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Review

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Molecular Investigations in Ornamental Floricultural Plants

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Introduction

Molecular research has indeed received a rousing welcome by the booming floriculture industry, which constantly seeks variety for its exotic ornamentals. But for a few exceptions like Germany, largely genetically modified (GM) food crops have been frowned upon (Anonymous 2004), viewed with suspicion and legislated against by agricultural economies. However, the anti GM pressure groups are non-existent in the floriculture industry. The insatiable hunger for variety drives the bloom lovers all over the world. Intervention of molecular research in ornamental plants and their potential of mass propagation provides the sought after value addition and real product differentiation. This deliverable capability of molecular research has opened new and lucrative vistas in floriculture. Thanks to GM, longer vase life and lasting fragrance add value while colour and chimera variance (Misra et al. 2003) continue to create its niche in the global floriculture trade. The resultant benefits of increased profits in commercial floriculture should attract molecular research to this cash rich and ever in demand industry. Though potential of gene technology is just as exciting in floriculture crops as in food crops (Wagstaff et al. 2002), till now, very few ornamental floricultural crops are being taken up for molecular research. Only genetically modified carnation for flower colour, has reached the market (Chandler 2003). The other popular cut flowers like roses, chrysanthemum, tulip, gerbera, lily and orchids are still in early days of molecular research.

Few reviews are available on molecular studies on ornamental plants. Notable among them are on biotechnological approaches for floricultural species by Woodson (1991), Hutchinson et al. (1992), Robinson and Firoozabadi (1993), Mol et al. (1995) and Pattanayak and Kumar (2000). Recently, a review on commercialisation of genetically modified ornamental plants is published by Chandler (2003). A review on genetic manipulation of floral pigmentation genes by Mol et al. (1989, 1999) depicts the detailed knowledge of the genetics and biochemistry of pigment synthesis in floricultural crops. Strategies to use genetic modification to improve vase life of flowers have been reviewed and very well documented by Van Altworst and Bovy (1995). A book on biotechnology of ornamental plants has also been published by Geneve et al. (1997).

In the present review, we have discussed the molecular approaches used to genetically modify ornamental floricultural plants utilizing genetic engineering through recombinant DNA technology (Tanaka et al. 1998a), *Agrobacterium*-mediated genetic transformation and gene transfer using gene gun through microprojectile bombardment (Knapp et al. 2002; Elomaa et al. 2003). The transfer of green fluorescence protein in transgenic plants (Stewart 2001, Sergei et al. 2002, Richards et al. 2003) and their use in the study of insect resistance (Zhu et al. 2003), gene silencing (Stam et al. 1997; Wassenegger and Pelissier, 1998; Ben-Ari 1999; Vaucheret and Fagard 2001; Yu and Kumar 2003), production of dwarf plants by genetic engineering (Petty et al. 2003), manipulation in floral pigment by antisense chalcone synthase gene by inhibiting flower pigmentation (Krol et al. 1988) or by chalcone synthase gene (Nakatsuka et al. 2003) and genetic manipulation of delphinidine-based anthocyanins in the flowers of transgenic plants (Mol et al. 1989; 1999; Holton et al. 1993; Holton and Tanaka 1994; Yoshida et al. 1995; Tanaka et al. 1998b; Abe et al. 2002; Ueyama 2002) have been discussed in light of the current literature available on the subject. Programmed cell death (Panavas et al. 1999; 2000; Solomon et al. 1999; Beers et al. 2000; Rubinstein 2000; Hunter et al. 2002) and anti-senescence gene ACC- to increase vase life (Van Altvorst and Bovy 1995) of carnation (Chandler, 2003) have also

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been discussed. An altered flower morphology (Jordan et al. 1996; Mol et al., 1998), and control of flowering (Putterill et al. 1995; Levy and Dean, 1998; Cerdan and Chory 2003; Hsu et al. 2003) are also the important topics ineluded in this review. Work is in progress on floral introduction of fragrance into cut flowers and pot plants by genetic modification. Various genes responsible for this fragrance have been identified (Vainstein et al. 2001) but not commercialised. A compact review of molecular studies on different floriculture crops has been presented in Table 1.

Table 1. An overview of molecular studies on various plant species, their common names in parentheses.

