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Biotechnological approaches for improvement of flower crops

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Abstract
Unequivocally, horticultural industry has been revolutionized due to contribution by ornamental plants. Flowers and its products are globally traded commodities. Due to rising needs, ornamental plant industry requires new plant varieties with elite traits such as improved anatomical attributes, floral colour, pigments, fragrance and stress tolerance. Therefore, we witness transgenic plant varieties of high aesthetic and commercial value. This century is considered as the era of bio-economy lead by bioscience and biotechnology. Classical breeding strategies for developing new plant lines has some limitations and draw backs i.e. male sterility, degree of heterozygosity etc. Hence, biotechnological approach together with classical breeding methods has been eventually used to modify flower colour, fragrance, appearance of some novel trait, disease resistance etc. Techniques like genetic engineering (GE) has been broadly adopted as more feasible methods to deal with intrinsic obstacles of classical techniques and floral trait modification. Transgenic strategies possess immense potential to produce novel flower phenotypes that are not found in nature. The prime benefit in adopting GE is that any gene from other species gene pool could be introduced in ornamental plants.
Achieving a novel flower colour is considered as a chief commercial asset obtained from transgenic plants. Besides this several other commercial traits are also important which enhance the commercial value of flower cops commercially but a little information is available regarding successful transformation of other valuable horticultural characteristics. This review gives a summarized account of the work done on various aspects of floriculture using biotechnological approaches.

Keywords: biotechnological approaches, colour, fragrance, vase life

1. Introduction
Developing new ornamental cultivars with improved floral attributes is a major goal in floriculture. Biotechnological approach together with classical breeding methods has been used to modify floral colour, appearance as well as for increasing disease resistance. Transgenic strategies possess immense potential to produce novel flower phenotypes that are not found in nature. Adoption of Genetic engineering has supported the idea of floral trait modification. Ornamental plant attributes like floral colour, fragrance, disease resistance, and vase life can be improved by means of genetic manipulation. Therefore, we witness transgenic plant varieties of high aesthetic and commercial value. This review focuses on biotechnological advancements in manipulating key floral traits that contribute in development of diverse ornamental plant lines. Data clearly reveals that regulation of biosynthetic pathways related to characteristics like pigment production, flower morphology and fragrance is both possible and predictable. In spite of their great significance, small number of genetically engineered varieties of ornamental plants has been field tested. Today, novel flower colours production is regarded as chief commercial benefit obtained from transgenic plants. But certain other floral traits are much more important and have high commercial potential. Other than achievements such as novel architecture, modified flower colour, etc., very few reports are available regarding successful transformation of other valuable horticultural characteristics.

Biotechnology in Floriculture
The global flower industry thrives on novelty. 'Classical' flower breeding by continuous crossing and selection has its limitations; for example, no one had succeeded in breeding a blue rose or an orange petunia. Transgenic strategies possess immense potential to produce novel flower phenotypes that are not found in nature. Adoption of Genetic engineering has supported the idea of floral trait modification. Genetic engineering is providing a valuable means of expanding the floriculture gene pool so promoting the generation of new commercial

varieties. Due to rising needs, ornamental plant industry requires new plant varieties with elite traits such as improved anatomical attributes, floral colour, pigments, stress tolerance and disease resistance.

