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Biotecnologies for ornamental plants: some insights to the Brazilian productive chain

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Summary: The world industry of ornamental plants is under wide transformations regarding the changes in the cultivation, management, post-harvest technologies and the use of new (bio)technologies to assist the improvement and development of new varieties. This industry worth more than US$ 20 billion, but Brazil shares only 1% of this value. To compete at this market with permanent technological innovation, the country needs to be tuned in with the progresses in the biotechnologies applied to the productive chain of ornamental plants. In this revision, we analyze the Brazilian and world ornamental market emphasizing the role of the biotechnologies in the modernization and increase of the competitiveness of the sector.

Key words: Brazilian floriculture, flowers market, applied biotechnologies, genetic engineering

Introduction

In the wide sense, the industry of flower and ornamental plants comprises the cultivation of potted flowers and ornamental species, cut flowers and stocks of flower and ornamentals, including woody stocks species (*Marques & Caixeta-Filho*, 2002).

Between 4,000 and 6,000 non-woody species are commercialized in the worldwide market of ornamental plants. Some of them are directly collected in forests, but the vast majority is produced in modern commercial systems (*Iqbal*, 1993). The sustainable yield of non woody ornamental species can provide an important income source to local communities of farmers and, at the same time, maintain the natural populations of plants in their natural habitats (*Alves* et al., 2004, *Ticktin & Nantel*, 2004). However the overexploitation of several species resulted in an accentuated decline in the natural stands of these species, therefore, leading to the unsustainability (*Velasquez & Gentry*, 1989), or in a genetic erosion of such species in tropical forests, as is the case of the overexploitation of bromeliads in the Atlantic Rain Forest (*Pompelli & Guerra*, 2004). The cultivation and management of non woody forestry species are appropriate strategies since they provide an increment in the production and quality and reduce the pressure over natural wild remnants (*Alves* et al., 2004; *Rech Filho* et al., 2005).

The Atlantic Rain Forest is one of the most important and endangered biomes in the world containing over than 20,000 different species, 10,000 from which are endemic (*Schäffer & Prochnow*, 2002), its remnants do not exceed 7% of the original area (*Heringer & Montenegro*, 2000).

The biological diversity of Brazilian plants and the different environmental conditions as well as the strategic geographic position of the country are important aspects for the growth of this industry as compared to other regions (*Brazil*, 2004).

The market of flowers and ornamental plants

In 1985 the world market was estimated in US$ 12.5 billion, and in 1999, it raised up to US$ 31 billion (*Groot*, 1999). In 1994, the flower consumption *per capita* in Brazil was US$ 4.0 (*Barletta*, 1995) and in 1998, this value raised up to US$ 7.0 (*Castro*, 1998). It is interesting to compare these values with the *per capita* consumption in Argentina was US$ 24.0. In 2003 the value of the Brazilian industry of flowers and ornamental plants was US$ 1.2 billion and less than 2% of this was destined to the external market

(*Tarnowski Jr*, 2004). Comparatively, the exportation of Colombia was estimated in US$ 550 million and US$ 37 million yearly to USA and Canada, respectively (*Brasil*, 2004). It should be mentioned the case of Spain on which exported, only in the month of june 2003 exported US$ 15,536,540.0 in flowers and ornamental plants. The highest consumption of flowers in Brazil is concentrated is special dates as the mother's and father's days, Valentine's day, international woman's day, Christmas and dad's day. The regular offer of flowers without drastic oscillations could reduce the final price to the consumers thus increasing the consumption and allowing stability in this market (*Claro*, 1998; *Marques & Caixeta-Filho*, 2002). However, it is not enough to increase the flower production in order to fulfill the requirements of the market in specific times. It is necessary the establishment of strategic plans that result in a coordinate production, distribution, and offer all year long based on the different times of flowering of different species in the distinct regions of Brazil (*Agostini & Sazima*, 2003).