Antirrhinum majus	
Pigmentation	Sommer & Saedler (1986); Coen et al (1988); Almeida et al. (1989); Martin et al. (1991)
Genetic Transformation	Cui et al. (2003)
Flower morphogenesis	Sommer et al. (1990)
Azalea sp. (Rhododendron)	
Altered flower morphology	De Schepper et al. (2001); Knapp et al. (2002)
Chrysanthemum sp.	
Insect resistance	Annadana et al. (2002)
Pigmentation	Suzuki et al. (2004)
Dwarfness of plants	Petty et al. (2003)
Altered flower morphology	Teixeira& Fukai (2003)
Genetic Transformation	De Jong et al. (1995); Boase et al. (1998); Annadana et al. (2002); Outchkurov et al. (2003); Petty et al. (2003); Teixeira & Fukai (2003)
Chimera isolation	Misra et al. (2003)
Dianthus caryophyllus (carnation)	
Senescence/ Programmed cell death	Park et al. (1992); Savin et al. (1996); Jones and Woodson (1999); Rubinstein (2000);
Pigmentation	Zuker et al. (2002)
Genetic Transformation	Lu et al. (1991);
Forsythia sp.	
Pigmentation	Rosati et al. (2003)
Gerbera hybrida (gerbera)	
Pigmentation	Helariutta et al. (1993); Helariutta et al. (1995); Martens and Forkmann (1999); Elomaa et al. (2003)
Gladiolus sp. (gladiolus)	
Disease resistance	Raj et al. (2002)
Genetic Transformation	Babu and Chawla (2000)
Hemerocallis spp. (daylily)	
Senescence/ Programmed cell death	Valpuesta et al. (1995); Guerrero et al. (1998); Panavas et al. (1998; 1999; 2000)
Ipomoea tricolor cv. Heavenly blue (morning glory)	
Pigmentation	Inagaki et al. (1994); Yoshida et al. (1995); Durbin et al. (2000); Suzuki et al. (2000)
Lilium spp. (Asiatic hybrid lily)	
Pigmentation	Abe et al. (2002); Nakatsuka et al. (2003)
Genetic Transformation	Hoshi et al. (2004)
Lisianthus spp.	
Pigmentation	Nielson et al. (2002)
Lotus japonicus	
Genetic Transformation	Jiang and Gresshoff (1997); Szezyglowski et al. (1998); Lombari et al. (2003); Pacîos-bras et al. (2003)
Nod factor	Dēnariē et al. (1996); Spaink (2000); Mathesius (2001); De Long et al. (2002);
Green fluorescence protein	Quaedvlieg et al. (1998); Pacîos-bras et al. (2003)

Table 1. An overview of molecular studies on various plant species, their common names in parenthesis. (Continue)

Narcissus spp. (daffodil)	
Senescence/ Programmed cell death	Hunter (2002)
Orchids a) ***Cymbidium hybrida***	
Pigmentation	Johnson et al. (1999)
b) ***Bromheadia finlaysoniana***	
Pigmentation	Liew et al. (1998a;b)
c) ***Dendrobium nobile*** and ***D. phalaenopsis***	
Genetic Transformation	Yu et al. (2001); Men et al. (2003a;b)
d) ***Oncidium***	
Disease resistance	Liau et al. (2003)
Altered flower morphology	Hsu et al. (2003)
Genetic Transformation	Liau et al. (2003)
e) ***Phalaenopsis***	
Senescence/ Programmed cell death	Do and Huang (1997)
Petunia hybrida (petunia)	
Pigmentation	Meyer et al. (1987); van der Krol et al. (1988); Chuck et al. (1993); Huits et al. (1994); Jorgensen et al. (1996); Bradley et al. (1998); Yamazaki et al. (2002); Mori et al. (2004)
Senescence/ Programmed cell death	Tang et al. (1994); Shaw et al. (2002); Chang et al. (2003); Clark et al. (2004)
Rosa hybrida (rose)	
Pigmentation	Holton and Tanaka (1994); Tanaka et al. (1995)
Torenia fournieri (torenia)	
Genetic Transformation	Suzuki et al. (1997; 2000); Aida et al. (2000)
Pigmentation	Ueyama et al. (2002)

