Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5192

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (1):** 442-451 (2018)

Review Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Genetic and Molecular Basis of Petal Pigmentation in Floriculture Crops: A Review

Sunita Dhakar[1*] and Anjali Soni[2]

[1]Division of Floriculture and Landscaping, ICAR- IARI, New Delhi 110012, India

[2]Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi 110012, India

*Corresponding Author E-mail: sunita.dhakar214@gmail.com

Received: 11.07.2017 | Revised: 19.08.2017 | Accepted: 26.08.2017

ABSTRACT

Flower colours are of paramount importance in the ecology of plants and their ability to attract pollinators and seed dispersing organisms. The primary pigments occurring in plants are chlorophylls and carotenoids accumulated in plastids, anthocyanins and betalains, which are dissolved in vacuolar sap. Flavonoids and carotenoids are widely distributed in plant pigments. Among the factors influencing flower colour, anthocyanin biosynthesis has been the most extensively studied. Each plant species usually accumulates limited kinds of anthocyanins and exhibits limited flower colour. For example, roses and carnations lack violet/blue colour because they do not accumulate delphinidin-based anthocyanins and petunia and cymbidium lack brick red/orange colour due to the lack of pelargonidin-based anthocyanins. Different approaches are used for development of novel flower colour in floricultural crops like hybridization, mutation and genetic engineering. Advances in molecular biology and plant transformation technology have made possible the engineering of an anthocyanin biosynthetic pathway by the overexpression of heterologous flavonoid biosynthetic genes or the down-regulation of endogenous genes in transgenic plants. Transgenic carnations and a transgenic rose that accumulate delphinidin as a result of expressing a flavonoid 3', 5'-hydroxylase gene and have novel blue hued flowers have been commercialized.

Key words: *Carotenoids, Flavonoids, Flower colour, Hybridization, Mutation, Genetic engineering*

INTRODUCTION

There are many beautiful flowers in nature and they show a variety of shapes and colours. Such diversity is acquired through evolutionary processes to ensure successful reproduction by attracting pollinators or by promotion of wind pollination[13]. Flower and fruit colour are important in the attraction of pollinators and organisms for seed dispersal and thus for critical factors in plant survival organisms[26].

Cite this article: Dhakar, S. and Anjali Soni, A., Genetic and Molecular Basis of Petal Pigmentation in Floriculture Crops: A Review, *Int. J. Pure App. Biosci.* **6(1):** 442-451 (2018). doi: http://dx.doi.org/10.18782/2320-7051.5192

From a horticultural point of view, flower colour is one of the most important targets for plant breeders and many different coloured cultivars have been bred using natural mutants or genetically related species. Ornamentals were among the first plants to be hybridized to alter specific colour traits, and fruit and flower colour have contributed to make clear fundamental genetic principles. Today, the market for ornamental plants and cut flowers is rapidly expanding and totals over $70 billion in annual sales. Although increasing postharvest life, altering scent, and modifying flower shape are areas where progress is being actively pursued, much of the novelty in the cut flower industry continues to be targeted toward the generation of new colours. Generally speaking, flower colour is predominantly determined by two classes of pigments: flavonoids and carotenoids. Flavonoids and their coloured class anthocyanins are the most common flower pigments contributing to a wide range of colours are yellow, orange, red, magenta, violet, purple, blue. Carotenoids are ubiquitously distributed in plant as essential components of photosynthesis and confer a red, orange and yellow colour on flower when they are present. These lipid-soluble pigments found embedded in the membranes of chloroplasts and chromoplasts. In addition, a third class of pigment is betalains, it contribute red and yellow colour. Betalains can be found only in certain plants from 10 families of the order Caryophyllales (including beetroot and several important genera such as *Amaranthus, Celosia, Gomphrena, Iresine,* etc.) and in some higher fungi such as fly agaric (*Amanita muscaria*)[6].

Major plant pigments

Primary function of pigments in flowers is to attract insects and other animals which help in cross pollination. The different roles of colour other than attraction of pollinators are function in photosynthesis, protecting tissue against photo oxidative damage, provide resistant to biotic and abiotic stress and symbiotic plant-microbe interaction. It acts as intermediary for other compounds and also shows antibacterial, antifungal, antitumor and anti-inflammatory properties. Chlorophylls and Carotenoids are synthesizing in the Plastid of the cell while Anthocyanins and Betalins are synthesize in vacuole of plant cell.