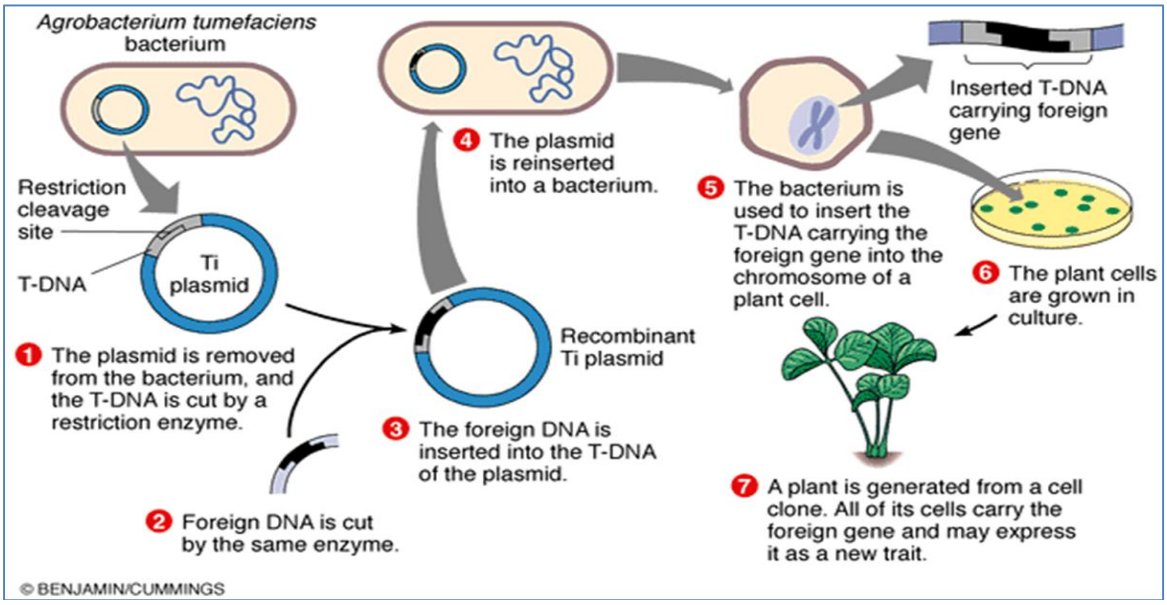
Why floral traits modifications are of special concern?

Ornamental flowers face problems because of troublesome sexual hybridization because of heterozygosity, high chromosome number, inadequate gene pool and elevated sterility; e.g. Being alloploid chrysanthemum has chromosome number 36–75 rather than basic chromosome number 9. Anthurium sp. have life cycle of about 3 years. Developing new cultivar may require long time span of about 8–10 years. Similarly, most of the carnation lines are self-fertile and unable to produce seeds. Transgenic crops are important because of limitations of conventional breeding for attaining the desirable traits and ddevelopment of organisms that express a "novel" trait: normally not found in the species.

Breeding objectives

- New Colours
- Long Vase Life
- Resistant to Biotic and Abiotic Stresses
- Improved Shape
- Improved Floral Scent
- Improved Size

Genetic Engineering: Manipulation of plant genome through recombinant DNA technology to alter plant characteristics. Genetic modification can be used to transfer new specific traits into the plant.