Molecular investigations for floral colour modification

Genetic engineering offers potential to generate new cultivars with altered flower colour. Flower colour is due to the presence of carotenoids and anthocyanins. Carotenoids are responsible for yellow to orange colour of flowers, while anthocyanins, like, pelargonidine (more orange-like), cyanidine (more reddish) and delphinidines (more bluish) are responsible for derived pink, red, mauve, purple and blue shades of flowers. Many regulatory genes for anthocyanin biosynthesis have been identified in several plants. The change in colour is due to the novel production of delphinidine-based anthocyanins in the flowers of transgenic plants (Martin and Gerats 1992; Holton et al. 1993; Yoshida et al. 1995; Tanaka et al. 1998b; Mol et al., 1999). There are a few reviews describing flavonoids and anthocyanins biosynthetic pathway (Mol et al. 1989; Stevensen 1991; Holton and Cornish 1995; Davies and Schwinn 1997). The early critical enzyme of this pathway is chalcone synthase. The final colour of the flower is mainly determined by (1) the particular anthocyanin, (2) vacuolar pH (the site of pigment accumulation), (3) the presence and conc entration of flavonols and other co-pigments and (4) formation of metal complexes (Wiering and de Vlaming 1984; Yoshida et al. 1995).

The first flower colour manipulation by recombinant DNA and gene transfer technology was done in Max Planck Institute, Cologne (Meyer et al. 1987) by transferring a maize DFR gene capable of producing pelargonidine in *Petunia* plant producing brick red flowers. This gene created a novel pigmentation pathway in heterologous species. The second successful work was done in Amsterdam by van der Krol et al. (1988) in *Petunia*, blocking the key enzyme chalcone synthase of flavonoid biosynthesis by 'antisense genes'. The reduction in enzymatic activity led to fainter colours. Variegation in regular rings or sectoral patterns were also observed in some transformants. These effects were not limited to homologous plants. Cloning and molecular characterization of chalcone synthase enzyme was done by Koes et al. (1989). Yamazaki et al. (2002) isolated cDNAs encoding 5-GT and 3-GT from *Petunia hybrida* by hybridisation screening with heterologous probes. The function of these isolated cDNAs was identified by catalytic activities for

3-GT and 5-GT exhibited by recombinant proteins produced in yeast (*Saccharomyces cerevisiae*) as a host. They studied substrate specificities of recombinant 3-GT and 5-GT, and expression pattern during flower development of *P. hybrida*. Recombinant PGT 8 protein could convert only anthocyanidins but also flavols into corresponding 3-o-glucosides. The mRNA expression of both 5-GT and 3-GT increased in early developmental stages of *P. hybrida* flower, reaching maximum at stage before flower opening. Southern analysis of genomic DNA by Yamazaki et al. (2002) showed that both 5-GT and 3-GT genes exist in two copies in *P. hybrida*. An understanding of the biochemistry and genetics of flavonoid biosynthesis was largely derived from *Petunia* spp. (Chuck et al. 1993; Huits et al. 1994; Jorgensen et al. 1996; Bradley et al. 1998; Mori et al. 2004).

With the same 'antisense gene' bluer flowers of transgenic *Torenia* were obtained (Aida et al. 2000), while redder flowers were obtained (Ueyama et al. 2002) by co-suppression of flavonoid 3'-5'-hydroxylase gene producing increased amount of cyanidine-type anthocyanins. Co-suppression of the flavone synthase II gene results in decreased amount of flavones and an increased amount of flavonones, yielding pale flower colour.

Anthocyanins are major pigments contributing to carnation flower colour. Many carnation varieties are sterile and therefore molecular breeding is a very attractive approach to create novel colours in this commercially very important crop. The genetically modified moon series of carnation varieties produce mauve, purple or violet flowers. These varieties were developed by an *Agrobacterium*-based transformation method (Lu et al. 1991) from carnation varieties that produce cream coloured flowers. Addition of a *Petunia* dihydroflavonol- 4-reductase (DFR) enzyme ensures that only delphinidine-based pigments are produced in the petals, having unique colours. So far, six commercial varieties of carnation have been developed using this strategy (Chandler 2003). Zuker et al. (2002) have shown that flower colour in carnation cv. Eilat could be changed by using antisense suppression to block expression of structural genes coding flavonone-3'-hydroxylase, an important step in the anthocyanin pathway. The control untransformed flower only accumulates orange-red pigment pelargonidine, whereas genetically transformed plants show flower colour modification upto complete loss of original orange-red colour. These new phenotypes were more fragrant than the untransformed ones and stable for more than 4 years of vegetative propagation.

