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Review Article

TISSUE CULTURE ASPECTS OF ORNAMENTAL PLANTS

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ABSTRACT
Ornamental plants are produced primarily for their artistic value, thus the propagation and improvement of quality attributes and the creation of novel variation are important economic goals for floriculturists. Micropropagation, Clonal reliability and Conservation are important aspects which should be considered. Moreover these partial processes are amenable to controlled investigations and successful in vitro propagation of ornamental plants is now being used for commercialization. The advent of in vitro tissue culture technique has offered a new approach to the morphogenetic investigations.

Key Words: *Tissue Culture Aspects, Ornamentals, Micropropagation, Clonal Reliability, Conservation*

INTRODUCTION
In general, the term "ornamental plant" or "ornamental" is used to describe those species primarily cultivated for their aesthetically-pleasing characteristics such as form, bark, leaves, flowers, fruit or some combination thereof. The viable production of ornamental plants is increasing worldwide (Jain, 2002). In UK, the trade in ornamental and floriculture industry is estimated to be around £2.1 billion in 2005, while the international trade is around £60-75 billion. Its economic value has drastically increased over the last two decades and there is a great prospective for continued further growth in both marital and intercontinental market (Franken Berger *et al.,* 1981). Tissue culture system in ornamental flowers like roses has been established (Hsia and Korban, 1996; Kintzios *et al.*, 1999; Ibrahim and Debergh, 2001; Rout *et al.,* 2006; Hameed *et al.,* 2006; Drefahl *et al.,* 2007; Previati *et al.,* 2008). Recently, *in vitro* flower induction in roses was demonstrated (Wang *et al.,* 2002; Vu *et al.,* 2006). Tissue culture techniques are applied for micropropagation and production of pathogen-free plants. (Kaviani *et al.,* 2011)

Plant tissue culture has emerged as an approaching tool and forms the backbone of plant biotechnology. The better quality planting material is a basic need of growers for boosting productivity. (Chebet *et al.,* 2003) The success of the clonal propagation method depends on numerous factors like genotype, media, plant growth regulators and type of explants, which should be experiential during the process (Kim *et al.,* 2003). The most often used growth regulators for micropropagation of ornamental plants by organogenesis, embryogenesis and axillary proliferation are naphthalenacetic acid (NAA), and benzyl adenine (BA). Studies have been conducted in thin layer cultures of hybrid seed geranium (*Pelargonium*) to assess the high frequency direct emryogenesis (Gill *et al.,* 1992).

Plants raised through micropropagation are of uniform quality, Pathogen free, Can be produced much more rapidly as new cultivars could become commercially available within 2 to 3 years from development rather than 5 to 10 years needed using conventional propagation, Produce uniformly superior seeds, Show improved vigor and quality. Propagation through meristem has been identified as mechanism of rapid regeneration in Caladium and the plants produced through this technology provide much export potential as they are shipped internationally with limited quarantine restrictions and it has the prospective for developing new cultivars of the species (Hyndman, 1987). In more recent years, several researches were carried out in order to develop semi-automatic systems that use the principle of the growth in temporary immersion with the aim to avoid the tissue hyperhydricity. Some systems of temporary immersion (TIS) have been employed on tropical plants (Alvard *et al.,* 1993; Lorenzo *et al.,* 1998; Escalona *et al.,* 1999) and fruit trees (Damiano *et al.,* 2002).

Review Article

In-Vitro Propagation of Ornamentals

In vitro culture is one of the input tools of plant biotechnology that exploits the totipotency character of plant cells, a concept unambiguously confirmed for the first time by (Steward *et al.* 1958). Ornamental industry has applied massively in vitro propagation loom for large-scale plant development of selected better varieties. As a result, hundreds of plant tissue culture laboratories have come up wide-reaching especially in the developing countries due to inexpensive labor expenses.

Micropropagationvia Meristem Culture

The most significant method of micropropagation, reported by various researchers, is meristem proliferation in which apical buds or nodal segments having an axillary bud are cultured to regenerate several shoots without any intervening callus phase. Many commercial ornamental plants are being propagated by in vitro culture on the culture medium containing auxins and cytokinins. The Photoautotrophic micropropagation of ornamental plants has been reviewed (Kozai *et al.*, 1988; Kozai, 1990) and is suggested to use for reducing production costs, and automation to use robots for micropropagation process (Kozai *et al.*, 1988; Kozai, 1991). 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). (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* (Schwenkel, 1991; Dillen *et al.*, 1996), *Heucherasanguinea* (Hosoki and Kajino, 2003), and *Begonia* (Takayama, 1983).

Micropropagationvia Somatic Embryogenesis

Somatic embryos, which are bipolar structures, arise from individual cells and have no vascular connection with the maternal tissue of the explants (Haccius, 1978). 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). 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 (Castillo and Smith, 1997). Somatic embryos from hypocotyl tissues of *E. pulcherrima* on MS medium supplemented with 2.0 mg/l IAA (Osternack *et al.*, 1999). Plant regeneration via somatic embryogenesis of *C. persicum* and maintained the regeneration ability for prolonged period (Pueschel *et al.*, 2003). There are advantages and disadvantages of somatic embryogenesis in large-scale plant multiplication (Jain, 2002). Somatic embryogenesis in ornamental pot plants like chrysanthemum (*Dendrathemagr and iflorum*) (May and Trigiano, 1991; Tanaka *et al.*, 2000), *Cyclamen persicum* (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).