Table 1: Major plant pigments and their occurrence

Pigment	Compound Types		Compound Examples	Typical Colours
Chlorophylls	Chlorophyll		Chlorophyll a and b	Green
Carotenoids	Carotenes		Lycopene, α-carotene, β-carotene, γ-carotene etc.	Yellow, Orange, Red
	Xanthophylls		Lutein,, Zeaxanthin, Neoxanthin, Violaxanthin etc.	Yellow, Orange,
	Anthocyanins		Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin	Red, Orange, Blue, violet
Flavonoids	Flavones	Flavonol	Luteolin, Apigenin, Tangeritin	Yellow
		Flavanone	Quercetin, Kaempferol, Myricetin	Yellow
		Flavanonol	Hesperetin, Naringenin, Eriodictyol , Homoeriodictyol	Colour less co- pigments
		Flavone	Taxifolin , Dihydrokaempferol	Colour less co- pigments
Betalains	Betacyanins			Red - Violet
	Betaxanthins			Yellow -Orange

Each plant species usually accumulates limited kinds of anthocyanins and exhibits limited flower color by the expression of a specific set of biosynthetic genes, the substrate specificity of key enzymes and/or the temporal and spatial regulation of the biosynthetic genes. Therefore, it is rare for a single species to have the entire spectrum of flower color, although floricultural breeders have always sought novel flower colours[26]. For example, roses, carnations and chrysanthemum, which are the most important floricultural crops, do not accumulate delphinidin-based anthocyanins. Thus, they lack violet/blue varieties. This is attributed to their deficiency of flavonoid 3', 5'-hydroxylase (F3'5'H), a key enzyme in the

synthesis of delphinidin. On the other hand, petunia and cymbidium lack brick red/orange varieties due to the lack of pelargonidin-based anthocyanins because their dihydroflavonol 4-reductases (DFRs) do not utilize dihydrokaempferol as a substrate. Some species that usually accumulate delphinidin-based anthocyanins and do not accumulate pelargonidin-based anthocyanins in their petals, such as iris and gentian, are likely to have DFRs with a similar substrate specificity to that of petunia DFR. Rose DFR can utilize dihydromyricetin as a substrate on the basis of feeding of dihydromyricetin to rose petals. So for the breeding for colour various approaches have been followed.

Flavonoids

Flavonoids are the most common pigment in flower tissue. Flavonoids are derived from phenyl-propanoid pathway with precursor amino acid phenyl-alanine. Though hundreds of anthocyanins have been reported, they are primarily based upon six common anthocyanidins; pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. In terms of biosynthesis, since peonidin is derived from cyaniding and petunidin and malvidin are both derived from delphinidin, there are only three major anthocyanidins; pelargonidin, cyanidin and delphinidin (Figure- 1). Flavonoid biosynthesis has been well characterized in the flowers of petunia and snapdragon, and in the kernels of maize, and is therefore one of the most well studied pathways among plant secondary metabolisms[20,16,29]. Blue flowers tend to have delphinidin and intense red flowers tend to have pelargonidin as the anthocyanidin base. An increase in the number of hydroxyl groups on the B-ring imparts a bluer colour to the anthocyanins derived from the anthocyanidin, while methylation of the 3’ or 5’-hydroxyl group results in a slight reddening.