The production of tropical flowers is an industry in expansion in Brazil. This activity is considered as one of the most profitable in the agriculture and tailor made for the small-scale agriculture in Brazil (*Tarnowski Jr*, 2004).

The most preferred flower in Brazil is the rose that is the leader in the CEAGESP-SP market. However when considering the aggregate values, the most profitable plants are the violets. This species are normally produced with low costs and obtains a good market value (*Marques & Caixeta-Filho*, 2002). Besides of roses and violets, Heliconia, orchids and bromeliads comprises the most popular ornamental and flower species in the Brazilian market (*Carneiro* et al., 1998).

The market of flowers and ornamental plants in the State of São Paulo (Brazil), was equivalent to US$ 228 million in the cycle 1995–1996. These values are higher than the ones observed for other important agricultural products as mango (US$ 215 million), tangerine (US$ 208 million), non-processed cotton (US$ 106 million), non-processed rice (US$ 31 million), tomato for industry (US$ 16.5 million). Those values are quite similar to the market value of other traditional agricultural products as coffee (US$ 297 million), bean (US$ 259.5 million), and salad tomato (US$ 245 million). It is a rather than strong evidence of the dynamism of the industry of flowers and ornamental plants in the São Paulo State. In 2001, this market increased its production with a sharing of US$ 315 million, considering only the three most important markets of flowers and ornamental plants in Brazil: Veiling Holambra, CEAGESP and CEASA/Campinas (*Kiyuna*, 2002).

For a long time, the States of São Paulo, Minas Gerais and Rio de Janeiro shared 75% of the total production of flower and ornamental plants in Brazil (*Castro*, 1998). This scenario is now under drastic changes due to the establishment and consolidation of several new poles of production, namely in the States of Pernambuco and Ceará (Northeast Brazil). These two states became specialized in tropical flowers, as well as in Santa Catarina and Rio Grande do Sul States (*Perassoli*, 2004). In 2004, the State of Ceará exported nearly US$ 3 million, mostly roses, and the Netherlands was the main destination. In 2003, 72% of the roses produced in Ceará State were sent to Holland. The remaining 20% of the production have been exported to Portugal over the last four years. Other ornamental plants, as helicons, alpinias, anthuriums, zinziber, chrysanthemums, gladiolus, and others, were also exported to European countries (*Mathias*, 2004).

Key points in the industry of flower and ornamental plants

Production technology, including plant protection and the new biotechnologies, transport, post-harvest, conservation and distribution are key points in the productive chain of flower and ornamental plants. Handling and transport strongly affect the final product quality and they are the most expensive components in the production costs (*Perassoli*, 2004). In Brazil, it is important to note that approximately 90% of production and consumption of flower and ornamental plants occur in a distance of 500 km each other (*Kras*, 1999).

The production technology is affected by several factors among them the structure of the plant, the mode of propagation, the nutrition, the plant protection, the photosynthetic capacity, and the specific management. Such information is based on advanced knowledge regarding to the biology of the species to be explored (*Arditti & Ernst*, 1993).

Another aspect to be considered in the production of flower and ornamental species regards the toxicity to humans and animals as it is the case of the species *Codiaeum variegatum*, *Nerium oleander*, *Diefembachia* spp., *Euphorbia milli*, *Caesalpinea pulcherrima*, *Euphorbia leucocephala*, *Brugmansia suaveolens*, and *Monstera deliciosa* among others.

Biotechnologies to the industry of flower and ornamental plants

Plant breeders are continually striving to improve many aspects of crop production. These include increased crop yield, nutritional value, and efficient use of water, improved post-harvest crop quality, and resistance to pests. A number of new varieties of flower and ornamental plants are continuously developed in response to the new demands of the market. Plants with altered morphology and pigmentation, flowers with differential fragrance and with prolonged shelf life are the targets by the market point of view. On the other hand the farmers are interested in plants with agronomic traits associated with resistance to biotic and abiotic stresses, as well as with the exploitation profitability.

