants are presented in Table 3.

Thin cell layer (TCL) technology can be used for genetic engineering and crop improvement. The untransformed cells can be eliminated by using selective antibiotic medium, where the transformed cells will grow faster. The cells harbouring selector gene within their genome proliferate on the selective medium. The reports on the use of a thin cell layer system as an initial explants for gene transfer are few, but those that exist demonstrate the effectiveness of introducing a gene into an explants with defined cellular structure and with a controlled regeneration program, allowing for the formation of non-chimeric transgenic plants (Teixeira da Silva, 2003a). Successful transformation of *D. grandiflora* was done by using stem tTCLs or leaf ITCLs as an initial explants (Teixeira da Silva and Fukai, 2002a,b).

6. Conclusion

Ornamental plants are produced mainly for their aesthetic value, thus the propagation and improvement of quality attributes such as leaf types, flower colour, longevity and form, plant shape and architecture, and the creation of novel variation are important economic goals for floriculturists. Successful *in vitro* propagation of ornamental plants is now being used for commercialization. Many commercial laboratories and national institutes worldwide use *in vitro* culture system for rapid plant multiplication, germplasm conservation, elimination of pathogens, genetic manipulations, and for secondary metabolite production (O'Riordain, 1999). Annually, millions of ornamental plants are routinely produced *in vitro*. The great potential of micropropagation for large-scale plant multiplication can be tapped by cutting down the cost of production per plant by applying low-cost tissue culture, which is to adopt practices and proper use of equipment and resources to reduce the unit cost of micropropagule and plant production without compromising the quality. Bioreactor technology may cut down the cost of plant production provided proper precautions are taken to prevent contamination. Somatic embryogenesis facilitates cryopreservation, synseed development, mutations, and genetic transformation. Plant transformation methods and enhanced gene silencing technology can effectively be used to evaluate and authenticate newly discovered endogenous genes to characterize their function in plants as well as to genetically manipulate trait quality and productivity (Dandekar, 2003). Recent progress in genetic manipulation of plant cells has opened new possibilities for improvement of ornamental pot plants. In 2001, an Australian company Florigene became the first company in the world to sell genetically modified plants by mail order to the general public for home garden use (Lu et al., 2003). They are still selling transgenic products of two carnation types in Australia, Japan and USA.

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