Elomaa et al. (2003) have identified an R2R3-type MYB factor, GMY B10, from *Gerbera hybrida* (Asteraceae) that shares high sequence homology to and is phylogenetically grouped with previously characterized regulators of anthocyanin pigmentation in *Petunia hybrida*. GMY B 10 is able to induce anthocyanin pigmentation in genetically engineered tobacco (*Nicotiana tabacum*), particularly in vegetative parts and anthers. In *G. hybrida* GMY B10 is involved in activation of anthocyanin biosynthesis in leaves, floral stems and flowers. In flowers of gerbera its expression is restricted to petal epidermal cell layers in correlation with anthocyanin accumulation pattern. They have also shown, using yeast (*Saccharomyces cerevisiae*) two-hybrid assay, that GMY B10 interacts with previously isolated bHLH factor GMYC1. Particle bombardment analysis was used to show that GMY B10 is required for activation. Of a late anthocyanin biosynthetic gene promoter, PGDFR2, cis-analysis of target PGDFR2 showed a sequence element with a key role in activation by GMY B10/ GMY C1. This element shares high homology with anthocyanin regulatory elements characterized in maize (*Zea mays*) anthocyanin promoter, suggesting that regulatory mechanisms involved in activation of anthocyanin biosynthesis have been conserved for more than 120 million years, not only at level of transcriptional regulators but also at level of biosynthetic gene promoters (Grotewold et al. 1998).

As novelty in flower colour has immense potential for commercial floriculture trade, few other crops are also being tried for genetic manipulation in floral pigment, e.g. *Antirrhinum majus* (Coen et al.1988; Almeida et al. 1989; Martin et al. 1991), morning glory (Inagaki et al. 1994; Yoshida et al. 1995; Durbin et al. 2000), rose (Holton and Tanaka 1994; Tanaka et al. 1995), *Torenia* sp. (Suzuki et al. 1997; 2000; Aida et al. 2000; Ueyama et al. 2002), Asiatic hybrid lily (Abe et al. 2002; Nakatsuka et al. 2003) and orchids (Liew et al. 1998a; 1998b; Johnson et al. 1999). *Forsythia* genotykes, early spring, and yellow flowering shrub are widely grown for high ornamental value and hardiness. Flowers of all *Forsythia* genotypes are yellow because of the presence of carotenoids, xanthophylls and lack of anthocyanin petals, a result of organ specific regulation of flavonoid pathway, since anthocyanins are synthesized in sepals and vegetative organs, e.g. stem and senescent leaves. Rosati et al. (2003) modified flower colour in *Forsythia* by inducing anthocyanin synthesis in petals through *Agrobacterium*-mediated genetic transformation with dihydroflavonol 4-reductase from *Antirrhinum majus* (AmDFR) and anthocyanidine synthase from *Malthiola incana* (MiANS) genes. This is the second report of flower colour modification of an ornamental shrub, after rose and first time an ANS gene was used for this study. Rosati et al. (2003) have checked presence of a *Forsythia* FGT (FiFGT) transcript in petals and FiFGT activity on anthocyanidine prior to genetic transformation with an ANS gene from *Malthiola incana* (MiANS) driven by CaMV 35S promoter into fix intermedia cv. Spring Glory wild type (wt) and previously obtained

genetically engineered plants for a DFR gene from *Antirrhinum majus* (AmDFR). Double AmDFR + MiANS transformants showed a novel bronze-orange coloured transgenic flowers, given by *de novo* synthesis of red anthocyanins in petals over yellow carotenoid background. Further analysis showed that qualitative and quantitative modification of flavon-3-ol pools in leaves and petals of transgenic *Forsythia* plants, and no substantial variation of major flavonoid pools upstream the transgenes. *Lisianthus* (*Eustoma grandiflorum* Grise.) is available only in limited range of flower colours including pink, purple and mauve. Nielson (2002) has generated a number of genetically engineered *Lisianthus* flowers with modified flavonol biosynthesis. These genetically engineered plants showed an altered flower with novel red pigmentation (magenta) as a result of modification of flavonol : anthocyanin ratio. The novel flower colour phenotype was stably inherited in two transgenic generations and was apparent under both glasshouse and field conditions.