In the last years, the advances of new biotechnologies were progressively incorporated in the industry of flower and ornamental plants. The development and application of molecular tools allowed the better understanding of the genetic structure of several species. The techniques of genetic markers may allowed to better design the breeding programs and the technology of recombinant DNA may allow the modification of several traits through the native or foreign genes transference.

Transformation of ornamental and flower plants

Methods of transformation allow the introduction in the genome of the host plants of specific traits. The *in vitro* regenerative competence is an essential step for the application of transformation techniques. Even considering that some species are recalcitrant to *in vitro* culture and others may be unstable (*Cassells,* 2002), several methodologies of genetic transformation are well established for ornamental and flower plants (*Zuker* et al., 1998). Petunia was the first transgenic ornamental plant (*Meyer* et al., 1987), and since then several transformation techniques have been employed in ornamental plants. At least 30 ornamental species were transformed including anthurium, begonia, clover, chrysanthemum, gerbera, orchids, iris, petunia, saintpaulia, gentiana, lavender, and roses (*Mishiba* et al., 2000; *Kushikawa* et al., 2001; *Deroles* et al., 2002; *Teixeira da Silva,* 2004; *Rout & Jain,* 2004, and references therein).

Recently, several genes coding to specific traits were isolated. Among them, there are included those related to the flowering and plant architecture, as well as genes associated with the biosynthesis of pigments, of compounds that confergrafrance perfume, and those involved with the increase in the shelf life, specially for cut flowers.

Control of flower development

The control of development and flowering has been subject of several studies. Some studies showed the existence of network of internal signaling pathways that operates in response to endogenous and environmental signals. This network enhances the activation of genes associated to flower identity, resulting in the developmental control of floral organs (*Mouradov* et al., 2002; *Sung* et al., 2003). Studies revealed that at least four different pathways (photoperiodism, vernalization, and gibberellins) control the flowering time in *Arabidopsis* and that these pathways integrate in a specific transcriptional regulator point of the genes *LFY*, *FT* and *SOC1*. Those genes regulate positively other genes that define the identity of the floral meristem (*Mouradov* et al., 2002). Orthologs genes associated with the transition to flowering were not yet isolated in ornamental plants.

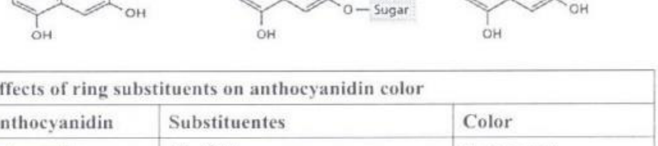
The identity of the floral meristem is promoted by interactions occurring among *LFY* and three transcriptional factors: *AP1, CAL* and *FUL.* Those genes repress the expression of *TFL1*, which maintain the apical meristem of the inflorescence in undetermined state with unlimited growth. It is interesting to note that *tfl1* and *cen* exhibit determined inflorescence with limited growth (*Sung* et al., 2003).

The floral structures, the color and the fragrance are critic factors for the pollinator attraction as well as for the consumers shopping decision. The wide diversity in forms is greatly influenced by changes that can occur in their main organs that keep their identity under the control of homeotic genes. The genes that control the identity of floral organs are classified as the followings: type "A", which controls the identity of sepals and petals; type "B", which control petals and stamens; type "C", which controls stamen and carpel (*Coen & Meyerowitz,* 1991). In Arabidopsis AP1 and AP2 are type "A" genes; AP3 and PI are type "B" genes, and AG is the only one known gene of type "C" (*Vishnevetsky & Meyerowitz,* 2002). Orthologs of genes *ABC* have been isolated in *Antirrhinum* (*Ma & Depamphilis,* 2000), petunia (*Van der Krol & Chua,* 1993) and gerbera (*Yu* et al., 1999). Homeotic mutants of primula have been intensely studied (*Webster & Gilmartin,* 2003). Additional studies on different species can yield new insights that may elucidate the mechanisms controlling the flowering architecture.