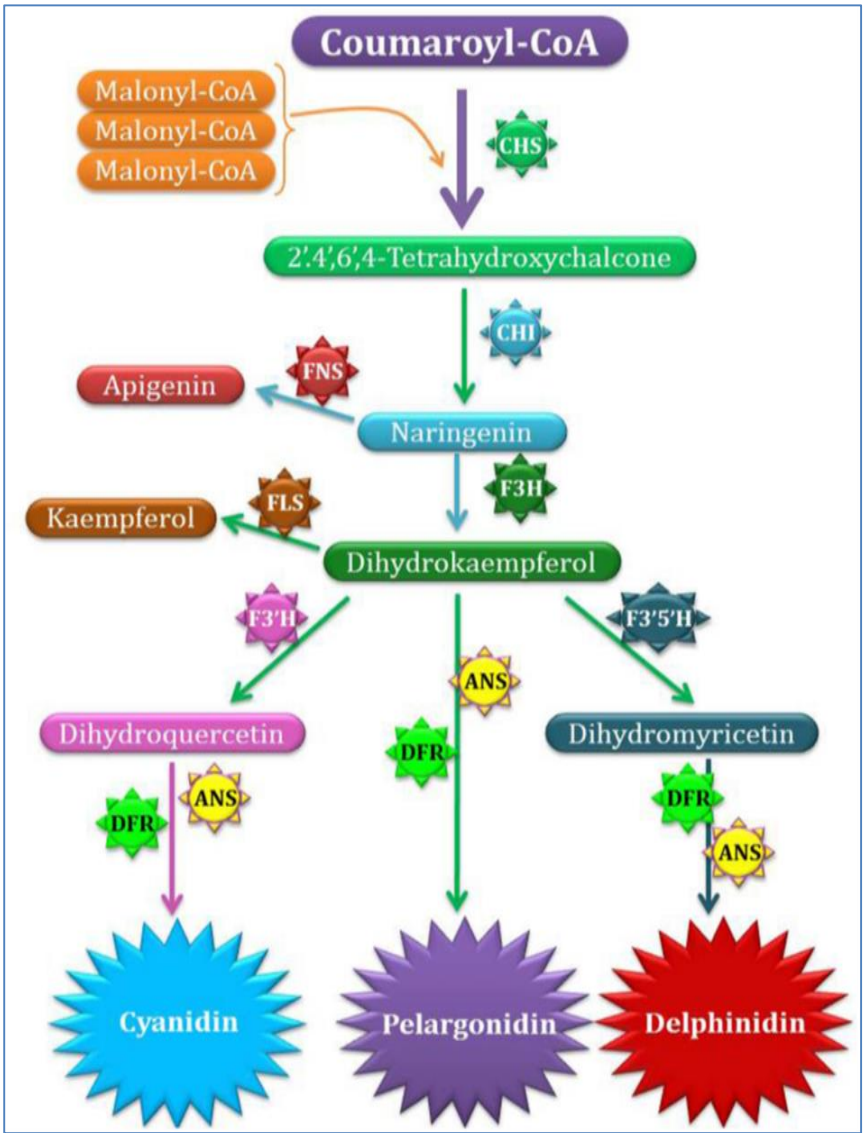
Gene transfer methods	
Indirect	**Direct**
A. Agrobacterium mediated gene trasfer	A. Particle bombardment or micro projectile
1. Most widely used	B. Direct DNA delievery by Microinjection or PEG mediated uptake
2. More economical	C. Ultrasonication
3. More efficient	D. Electroporation
4. Transformation success is 80-85%	E. Electroporotic uptake

Importance of flower colour

Generally, traditional plant breeding strategies are applied to perk up attractiveness as well as effectiveness of ornamental plants. But these strategies face limitations in terms of genes pool and some other characteristics reported in sexually resembling species. During last 20 years, biotechnology has produced innovative and exclusive characters in ornamentals by adopting genes from different plant species. Floriculturists and related entrepreneur are always eager to introduce innovations in flower colours. The major pigments responsible for attractiveness of flower colours are anthocyanins, flavonoids, carotenoids, and betalains. These pigments are primarily based upon six anthocyanidins types i.e., cyanidin, delphinidin, peonidin, petunidin, malvidin, and pelargonidin. Three of the described anthocyanidins i.e., delphinidin, cyanidin and pelargonidin are regarded as major types. Blue flowers tend to have high level of delphinidin and derivatives while intense red flower colour is due to pelargonidin working as anthocyanidin base. Flower colour determines the market value in ornamental plants, act as an attraction of pollinators, function in photosynthesis, act as antioxidants and precursors of vitamin A in human health, helps in seed dispersal. Besides this, flower colour also helps in protecting tissue against photo oxidative damage and shows resistance to biotic and abiotic stresses.

Why we need Modification in colour?

- Modification in flower colour of a variety with desirable agronomic or consumer characteristics e.g. a white carnation from preferable red-flowering variety.
- Flower colour modification is an obvious application of the technology as flower colour is one of the most important traits for the flower breeders and in many species the whole spectrum of flower colour is not available e.g. blue colour in rose, carnation, orchids.
- Change in trend for colour varies from season to season, year to year according to the market demand.
- Novel colour fetches a higher price e.g. price for a single blue rose is about $22 to $33.