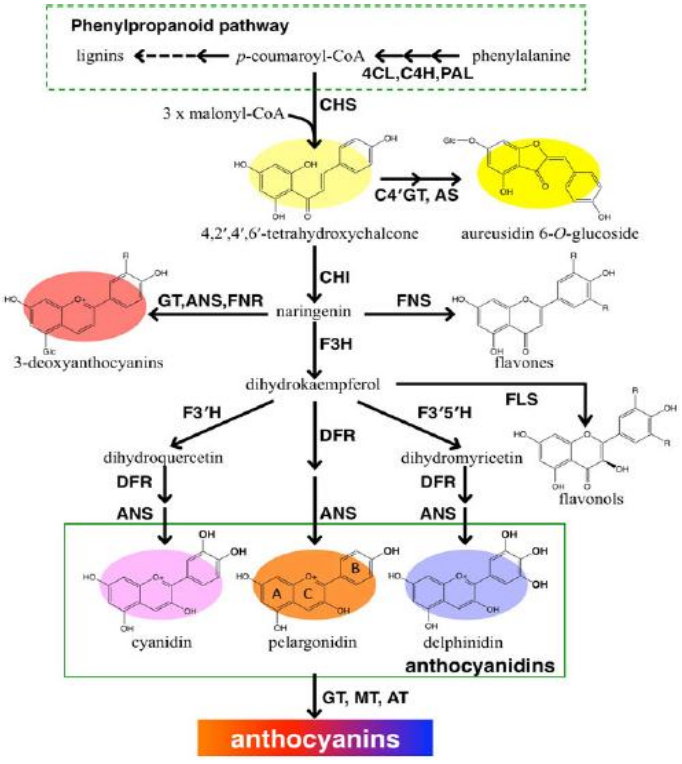

Fig. 1: Flavonoid biosynthetic pathway and flavonoid compounds accumulated in flowers. A simplified pathway derived from several plant species is depicted for ease of explanation. The painted colours shows image of each compound. ANS (Anthocyanidin synthase), AS (Aureusidin synthase), AT (Acyltransferase), C4’GT (Chalcone 4’-Oglucosyltransferase), C4H (Cinnamate-4-hydroxylase), CHI (Chalcone isomerase), CHS (Chalcone synthase), 4CL (4-coumarate:CoA ligase), DFR (Dihydroflavonol 4-reductase), F3H (Flavanone 3-hydroxylase), F3’H flavonoid 3’-hydroxylase, F3’5’H (Flavonoid 3’,5’-hydroxylase), FLS (Flavonol synthase), FNR (Flavanone 4-reductase), FNS (Flavone synthase), GT (Glycosyltransferase), MT (Methyltransferase), PAL (Phenylalanine ammonialyase)[23].

Table 2: Genes involve in pigment synthesis

Enzyme	Gene	Species
CHS (Chalcone synthase)	*Chs*	Antirrhinum, Chrysanthemum, Orchid, Rosa, Dianthus
CHI (Chalcone isomerase)	*Chi*	Antirrhinum, petunia, Dianthus
F3H (Flavone 3-hydroxylase)	*F3h*	Antirrhinum, Orchid, Dianthus, Chrysanthemum
F3'H (Flavonoid 3' hydroxylase)	*F3'h*	Antirrhinum, Petunia
F3'5'H (Flavonoid3',5'-hydroxylase)	*F3'5'h*	Calistephus, , Petunia
FLS (Flavonol synthase)	*Fls*	Petunia, Rosa
FNS (Flavone synthase)	*FnsII*	Antirrhinum, Gerbera
DFR (Dihydroflavonol-4-reductase)	*Dfr*	Antirrhinum, Gerbera, Petunia, Dianthus, Orchid

Carotenoids

Carotenoids are a widespread family of plant pigments, like flavonoids, play vital roles in plant photosynthesis in chloroplasts. Carotenoids are also precursors for the synthesis of the plant hormones abscisic acid, strigolactone and gibberellin[11,27]; therefore, their genetic manipulation is rather difficult. Carotenoids are generally C_{40} terpenoid compounds formed by the condensation of eight isoprene units. At the center of the molecule, the linkage order is reversed, so the molecule as a whole is symmetrical. A set of conjugated double bonds is responsible for the absorption of light in the visible region of the spectrum. Carotenoids protect photosynthetic organisms against potentially harmful photooxidative processes and are essential structural components of the photosynthetic reaction. Carotenoids are responsible for most of the yellow to orange flower colors in ornamentals that include marigold (*Tagetes*), daffodil (*Narcissus*), *Freesia*, *Gerbera*, *Rosa*, *Lilium*, and *Calendula*. More important and less recognized is the ability of carotenoids to coexist with red or purple anthocyanins, resulting in brown and bronze hues that neither pigment would be able to provide by itself[7]. Classical geneticists identified carotenoid mutants in maize, tomato, and Arabidopsis during the early part of this century. However, difficulties with the biochemical properties of the enzymes of the pathway and the generally poor viability of mutants affected in carotenoid biosynthesis during early stages of development conspired to delay development of the field. Genetic studies of carotenoid biosynthesis in bacteria have played a fundamental role in the cloning and characterization of plant carotenoid genes.