Development of new colors

The colors of the flowers, leaves, and fruits are important traits in the ornamental plants. Pigments like anthocynanins, which are a class of flavonoids, are responsible by the red, violet and blue colors (*Forkmann,* 1991). Naturally, in many species (e.g. violaceae family) the flower ontogeny undergoes a dramatic developmental color change (*Farzad* et al., 2002, and *Farzad* et al., 2005).

The synthesis of pigments may be altered by the modulation of genes involved in the biosynthetic pathways *(Fig. 1)*. In the synthesis of flavonoids, the enzyme chalcone synthase (CHS) is very important, once it is catalyzes the condensation of three acetate residues from malonyl-CoA with *p*-coumaroyl-CoA to form naringenin chalcone, which is the first committed step of the phenylpropanoids pathway leading to other side branches to produce flavonoids,



Effects of ring substituents on anthocyanidin color		
Anthocyanidin	**Substituentes**	**Color**
Pelargonidin	4' – OH	Orange red
Cyanidin	3' – OH; 4' – OH	Purplish red
Delphinidin	3' – OH; 4' – OH; 5' – OH	Bluish purple
Peonidin	3' – OCH_3; 4' – OH	Rosy red
Petunidin	3' – OCH_3; 4' – OH; 5' – OCH_3	Purple

Figure 1 The structures of anthocyanidins (a), anthocyanin (b) and pelargonidin (c). The color of anthocyanidins depend in part on the substituents attached to ring B. Note that an increase in the number of hydroxyl groups shifts absorption to a longer wavelength and gives a bluer color. Replacement of a hydroxyl group with a methoxyl group (OCH_3) shifts absorption to a slightly shorter wavelength, resulting ina redder color.

isoflavonoids, anthocyanin and others in plants (*Kreuzaler & Hahlbrock*, 1975).

One of the important objectives when breeding ornamental flowers is to obtain a wide variety of flower colors, which is the result of an accumulation of secondary metabolites, such as flavonoid, carotenoid and betalain compounds. Among these last ones, there have been extensively studied the flavonoid pigments, which are widespread in higher plants.

Until now, several *chs* genes have been cloned from monocot, dicot and some gymnosperm species (e.g. *Zea mays*, *Sorghum bicolor*, *Bromheadia finlaysoniana*, *Petunia hybrida*, *Ginkgo biloba*, Arabidopsis, snowdragon, leguminous species and pines) (*Pang*, 2005, and references therein). An application of these techniques is the introduction of the native *chs* gene in anti-sense orientation and lead to a decrease in the levels of anthocyanin. *Ben-Meir* et al., (2002) have cloned *chs* gene and though modified a very important feature in floriculture, the original color of rose, gerbera, torenia and chrysanthemum.

Anthocyanins are glycosides that have sugars at position 3 *(Fig. 2)* and sometimes elsewhere (*Gershenzon*, 2004). Without their sugars, anthocyanins are known as anthocyanidins *(Figure 2)*. Anthocyanin color is influenced by many factors, including the number of hydroxyl and methoxyl groups in ring B of the anthocyanidin *(Fig. 2)*, the presence of aromatic acids esterified to the main skeleton, and the pH of the cell vacuole in which these compounds are stored. In the biochemical conversion of one anthocyanidin in other, some reactions is envolved. For instance, in convertion of dihydrokaempferol in pelargonidin are envolved two enzymes: (i) dihydroflavonol-4-reductase (DFR), which converts dihydrokaempferol to leucopelardonidin and (ii) anthocyanidin synthase (ANS), which convert leucopelardonidin to pelargonidin (*Croteau* et al., 2000). In this context, the biotecnologies techniques can be useful, once with the over expression or repression of one of the genes involved in the biosynthetic routes of those compounds the biosynthesis it can be taken of other with drastic changes in the coloration of flowers and fruits *(Fig. 2)*.