CHI- chalcone isomerase
FLS-flavonol synthase
F3H-flavanone 3-hydroxylase
F3'H- flavanoid 3'-hydroxylase
F3'5'H- flavanoid 3' 5'-hydroxylase
DFR- dihydroflavonol 4-reductase
ANS- anthocyanidin synthase
FNS- flavone synthase
CHS- chalcone svnthase

Fig 1: Anthocyanin biosynthetic pathway

Biosynthesis of anthocyanidin: CHS catalyze the formation of Tetrahydroxy calchone. Later on, different enzymes such as CHI, F3H, DFR, ANS catalyze other steps of pigment production. The methyl groups are only added to anthocyanins not to anthocyanidins. The actual pigment colour production is not solely dependent upon the enzyme catalyzing reactions but also depends upon other factors. CHS, chalcone synthase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′, 5′-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; MT, methyltransferase, GT, glucosyltransferase; AT, acyltransferase; FNS, flavone synthase; FLS, flavonol synthase.

Flavanoides and their coloured class compounds anthocyanins are major contributors to flower colour. It is possible to alter flower colour by overexpressing or downregulating these native genes or by introducing a foreign gene to allow new branching of pathway. Roses, Carnations and Chrysanthemums lack blue coloured varieties because of deficiency of flavonoid 3',5'-hydroxylase therefore, they do not accumulate delphinidin based anthocyanins. Petunia and Cymbidium lack brick red/orange varieties due to lack of pelargonidin- based anthocyanins as their dihydroflavonol 4-reductases (DFRs) do not utilize dihydrokaempferol as a substrate. Similarly, Iris also is likely to have DFRs with a similar substrate specificity to that of petunia DFR because it Accumulate delphinidin-based anthocyanins and do not accumulate pelargonidin-based anthocyanins in their petals. Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin.

Genetic Engineering for blue colour

Blue Rose

According to a study conducted by Katsumoto *et al.* 2007 [6] found why roses fundamentally lacked the components required to yield violet/blue flowers? It is because a) "Flavonoid 3', 5'- hydroxylase" (F3'5'H) is deficient in rose b) other factors such as co-pigments and vacuolar pH also affect flower colour and c) the vacuolar pH of rose petal epidermal cells is low (from pH 3.69 to 5.78). The criteria for the selection of possible host cultivars to achieve flower colour modification towards blue by delphinidin production were i) they accumulated flavonols that were expected to be co-pigments ii) they had a higher vacuolar pH iii) ideally, they did not have F3'H activity and iv) they accumulated pelargonidin rather than cyanidin.

In this study, selection of rose cultivars that were suitable for delphinidin production and colour change toward blue was done. Constitutive expression of the viola F3'5'H gene

successfully resulted in delphinidin accumulation and colour changes with a blue hue that has commercial value as far as novel flower colour is concerned. More efficient and dominant modification of the flavonoid pathway toward delphinidin was achieved by functional replacement of DFRs, i.e. the down-regulation of the endogenous DFR gene and overexpression of the iris DFR gene in vivo, in addition to the overexpression of the viola F3'5'H gene. The ability for the exclusive accumulation of delphinidin was heritable by progeny, indicating that the flavonoid pathway was consistently modified toward delphinidin production.

Fig 2: Flower colour changes by delphinidin production: Rose Varieties transformed with pSPB130 and their flower colour changed are shown (left, host; right, a transformant) the production of delphinidin and myricetin indicates that the introduced F3'5'H gene functioned in transgenic roses

Blue-Violet Carnation

The blue gene was isolated in 1991 and patented in 1992. In 1996, Florigene developed mauve-coloured carnation, FLORIGENE Moondust: world's first genetically modified flower on sale. In 1997, developed second genetically-modified carnation, FLORIGENE Moonshadow with a richer and true purple colour. Successfully developed a range of transgenic violet carnations by introduction of a F3′5′H gene together with a petunia DFR gene into a DFR-deficient white carnation. In carnation, the overexpression of the F 3'5'H gene alone was insufficient to convert the metabolic flux fully toward delphinidin biosynthesis. White carnation cultivars that specifically lacked the DFR gene were transformed with the petunia DFR and F 3'5'H gene (Holton 1996 and Fukui *et al* 2003) [4, 3].