Malonylation of Anthocyanins

Suzuki et al. (2004) identified enzymes that are responsible for these malonyltransferase activities and established enzymology of biosynthesis of dimalonylated anthocyanin in chrysanthemum flowers. They have identified two cDNAs coding for enzymes catalysing malonyl transfer to anthocyanins : one of them catalyses monomalonylation through malonyltransferase (Dm3MaT1) and other catalyses dimalonylation through dimalonyltransferase (Dm3MaT2). Dm3MaT2 is the first example of an anthocyanin acyltransferase that can catalyse consecutive acyl transfer. Suzuki et al. (2004) have done cDNA cloning, heterologous expression in *Escherichia coli* cells and functional characterization of Dm3MaT1 and Dm3MaT2, which code for enzymes responsible for malonylation steps involved in biosynthesis of anthocyanin pigments.

Improvement of vase life of cut flowers and programmed cell death

A short post harvest longevity remains a major limiting factor for several crops. Separation from plant leads to senescence of flowers. In several species senescence is regulated by ethylene. Flowers rapidly deteriorate due to ethylene biosynthesis and xylem blockage while in vase. The genetic modifications to improve vase life are reviewed by Van Altvorst and Bovy (1995). Ethylene is produced in the flowers by the activity of two enzymes, ACC synthase and ACC oxidase. ACC synthase catalyses the conversion of s-adenosyl methionine to 1-aminocyclopropane-1-carboxylic acid and ACC oxidase (ethylene forming enzyme) catalyses the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Ethylene biosynthetic pathway (Kende 1989) and cloning and sequencing of ACC synthase (Nakajima et al., 1990) and ACC oxidase or ethylene forming enzyme (Spanu et al. 1991) are now well understood. Silver ions are thought to prevent ethylene perception by blocking the ethylene receptor, preventing the autocatalytic production by the flower. All the common formulations contain silver. The Florigene Limited, Australia has developed genetically modified carnation by inserting a c-DNA of petal specific carnation ACC synthase back into carnation, causing inhibition of the production of ACC synthase enzyme by co-suppression of the expression of c-DNA (Chandler 2003). This inhibits the production of ethylene, causing enhanced vase life without any chemical treatment. Till now no such type of carnation has been commercialised. Lots of work has been done on molecular cloning of ACC synthase (Park et al. 1992; Jones and Woodson 1999) and antisense ACC oxidase (Savin et al. 1996) to delay petal senescence in carnation (Rubinstein 2000). Petal senescence related gene ACC oxidase has been structured (Do and Huang 1997) in *Phalaenopsis* and expressed (Tang et al. 1994) in *Petunia*. Long-term solutions will most probably be based on genetically engineered plants with genes that either suppress ethylene synthesis or reduce sensitivity of ethylene. Later objective was targeted by Shaw et al. (2002), who reported action of boers, a mutant of an ERS. They reported Petunia with boers, a mutant allele of boers, an ethylene receptor sensor gene of *Brassica oleracea*. Genetically engineered *Petunia* flowers retained turgidity and pigmentation longer than the control flowers. Transgenic flowers were not affected by exogenous ethylene treatment.

It has also been shown that leaf senescence was delayed with the - tmr (tumor morphology gene, a Ti plasmid locus, encoding for isopentenyltransferase), a cytokinin production gene isolated from *A. tumefaciens* (Smart et al. 1991) in tobacco but delays corolla senescence in *Petunia* by the introduction of senescence-associated gene (SAG 12).

Programmed cell death is a process by which cells of a particular organism die. In animals cysteine proteases emerged as a key enzyme for programmed cell death. In soyabean cells the activation of cysteine proteases was also found instrumental in programmed cell death. An endogenous cystein protease inhibitor gene, inhibited cysteine protease activity and blocked programmed cell death triggered by oxidative stress (Solomon et al. 1999). Proteinase inhibitor genes and proteolytic enzymes have been found to play roles as modulators of programmed cell death in plants (Jones et al. 1994; Beers et al. 2000). In daylily (*Hamerocallis* spp.), several genes for proteolytic enzymes like thiolprotease

gene (Guerrero et al. 1998), nucleases (Panavas et al. 2000), cystein protease (Valpuesta et al. 1995) and several other senescence-associated genes were identified from petals (Panavas et al. 1998; 1999). In daffodil (*Narcissus pseudonarcissus*) several genes associated with perianth senescence were identified (Hunter et al. 2002).