Micropropagationvia Thin Cell Layer

Thin cell layer (TCL) is a simple but effective system that relies on a small size explants derived from a limited cell number of homogenous tissue. Organogenesis and somatic embryogenesis for plant regeneration and genetic improvement via transformation presented by (Teixeira da Silva, 2003). Explants consisting of 3–6 layers of sub epidermal and epidermal cells produced vegetative buds within 2 weeks of culture (Mulin and Tran Thanh Van, 1989). 100–200 shoots per tTCL (transverse thin cell layer) explants were obtained from petiole or lamina sections, respectively of *S. ionantha* within 4 weeks of culture (Ohki, 1994). Over 70,000 plants were produced from a single leaf within 3–4 months. tTCL hypocotyl explants of 1-weekold Geranium hybrid seedlings for induction of somatic embryogenesis (Gill *et al.*, 1992). Organogenic and embryogenic callus and subsequent regeneration from lTCL (longitudinally thin cell layer) explants derived from dormant bud floral stalks of *R. hybrida cv. Baccara* (Hsia and Korban, 1996). 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; Teixeira de Silva, 2003). 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. hybrid.

Clonal Reliability

In vitro raised cultures exhibit clonal variation that is commonly called somaclonal variation. This variation may be caused through pre-existing genetic variation occurred in the explants and the variation induced by the *in vitro* conditions. This variation may be genetic or epigenetic and is manifested in the form of DNA methylations, chromosome rearrangements and point mutations. Molecular techniques are powerful tool for assessment of genetic fidelity raised among *in vitro* regenerants. At present, Random Amplified Polymorphic DNA (Williams *et al.*, 1990) and Inter Simple Sequence Repeats have been successfully used for the assessment of geneticfidelity in various plant species.Such as banana, Lillium Ginger *Robinaambiguna* and medicinal herb *Swertiachirayita.*Somaclonal variations were also recorded in *vitro* propagated plants of *Pinustremuloides* and*Actinidiadeliciosa* using PCR based RAPD and ISSR markers. In White Flowered variety *Pacifica* of Gladiolus, only one report of Roy *et al.*, (1992) was successfully reported for clonal fidelity of *in vitro* raised cormels and mother cormels through isozymes, RAPD and ISSR techniques.

Impact of Climate Change on Genetic Resources of Ornamental Plants

Changes to land use and agricultural management can affect biodiversity, both positively and negatively. Further, the intensification of agriculture has generated lot of pressure on plant genetic resources particularly on the traditional varieties, landraces and large number of crops wild relatives affecting therefore, crops productivity and biodiversity negatively.

Shift in Crops Suitability Areas

Climate change will cause shifts in areas suitable for cultivation of a wide range of crops and also geographic distribution of species whilst some regions considered marginal will gain suitability and others will lose. It also predicts that with rising temperatures and change in the rainfall regime the global suitability for crops does not per se decrease, but does shift geographically. For a given site, there is high likelihood that crops that are currently adapted to the conditions become mal-adapted, resulting in the need for new within-crop diversity to adapt to future conditions and under extreme conditions, new crops will be required

Migration and Extinction of Species

The climatic factors such as temperature and precipitation when change beyond the tolerance of a species phenotypic plasticity, the inward and outward movement of species causing change in species composition is inevitable. Though evidence of climate related biodiversity loss remains limited, a large number of plant and animal species are reported to be moving to higher latitudes and altitudes.

Alteration in Phenological Responses

Plants are finely tuned to the seasonality of their environment particularly temperature and photoperiod, and shifts in the timing of plant activity (i.e. phenology) provide some of the most compelling evidence that species and ecosystems are being influenced by climate change. For plant reproduction, timing is everything.

Effects on Regeneration of Species

The temperate species in general require chilling/ stratification (remained under snow for 2-3 months) of seeds to germinate. If such conditions are not met, the rejuvenation of species hammered largely. The data collected on the number of saplings and adult plants of *Quercusleucotrichophora, Rhododendron arboretum* and *Cedrusdeodara* in the Shimla forests showed considerable reduction in the number of saplings compared to adult trees. In the alpine region, big trees are noticed in their original distribution but not the saplings and the juvenile stages in case of *Prunuscornuta, Corylusjacquemontii* and *Pinusgerardiana*. The poor winter precipitation also hammers seed germination of many species.

Conservation of Ornamental

Some of ornamental plants are in danger of becoming extinct and Efforts are being made for conservation of them. Coordinated effort has been made up to date to conserve and protect the genetic resources of ornamental plants. One exception is the genebank of the "Ornamental Plant Germplasm Center" in USA. Seed banks are a common way of conserving plant genetic resources. In these, orthodox and sub-orthodox

seeds are stored at temperature of either -15°C to -20°C (cold tolerant species) or 0 to -5°C (temperate and tropical species). However, as the seeds do not represent the genetic profile of the mother plant, this approach cannot be used when endangered clonal germplasm is to be preserved, e.g., that of ancient cultivars (Ozudogru *et al.,* 2010). For vegetatively-propagated species, the conservation of clonal germplasm is made in field (clonal collections). However genotypes preserved only this way run the risks of biotic and a biotic stresses.