Genetic engineering for orange and red colour (pelargonidin):

Substrate specificity of dihydroflavonol 4-reductase (DFR) contributes to determine the hydroxylation pattern. Petunia and Iris lack orange/brick-red colour varieties because their DFR efficiently utilizes dihyromyricetin but does not utilize dihydrokaempferol (DHK); thus does not accumulate pelargonidin. Maize and Gerbera DFR can utilize DHK as substrate owing to deficiency of F3'H and F3'5'H genes. Expression of the maize DFR gene and mutant. Deficiency of competing enzymes (F 3'5' H and F' 3' H) against DFR is necessary for pelargonidin accumulation. Petunia does not accumulate pelargonidin since its DFR does not catalyse DHK. Meyer *et al* 1987 studied that the expression of the maize DFR gene and those of a few other species in a petunia mutant, deficient in FLS, F3'H and F3'5'H, resulted in transgenics with orange flowers, as a result of an accumulation of pelargonidin. The expression of a gerbera DFR gene resulted in the production of more pelargonidin than in the maize DFR gene.

Genetic engineering for yellow colour

The carotenoid content can be also altered by manipulating the degradation. Ohmiya *et al* (2006) [10] have demonstrated that in the white petals of chrysanthemum carotenoids were synthesized but subsequently degraded by carotenoid cleavage dioxygenase (CmCCD4a), resulting in the white colour. Suppression of CmCCD4a (Carotenoid cleavage deoxygenase) gene expression converted the petal colour from white to yellow. By contrast, overexpression of CmCCD4a altered petal colour from yellow to white.

Fragrance

According to a study conducted by Zuker *et al* (2002) [13] the flowers of carnation cv. Eilat have dark orange petals with reddish edges. HPLC and TLC analyses of anthocyanin revealed that only pelargonidin, but not cyanidin, accumulates in this cultivar. To modify carnation flower colour, F3H (flavanone-3-hydroxylase) activity was inhibited by antisense suppression of the corresponding gene: *f3h* was cloned in an antisense orientation under the regulation of the CaMV 35S promote into a binary vector and used to transform carnation cv. Eilat plants. Following transformation, 14 individual transgenic plants were regenerated and grown to flowering in the greenhouse. All plants developed and flowered normally, and six of them exhibited flowers with colour modifications ranging from attenuation to complete loss of their orange/reddish colour. Southern blot test confirmed presence of anti f3h DNA in transformed plants. The level of ISP was not increased in the anti-f3h transgenic plants. Yellow and cream colour of the transgenic plants might be due to unmasking of the yellow ISP (isosalipurposide) as a result of decreased pelargonidin levels. 4 year field tests showed that transgenic plants were more fragrant than control plants. Transgenic plants revealed 7 fold increase in methyl benzoate and other aromatic compounds. The results showed that production of methyl benzoate (responsible for fragrance) is regulated by anti-f3h gene. Fragrance affected by modulation of anthocyanin biosynthesis reveals an intriguing link

between the two secondary metabolic pathways. Fragrance affected by modulation of anthocyanin biosynthesis reveals an intriguing link between the two secondary metabolic pathways. To evaluate whether scent production originating from diverse metabolic pathways (e.g. phenylpropanoids and isoprenoids) can be affected by transcriptional regulators, Zvi *et al* (2012) [14] introduced Arabidopsis Production of Anthocyanin Pigment1 (PAP1) transcription factor into *Rosa hybrida*. The level of phenyl propanoid compound eugenol accumulated in transgenic was upto 20 fold higher than control plants. Level of major emitted volatile compound the sesquiterpene Germacrene D, were upto 8.5 fold higher in transgenic flowers. Emission of the norisoprenoid compound b-ionone was also dramatically increased (up to sixfold) in PAP1-transgenic flowers. By contrast, the internal pool levels of detected terpenoid compounds were similar in PAP1-transgenic and control flowers. Overall, the sum of emitted volatile compounds was up to approximately fourfold higher in flowers of PAP1-transgenic lines when compared with control flowers, whereas the level of all accumulated volatile compounds was not significantly different between the two.