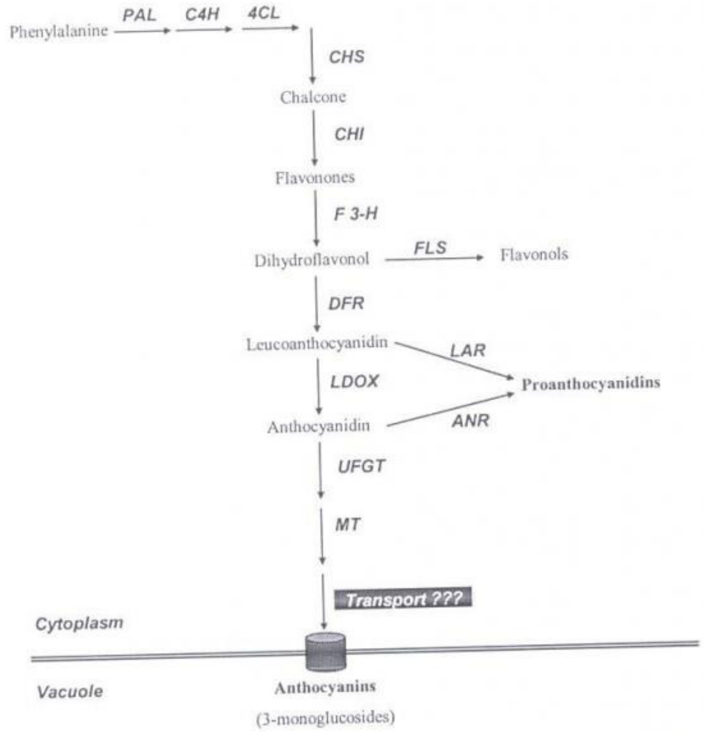


Figure 2 Simplified anthocyanin biosynthesis pathway. Key: PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHI, chalcone synthase isomerase; F 3-H, flavanone 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanidin dioxygenase; UFGT, UDP glucose:flavonoid 3-O-glucosyltransferase; MT, methyltransferase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase (For more details, see *Ageorges* et al., 2005).

Suppression of biosynthetic genes involved in flower color formation is an important approach for obtaining target flower colors. In *Torenia hybrida* the flower's color was successfully modulated by RNA interference (RNAi) against *chs*. By using each of the coding region and the 3'-untranslated region of the *chs* mRNA as an RNAi target, exhaustive and gene-specific gene silencing were successfully induced, and the original blue flower color was modulated to white and pale colors, respectively (*Fukusaki* et al., 2004). These results indicate that RNAi is quite useful for modulating flower colors of commercially important garden plants.

The carotenoids pigments are a class of isoprenoids responsible by the yellow, orange and red colors in several flowers and fruits. The genetic mechanisms controlling that biosynthetic pathway were already elucidated (*Cunningham & Gantt*, 2002). However, it was not yet observed any application in flowers to date, but in a near future possibly will observe many flowers with colors and forms altered through the biotechnology techniques will be observed.

Development of novel fragrance in flowers

The breeding programs plants resulted in a reduction in the fragrance of the flowers, revealing that the mechanisms governing that trait should be more studied. To date several genes directly involved in the production of fragrance were identified. The development of new varieties with modified composition of volatile compounds could increase the ornamental value of several species. However, this area requires additional efforts to deepen on the basic mechanisms associated to these traits.