Slow Growth Storage of Ornamentals:

In this method, depending on the species, sub culturing can be decreased to once in every several months. The most widely used approach is the coupling of a low temperature with the culture in the dark or low light intensity. In addition to cold storage, *in vitro* conservation can be achieved by modifying the medium compositions, i.e., by reducing the sugar and/or mineral concentration, using growth retardants (e.g., chlorocholin chloride and ABA) or osmotically active compounds (e.g., mannitol), and covering the explants with a layer of liquid medium or mineral oil to reduce the oxygen available to the plants (Withers and Engelmann, 1997). To date, only a limited number of reports, such as *Camellia* spp. (Ballester *et al.,* 1997), Humulusspp (Reed *et al.,* 2003), *Nerium oleander* and *Photiniafraseri* (Ozden-Tokatli *et al.,* 2008), *Splachnumampullaceum* (Mallón *et al.,* 2007), and *Rosa* (Previati *et al.,* 2008) deal with the *in vitro* conservation of ornamental plants, all based on the cold storage approach. Storage in total dark conditions is preferred for a better slowing down of cell metabolism; however, storage under low light intensity showed to be effective for shoot cultures of *Camellia* (Ballester *et al.,* 1997), and *Humulus*spp. (Reed *et al.,* 2003)

*Cryopreservation of Ornamentals***:**In ornamental species, one-step freezing techniques (such as vitrification, encapsulation-vitrification and ED) are widely preferred than slow cooling (Ozudogru *et al.,* 2010). Examples are *Chrysanthemum grandiflora* (Halmagyi *et al.,* 2004), *Humulus*spp. (Reed *et al.,* 2003), *Acer mono* (Park *et al.,* 2005), *Gentian* spp. (Tanaka *et al.,* 2004), *Dianthus caryophyllus* (Halmagyi and Deliu, 2007), and *Ribes*spp. (Johnson *et al.,* 2007) Four different sugars or sugar alcohols (i.e., sucrose, glucose, mannitol, and sorbitol) in preculture and tolerance to freezing of rose shoot tips were highest when sucrose was used (Halmagyi and Pinker, 2006). Pretreating explants at 10°C or below (cold hardening) is another approach for inducing freezing tolerance (Ozudogru *et al.,* 2010). For example, nodal segments of *Chrysanthemum* stored at 10°C and low light intensity for 3 weeks, shoot cultures of *Photiniafraseri*stored at 4°C in darkness for 2-3 weeks (Ozden-Tokatli *et al.,* 2008). Also *in vitro*-grown Gentian plants stored up to 50 days at 5°C and low light intensity (Tanaka *et al.,* 2004), and embryogenic callus of *Aesculushippocastamum*stored at 4°C for 5 days in darkness (Lambardi *et al.,* 2005), then plunged in LN. In ornamental plants, the PVS2 treatment ranges from 5 min (shoot tips of *Chrysanthemum grandiflora*) (Halmagyi *et al.,* 2004) to 3 h (encapsulated shoot tips of *Dianthus caryophyllus*) (Halmagyi and Deliu, 2007). Warming temperatures ranging from 20°C (Johnson *et al.,* 2007) to 45°C (Reed *et al.,* 2003; Lambardi *et al.,* 2005) have been proposed for ornamental species (Ozudogru *et al.,* 2010). Several orchids were cryopreserved using the ED and vitrification methods (Lurswijidarus and Thammasiri, 2004; Thammasiri and Soamkul, 2007). Some orchid seeds with moisture content lower than 14% can be conserved in LN (Wang *et al.,* 1998). Cryopreservation of *Liliumledebourii* (Baker) Bioss.germplasm by encapsulation-vitrification, and ED methods as well using sucrose and dehydration as pretreatment was performed (Kaviani *et al.,* 2008; 2009; 2010; Kaviani, 2010). Cryopreservation using sucrose and dehydration showed that survival seeds of lily after freezing were nil for control seeds (Kaviani *et al.,* 2009). Studies have shown that the best lily germplasm is seed and the best pretreatments for survival of lily germplasm after cryopreservation are 0.75 M sucrose and dehydration for 1 h (Kaviani *et al.,* 2008; 2009; 2010).

DISCUSSIONS

Ornamental plants are produced mainly for their arty 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. Many commercial laboratories, international 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. 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. Conservation of Ornamentals may cut down the cost of plant production provided proper precautions are taken to prevent contamination. Somatic embryogenesis facilitates cryopreservation, Clonal Reliability tests, synthetic seed development, mutations, and genetic transformation. Clonal Reliability tests and enhanced technology can effectively be used to evaluate and authenticate various factors and can be used to characterize their function in plants as well as to manipulate trait quality and productivity. Recent progress in tissue culture techniques of plant has opened new possibilities for improvement of ornamental pot plants.

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