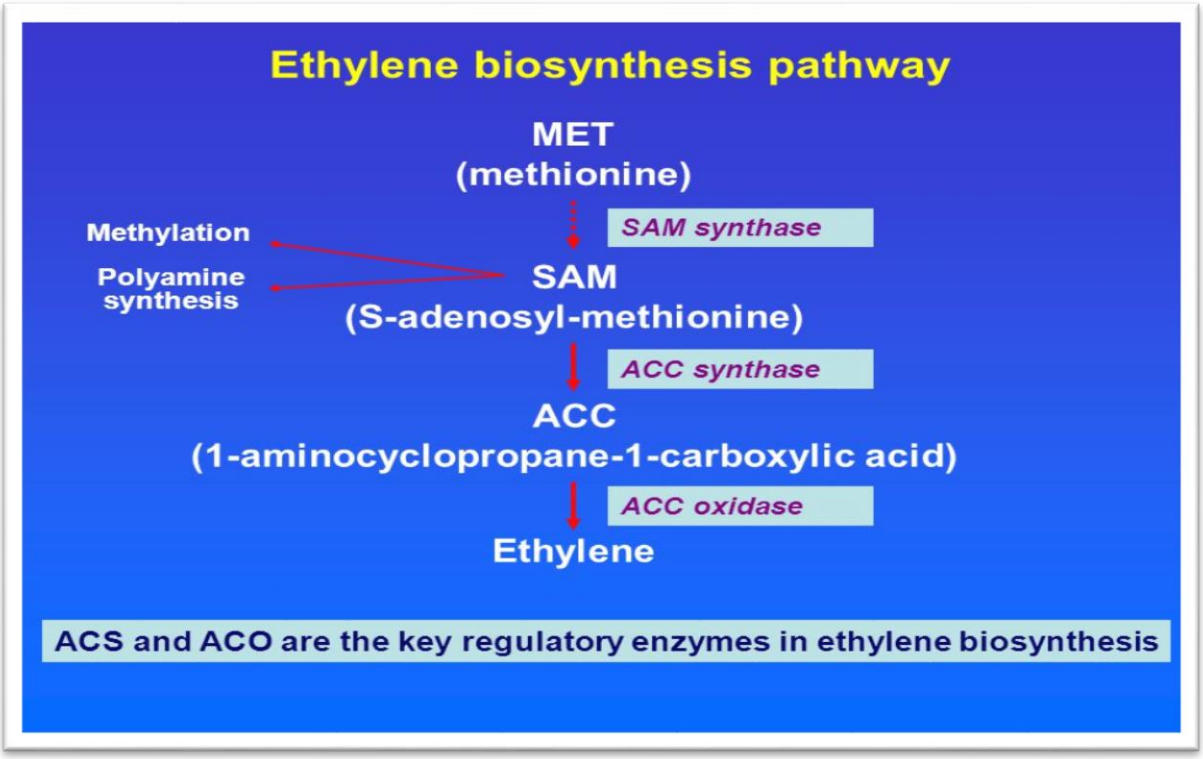


Fig 3: Genetic Engineering for Long Vase Life

- According to a study conducted by Kosugi *et al* 2002 [7] it was observed that when sACO transgene was incorporated in carnation resulted in formation of sACO-1 line with longer vase life as the transgenic plants produced negligible amount of ethylene during natural senescence. sACO-1 transgenic line had an average vase life of 9.5 days in contrast to control line which had 5.8 days. This means transgenic line had 1.6 fold prolonged vase life than control. Moreover, control line had ethylene causing senescence symptoms whereas transagenic lines had ethylene independent symptoms.
  Savin and his co-workers in 1995 [11] reported that When antisense ACO gene was incorporated in carnation cv. 'Scania' and 'White Sim' resulted in production of transgenic plants with enhanced vase life as the transgenic plants had low ethylen**e** production. The average vase life of transgenic carnation flowers was recorded 8-9 days compared to control which had 5 days of vase life. Moreover, control line had ethylene causing senescence symptoms whereas transgenic lines had ethylene independent symptoms. When ethylene was applied exogenously to the transgenic lines, there is induction of ACC synthase and ACC oxidase. As a result of which there was immediate inrolling of petals but the level was much lower than non-transgenic plants.