Genetically engineered orchid plants for resistance to fungal pathogen

Genetic engineering techniques can be used to introduce fungal disease resistance genes into orchid genepool. Ferredoxin like protein gene (pflp) is thought to function as a natural defence against infection due to its antimicrobial properties. Introduction of this gene produce *Oncidium* orchid plants resistant to *Erwinia carotovora*, causal agent for soft rot disease (Liau 2003). An expression vector with pflp cDNA, was transformed into protocorm like bodies of *Oncidium* orchid, using *A. tumefaciens* strain EHA 105. Transgenic orchid plants showed enhanced resistance to *E. carotovora*, even when entire plant was challenged with fungal pathogen (Liau et al. 2003).

Genetically engineered insect resistant chrysanthemum flowers

Chrysanthemum (*Dendranthema grandiflora*) is the second important floricultural crop, which is only next to global favourite roses. chrysanthemum is vulnerable to insects. This damage can cause reduction in flower quality and consequently lessened market value.

Two very important pests of chrysanthemum flowers are

1. Western flower thrips, *Frankliniella occidentalis* (Thysanoptera) (De Jager et al. 1995)
2. *Spodoptera exigua* (Lepidoptera: nocturdae)(Cuijpers and Spod-x 1994), which also severely damages other important floricultural plants.

Structural genes coding for *Bacillus thuringensis* (Bt) insecticidal crystral protein can be introduced by *Agrobacterium tumifaciens*-mediated genetic transformation in plants (Tuli and Srivastava 1994) for developing insect resistance (Tuli et al. 1997, 1998). Genes coding for Bt insecticidal crystal protein can also be introduced by particle bombardment in plants for developing insect resistance (Singh et al. 1996). These structural genes should be highly expressed in flowers and leaves of chrysanthemum plant. De Jong et al. (1995) has used 35S-CaMV (Cauliflower Mosic Virus) promoter for foreign gene expression in chrysanthemum plants, but Boase et al. (1998) observed low level expression of desired genes by using 35S-CaMV promoter. Annadana et al. (2002) have found that the enhanced d 35S-CaMV promoter fused to GUS showed bright blue colour but quantitative GUS assay showed low expression in all tissues.

The western flower thrips heavily damage petal tissues of ray florets in chrysanthemum flowers. To develop resistance to thrips in genetically engineered chrysanthemum plants, promoter should highly express insect resistance genes in floral tissues. Recently, Annadana et al. (2002) have found light harvesting cab promoter Lhca 3.st.1 as strong promoter for gene expression in chrysanthemum stems and leaves, but the expression of transgenes in chrysanthemum ray florets was 6 fold lower than in leaf tissue (chrysanthemum flower consists of disc and ray florets, ray florets are long florets). To obtain very high foreign gene expression in petal tissues of chrysanthemum ray florets, Annadana et al. (2002) cloned an endogenous UEP-1 (Ubiquitin extension protein) promoter and tested with GUS reporter gene. They compared gene expression levels with 4 heterologous promoters - 1) multicystatin (PMC) from potato, 2) eceriferum (CER6) from *Arabidopsis*, 3) zinc finger transcription factor (EPF2-5) from petunia and 4) chalcone synthase (chs-a) from petunia. They also compared expression levels of different constructs in chrysanthemum disc florets, ray florets and leaves. The highest mean expression in petal tissue of disc and ray florets of chrysanthemum was conferred by UEP1 promoter, followed by CER6 and EPF2-5. The UEP-1 promoter in chrysanthemum ray florets confers over 50-fold increase in expression as compared to CaMV35-S based promoters.