Improving floral scent is one of the goals of the US$ 20 billion per year horticulture industry. The aroma of a flower can contain as few as seven different oils, such as in snapdragon (*Antirrhinum majus*), or as many as 100 such as in orchids. In snapdragon flowers, the volatile ester methyl benzoate is the most abundant scent compound (*Vries*, 2005). Dudareva (2000) isolated a novel *S*-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase (BAMT), the final enzyme in the biosynthesis of methyl benzoate, and characterized its corresponding cDNA. Additionally, the complete amino acid sequence of the BAMT protein has only low levels of sequence similarity to other previously characterized proteins, including plant O-methyl transferases. For this same species, Tholl and co-workers (2004), discovered that *A. majus* possess a heterodimeric GPPS (geranyl diphosphate synthase) like that previously reported from *Mentha piperita* (peppermint). The *A. majus* cDNAs encode proteins with 53% and 45% amino acid sequence identity, respectively, to the *M. piperita* GPPS small subunit (GPPS.SSU). Expression of these cDNAs in *Escherichia coli* yielded no detectable prenyltransferase activity. However, ative GPPS large subunit (GPPS.LSU) was obtained when each of these cDNAs was coexpressed with the *M. piperita*, which shares functional motifs and a high level of amino acid sequence identity with geranylgeranyl diphosphate synthases (GGPPS), active GPPS was obtained. Using a homology-based cloning strategy, a GPPS.LSU cDNA also was isolated from *A. majus*. Analyses of tissue-specific, developmental, and rhythmic changes in the mRNA and protein levels of GPPS.SSU in *A. majus* flowers revealed that these levels correlate closely with monoterpene emission, whereas GPPS.LSU mRNA levels did not, indicating that the levels of GPPS.SSU, but not GPPS.LSU, might play a key role in regulating the formation of GPPS and, thus, monoterpene biosynthesis.

The recent progresses obtained in the development of molecular techniques and understanding of mechanisms of regulation of biosynthesis pathways of volatile will allow the obtention of flowers with modified scent. The increasing knowledge about floral scents is likely to have a bearing on the perfume industry as well as on flower sellers.

Post-harvest biotechnologies

Photosynthesis is one of the most important factors in plant growth and development. Starch and sugar synthesized during photosynthesis are stored in the stems, leaves and petals, and provide the essential food for flower opening and its maintenance during post-production. The post-harvest life of potted plants and cut flowers is often limited by their inability to maintain photosynthesis under the lighting conditions of the interior environment where they are held, so it is important to ensure high carbohydrate levels in plants at harvest time. This can be achieved by growing plant under optimum light conditions (*Serek & Trolle*, 2000).

The quality and post-harvest life of many plants and flowers are often reduced by the presence of ethylene in the environment. Ethylene has a variety of deleterious physiological effects, such as bud, flower or leaf abscission and senescence (*Zacarias & Reid*, 1990). Climateric flowers have short life due to the sensibility to ethylene (*Thompson & Wang*, 2002). In the ethylene biosynthetic pathway, there are two key enzymes: ACC-synthase and ACC-oxidase, which are involved in the ACC cyclization and subsequent conversion to ethylene (*Crozier* et al., 2000). It has been possible the modification in the levels of this hormone through the introduction of genes involved in the synthesis of ACC-synthase or ACC-oxidase in anti-sense orientation. Other possibility is the introduction of genes associated to ACC degrading enzymes thus interrupting the ethylene biosynthetic pathway (*Thompson & Wang*, 2002). Also, the effects of ethylene may be altered reducing the ability of the plant to perceive it (*Stearns & Glick*, 2003). Studies with clover showed that the suppression of ethylene activity occurred in transformed plants with the gene in anti-sense orientation. Thus flowers showed delayed petals senescence and a prolonged shelf life as compared to the control flowers (*Kosugi* et al., 2002).

Studies were realized aiming at to investigate the differential responses of flower opening to ethylene in rose cultivars. In these studies were cloned cDNA fragments of three Rh-ACSs (*Rosa hybrida* – ACC Synthase) and one Rh-ACO (*Rosa hybrida* – ACC Oxidase) genes that were designated as Rh-ACS1, Rh-ACS2, Rh-ACS3 and Rh-ACO1 respectively. Additionally, Northern-blotting analysis revealed that among three genes *acs*, ethylene-induced expression patterns of Rh-ACS3 gene corresponded to ACS activity and ethylene production in both cultivars. The results obtained suggests the existence of cultivars more sensitive to ethylene than others; and the changes of Rh-ACS3 expression caused by ethylene might be related to the acceleration of flower opening in the cultivars more sensitive cultivars and the inhibition in the more tolerants ones (*Ma* et al., 2005).