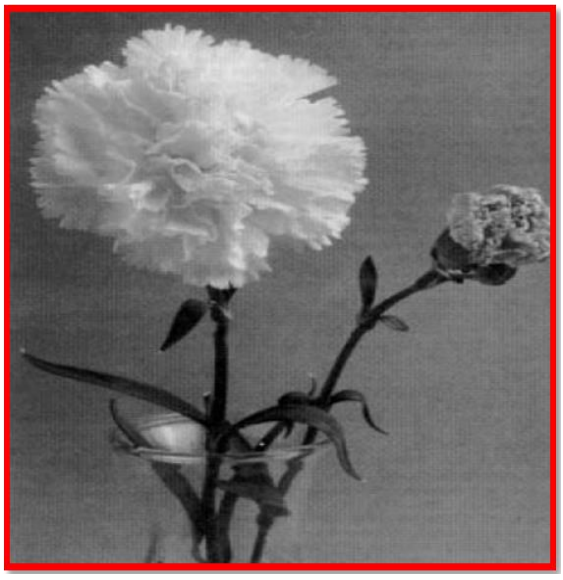


Fig 4: Transformed and control plant after 8 day of harvest

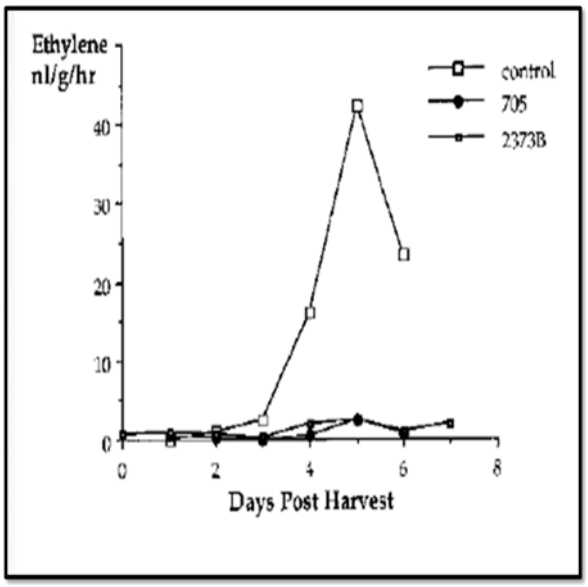


Fig 5: Ethylene production by control and transgenic lines

Other traits

Hong *et al* (2009) [5] studied the expression of At DREB1A gene in *D. grandiflorum* produced sturdy heat tolerance. Upon exposure to wild and transgenic plants exhibited a marked difference in survival rate.

Mariani (2016) [8] studied genetic transformation of *Phalaenopsis amabilis* with resistance to soft rot disease, herbicide and glowing in the dark by particle bombardment method. *Phalaenopsis amabilis* was transformed using particle bombardment method. Tungston coated with DNA (plasmid containing wabasi & bar gene alongwith co- plasmid containing luc gene). This coated DNA was shot into embroys of *Phalaenopsis amabilis.* The transgenic plants which were produced were resistant to soft rot disease and their leaves glowed in the dark.

Conclusion

Genetic engineering overcomes almost all the limitations of traditional breeding approaches.

Recent developments in genetic engineering (GE) for improvement of flower crops to modify flower colour, improve vase life, floral morphology, scent and disease resistance. Knowledge of flower colouration at the biochemical and molecular level has made it possible to develop novel colour. Selection of cultivars that have proper genetic background and flavonoid compositions and/or the artificial down-regulation of a competing endogenous pathway is necessary to obtain a desirable phenotype. GE has demonstrated the best examples such as 'Moon' series of transgenic carnations and transgenic blue rose marketed in North America, Australia and Japan. Advances in the isolation of scent biosynthetic genes have provided the basis and created the opportunity for the biotechnological manipulation of floral scent. Antisense ACS/ACO technology have led to enhanced vase life of flower crops. Worldwide increase in economic worth of flower crops is somehow a result of promising prospects of gene transformation. Novel transgenic flowers may therefore provide prospective benefits to growers and consumers by generating diverse floral appearance, novel colours, and improved fragrance. Genetic modification of ornamental plants is very pragmatic and successful scheme both in scientific and commercial perspective.

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