Genetic engineering in lotus

Leguminous plants are able to have a mutualistic symbiosis with many genera of bacteria of the family Rhizobiaceae under nitrogen deficiency conditions. This symbiosis starts with signal molecule interchange between plant and bacteria that ends in the formation of special plant organ called nodule on the root. One of the first detectable modifications, during formation of nodule on root, are differences in calcium distribution at cell membrane of root hair followed by root hair curling, and initiation of organized cell divisions in plant root cortex. These steps are induced by special rhizobial signal molecules called Nod factors that are secreted in response to plant signal molecules. The latter are flavonoids and isoflavonoids exuded by plant root (Denarie et al. 1996; Spaink 2000). Both plant flavonoids and microbial nod factors have been observed to be directly involved in regulation of distribution of indolacetic acid (IAA). IAA is natural auxin in plants. Rhizobial nod factors are thought to drive formation of root nodule through manipulation of plant IAA levels (Mathesius 2001). Auxin is not uniformly distributed in plant. Highest concentration of auxin

is found in plant cells undergoing cell division, elongation and redirectioning during tropisms like phototropism and gravitropism (Rinhardt et al. 2000). The exact mechanism of auxin transport, signalling and regulation is still not completely understood (De Long et al. 2002). Lotus (*L. japonicus*) has been used as a model leguminous plant because of its easy and rapid growth in lab conditions, self fertility, high level of seed production, small genome and easy transformation with *Agrobacterium tumifaciens* with very good expression of genes in transformants (Jiang and Gresshoff, 1997; Szezyglowski et al. 1998; Lombari et al. 2003). The fusion protein GFP/GUS has also been reported to be very good marker for investigations in lotus (Quaedvlieg et al. 1998).

For detail study, Pacîos-bras et al. (2003) developed genetically engineered plants of *Lotus japonicus* expressing a fusion reporter gene consisting of genes, green fluorescence protein (GFP) and β-glucuronidase (GUS), under control of soybean auxin responsive promoter GH3. These engineered lotus plants expressed GUS and GFP in vascular bundles of roots, leaves and shoots. Lotus root sections exhibited that in mature parts of roots, GUS was mainly expressed in vascular parenchyma and phloem of vascular cylinder. By detecting GUS activity, they described auxin distribution pattern in root of determinate nodulating legume *L. japonicus* during development of nodulation and also after inoculation with purified Nod factors, N- naphthylphthalamic acid (NPA) and indolacetic acid (IAA). Differently than clover, which forms indeterminate nodules, *L. japonicus* presented a very strong GUS activity at dividing outer cortical cells during first nodule cell divisions. This finding suggests different auxin distribution pattern between determinate and indeterminate nodulating legume plants that may be responsible for the differences in nodule development between these groups. By measuring GFP fluorescence expressed 21 days after treatment with Nod factors or bacteria, they were able to quantify the differences in GH3 expression levels in single living root. To link these new data with auxin transport, they measured auxin transport levels by an earlier reported radioactive method. At 48 h after inoculation with Nod factors, auxin transport showed an increase in middle root segment.

Genetic engineering for dwarfness of flowering plants

There is a distinct and a large variety-hungry market seeking compact and shorter cut flowers. Application of genetic engineering techniques to reduce stem length (plant height) avoids spraying of plant growth retardants - a very expensive and undesirable way of producing dwarf plants. *Arabidopsis* gai gene can induce desirable short stem length without affecting flowering time and leaf and flower morphology.

Dwarfism could be induced by transforming plants with the mutant *Arabidopsis* gai (gibberellic acid insensitive) gene to reduce the plant growth by *Agrobacterium*-mediated transformation (Petty et al. 2003). Several transgenic lines of chrysanthemum expressing the gai gene under its own promoter exhibited a range of dwarf phenotypes. In each transgenic line of chrysanthemum, the extent of dwarfism was related to the reduction in response to gibberellic acid.