Ethylene biosynthesis in higher plants is developmentally and environmentally regulated. In a study to investigate the regulation of ACC synthase gene expression, the promoters of *Arabidopsis thaliana* ACS genes, AtACS4, AtACS5, and AtACS7, were fused to a GUS reporter gene, and the recombinant transgenes were introduced into Arabidopsis to produce three groups of AtACS::GUS transgenic plants (*Wang* et al., 2005). The data on histochemical and fluorometric studies revealed that promoters of AtACS4, AtACS5, and AtACS7 were all active in dark-germinated seedlings. AtACS5 had the highest promoter activity in leaves of 2-week-old light-grown seedlings among the three AtACS genes studied. The promoter activities of all these AtACS genes were also found in the reproductive organs. AtACS5 and AtACS7 were highly expressed in petals, sepals, carpels, stamens, cauline leaves, inflorescence stems, and siliques, while AtACS4 expression was undetectable in the petals of open flowers (*Wang* et al., 2005). Accordingly, each AtACS gene had a unique expression profile during growth and development. It appears that at any developmental stage or any growth period of Arabidopsis, there is always a member of AtACS multigene family that is actively expressed.

Micropropagation-based biotechnologies applied to ornamental species and germplasm conservation

The floriculture industry is driven by novelty and plant biotechnology has opened up new ways for the production of crops with improved traits, and it is also a useful tool for the breeding of flowers. Concurrently, basic scientific research in recent years has provided a better understanding of plant regeneration, genetics, growth and development. While the fundamental techniques to achieve *in vitro* plant morphogenesis have been well established for a number of years, innovations in particular aspects continue to be made (*Phillips*, 2004). Such technical innovations comprise: (**i**) the manipulation of the gaseous and/or environment (*Zobayed* et al., 2004; *Kozai* et al., 2005); (**ii**) novel light sources provided by light-emitting diodes (*Hahn* et al., 2000; *Nhut* et al., 2003) and cold cathode fluorescent lamps (*Tanaka* et al., 2004); (**iii**) the use of film culture vessel made of fluorocarbon polymer Neoflon® PFA film (*Giang & Tanaka*, 2005; *Tanaka* et al., 2005) and alternative nylon film culture system (*Tanaka* et al. 1988; *Nhut* et al., 2005a); (**iv**) new bioreactor designs to facilitate economical large-scale production of plants (*Paek* et al., 2000; *Paek* et al., 2001; Etienne & *Berthouly*, 2002; *Fári* et al., 2004; *Ziv*, 2005); and (**v**) application of thin cell layer (*Nhut* et al., 2003) and synthetic seed (*Nhut* et al., 2005b) techniques. Surely, those technical innovations can significantly improve *in vitro* performance of ornamental species. Moreover, a number of candidate genes are being identified related to traits of interest such as flower color, floral organogenesis, morphogenic responses, fragrance, longevity, growth habit, variegation, disease and insect resistance and hardiness have been focused during biotechnology-based breeding program (*Mercuri* et al., 2001; *Clark* et al., 2003; *Lu* et al., 2003; *Lewinsohn* et al., 2003; *Phillips*, 2004). *In vitro* plant regeneration is often the most important step for successful implementation of various biotechnological tools used for plant improvement programs. However, it is known that due to genotype-specificities cultivar selection has led on the suitability for transformation (reasonable regeneration and gene transfer frequency, acceptable genetic background) and the commercial value of the cultivar that is well established in the global marketplace and has excellent grower characteristics (*Lu* et al., 2003). The continuous development of new varieties of flower and ornamental plants in response to the new demands of the market demanded efficient clonal propagation strategies.

Although White first successfully cultured plant tissues in 1934, Morel's success with rapid *in vitro* propagation of orchids in 1960 was a major stimulus to the application of tissue culture techniques to the propagation of floriculture crops (*Sagawa & Kunisaki*, 1983). The subject has been extensively reviewed (*Sagawa & Kunisaki*, 1983; *Cassells*, 2002; *Rout & Jain*, 2004; *Teixeira da Silva*, 2004). Cell and tissue culture-based methods for ornamentals have been developed and have rapidly assumed economic importance, and now, they are used widely and routinely. The major applications of *in vitro* techniques to ornamental species include the large-scale propagation, preservation of genetic resources, and the recovery of transgenic plants. In this particular micropropagation is the technique of widest application in ornamental plant industry. Historically, as judged by the success of transferring laboratory results to actual commercial application, micropropagation of flowering and ornamental plants was the first (both for large-scale clonal propagation and production of pathogen-free stocks), followed by some vegetable crops (mainly for elimination of viral and bacterial diseases), and only later for fruit trees and plantation crops (for virus elimination, commercial clonal and rootstock propagation) (*Altman & Ziv*, 1997).

As mentioned, the flower production in Brazil has a huge potential, and tissue culture-based techniques have been applied by private and public sectors (companies, universities and research institutions) for several species, among them: *Anthurium*, ferns, African violet, orchids, bromeliads, limoniun, begonia, alstroemeria, ammarilis, roses and *Zantedeschia*. Since late eighties, several tissue culture companies were set up in Brazil with major emphasis on internal market. However, there are several constraints there still need to be overcome. Due to phytosanitary restrictions related to diseases, there is an increasing demand for indexed propagative material. Therefore, commercial or public micropropagation laboratories in Brazil still face problems related to low efficiency production systems, reinforcing the need to adopt and adequate propagation technologies based on liquid systems, in order to increase competitiveness. However, some private companies such as ProClone (Holambra, São Paulo) and BIONOVA (Ribeirão Preto, São Paulo State) have also been applying bioreactor-based technologies to produce high quality and certified micropropagated material, some of them directed to external market.

Tissue culture also offers a promising alternative for germplasm storage, since small pieces can be stored *in vitro* in minimal space and under different environments such as reduced temperatures or altered media. Storage germplasm *in vitro* either by cryopreservation or by alternation of media appears to be highly promising. Cryopreservation has been considered the only safe and cost effective option for long-term storage system for *in vitro* cultured plant materials, ensuring genetic and physiological stability (*Engelmann*, 1997; *Engelmann*, 2004). Indeed, germplasm preservation techniques are highly demanded in order to enable the conservation of elite genotypes. For instance, in Brazil several efforts have been conducted in order optimize bromeliad tissue culture for ornamental purpose and conservation purposes (*Mercier & Kerbauyi*, 1993; *Mercier & Kerbauyi*, 1997; *Alves & Guerra*, 2001; *Pompelli & Guerra*, 2005; *Pompelli* et al., 2005).

The manipulation of ploidy level is also a valuable tool in improving crops. Polyploids often generate variants that may contain useful characteristics. In addition, by doubling the gene products, polyploids provide a wider germplasm base breeding studies (*Thao* et al., 2003). For ornamental species colchicine has been successfully applied to induce polyploids in *Phalaenopsis* (*Griesbach*, 1981) and cyclamen (*Ishizaka & Uematsu*, 1994); oryzalin has also efficiently induced polyploidy in *Alocasia* (*Thao* et al., 2003), *Lilium* and *Nerine* (*van Tuyl* et al., 1992) and *Gerbera* (*Tosca* et al., 1995).

Concluding remarks

The world market of ornamental plants is under constant changes. In this context there are an increasing demand for new products and novel technologies. The biotechnologies comprise several tools that may be applied for the characterization, domestication, conservation, mass propagation and improvement of ornamental plants. The world ornamental plant productive chain is highly competitive and open to new technologies. This industry was the first sector in agriculture to massively include the biotechnologies in its productive chain. Considering the huge plant genetic diversity and the different climatic conditions in Brazil there are several windows of opportunities for the modernization of this productive chain looking at the internal and external markets. Moreover, the development and application of new tools in cell and molecular biology are key components in this industry.

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