Genetic engineering for ultra new fragrance of flowers

Various genes responsible for fragrance are being identified (Vainstein et al. 2001), and commercial application is yet to happen. Work is going on in USA, Israel and NewZealand on the development of novel perfumes, or introduction of perfumes into cut flowers and pot plants by genetic engineering. Rose and carnation flowers that smell like citrus plant or freesias or terpene producing pot plants that could act as natural air freshners are the latest areas of research (Chandler 2003). Lavy et al. (2002) transformed a carnation variety, lacking detectable levels of monoterpenes with *Clarkia breweri* lis gene. Molecular and fragrance analysis revealed that ectopic expression of lis leads to production of linalool and its derivatives cis- and trans-linalool oxide in genetically engineered flowers. CaMV 35S constitutive promoter was used in this study. Zuker et al. (2002) have developed genetically engineered carnation plants with several colour modification and more fragrance than untransformed plants. GC-MS head space analyses showed that genetically engineered anti-f3h flowers emit higher levels of methyl benzoate. The new phenotype was stable for more than 4 years of vegetative propagation. Cloning of genes for monoterpene synthesis and their modification (Dudareva et al. 1996) will be of great help in developing floral fragrance.

Gene silencing

Gene silencing is the phenomenon where a single copy of the transgene or some of the copies or multiple copies get silenced/inactivated and results in loss of expression. The endogenous gene having homology with the transgene (Stam et al. 1997; Ben-Ari 1999) also gets silenced resulting in loss in expression of transgene. When multiple copies of a transgene are inserted, such a transgene is likely to be inactivated, the best example is of white flowering mutant of *Petunia hybrida*, where A1 gene was introduced. It was seen that insertion of a single copy gene gave coloured flowers, while insertion of multiple copies gave white flowers

(Finnegan and McElroy 1994; Pattanayak and Kumar 2000), although this inactivation is influenced by the properties of the foreign gene. Gene silencing in plants may occur transcriptionally (Vaucheret and Fagard 2001) or post transcriptionally (Wassenegger and Pelissur 1998; Yu and Kumar 2003) but silencing of endogenous genes seems predominantly post transcriptionally.

Control of flowering

Genetic manipulation of floral timing in ornamental plants has been reviewed by Jordan et al. (1996). The flowering in plants is mainly controlled by the environment. Daylength and temperature mainly control the flowering (Reeves and Coupland 2000; Wallerstein et al. 2002) but it is regulated by light quality also (Levy and Dean 1998; Cerdan and Chory 2003). However, the CONSTANS gene of *Arabidopsis* has been found to promote flowering (Putterill et al. 1995), while a gene (AGL-6) expressed in an orchid *Oncidium* promotes flowering time genes in *Arabidopsis thaliana* (Hsu et al. 2003).

Flower variegation and altered flower morphology

The genetic manipulations of flower morphology in ornamental plants has been discussed by Jordan et al. (1996) and Mol et al. (1998). Flower variegation is a complex phenomenon involving a correlation between somatic polyploidy, (flavonoid) gene expression and flower morphology, as it was seen in azalea (De Schepper et al. 2001). The molecular basis for flower morphology may create novel flowers. The best example for altered morphology are carnations and roses. The deficiens gene from *Antirrhinum majus* (Sommer et al. 1990) and the agamous gene from *A. thaliana* (Yanofsky et al. 1990) are the two genes responsible for control of flower morphology (Hutchinson et al. 1992). The deficiens gene is responsible for replacement of petals by a ring of sepals and conversion of stamens to carpel. Whereas, the agamous gene is responsible for the stamens to develop a whorl of petals and the carpel whorl into repeat mutant flowers (Bowman et al. 1991).

Conclusion

There can be little doubt that during the last decade certain path breaking researches in molecular biology have begun addressing critical issues in respect of ornamental floriculture plants. On one hand, the researchers engaged in ushering attractive varieties through genetic manipulation and on the other hand value addition was successfully achieved by delving deeper into issues such as delayed senescence and disease resistance. The outcomes of such researches have immense commercial value directly affecting the supply side of a huge global market of ornamentals. However, there are many challenges yet to be taken up meaningfully. The grave scenario of unabated global warming threatens the flower varieties of temperate regions and therefore, the intervention of molecular biologists towards strengthening of stress resistance, especially heat and temperature resistance, of temperate floriculture crops is the need of the hour. Apart from insuring this gene pool from a potential catastrophe, the researches in this direction can deliver contemporary commercial success too. Needless to mention that the flowers that naturally grow in temperate regions are most demanded globally. The vast fertile land of sub-tropics and tropical areas could well be used for high value floriculture of temperate crops if these can indeed be made hardy with molecular intervention. The endeavour of the researchers in this area must also be to conquer the last mile - laboratory to the field for it to remain meaningful.

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