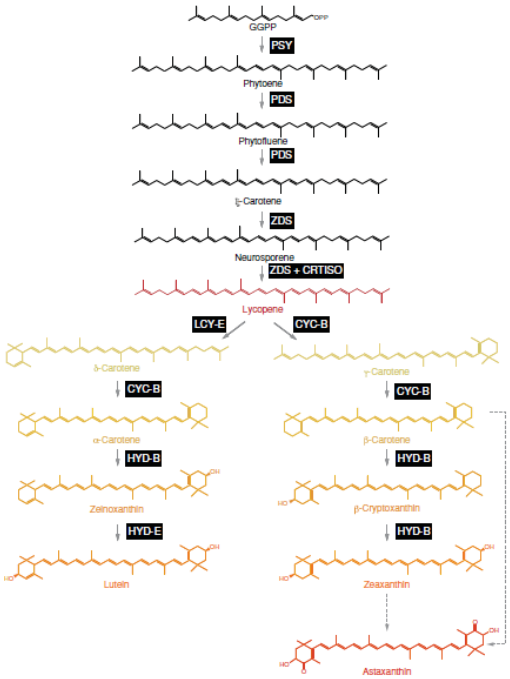

Fig. 2: Carotenoids biosynthetic pathway[11]

A simplified biosynthetic pathway. The colours indicate the prevalent colour of the compounds in nature. Compounds before ζ-carotene do not absorb light in the visible region of the spectrum. The colour of compounds in nature does not necessarily correspond to the colours of purified compounds in solution due to interactions with other components of chromoplast membranes and to concentration effects. The first steps of the pathway are condensation reactions that result in the formation of geranylgeranyl diphosphate (geranylgeranyl pyrophosphate, GGPP). Phytoene synthase (PSY) catalyzes the condensation of two molecules of GGPP into prephytoene pyrophosphate and then into phytoene (Figure-2). A series of desaturation reactions results in the synthesis of lycopene, which is then cyclized into carotene. Zeaxanthin and violaxanthin, the two main compounds of the xanthophyll cycle.

Breeding approaches to change flower colour

1. Hybridization
2. Mutation
3. Interspecific Hybridization and Polyploidy
4. Genetic Engineering

Interspecific and Polyploidy breeding:

Interspecific hybridisation and polyploidy are recognized as the most important sources of evolution and domestication of flowering plants. In ornamental plant breeding these phenomena go hand in hand and can be observed in the breeding history of many ornamental crops (*Rosa, Chrysanthemum, Gladiolus, Alstroemeria, Lilium,* orchids etc). The role of interspecific hybridisation is the most important source for variation in ornamental breeding. Interspecific hybrids have the potential to capture hybrid vigour as well as combine traits that do not occur within a single species. Because breeder rather than geneticist always want to add a new type of characteristics to the current cultivars, interspecific hybridisation is indispensable to combine diverse gene pools. Red gentian cultivars have been made by interspecies hybridization. It is also used in transferring yellow flower colour from yellow-flowered carnations (2n.2x.30) and *Dianthus knappii* (2n.2x.30) to a white-flowered cultivar of the garden pink, *Dianthus plumarius* (2n.6x.90). These hybrids were difficult to make but a small number were produced from both cross combinations. All the progeny from the crosses with carnations were pink but those from crosses with *D. knappii* were pale cream-yellow, with some variation in intensity between plants. Analysis of the flower pigments showed that the yellow flower colour of *D. knappii* resulted from the presence of high levels of flavone and flavonol glycosides whereas those of yellow carnations were chalcones[9].

Mutation Breeding

Ornamental plants appear to be ideal systems for application of mutation induction techniques as many characters of economical interest that is flower traits or the growth habit are easily monitored after mutagenic treatment. Furthermore, many ornamental species are heterozygous and often propagated vegetatively thus allowing the detection, selection and conservation of mutants within the M1-generation. Since the beginning of practical mutation breeding in the 1930s, this technique has been applied to ornamental plants. One of the first officially released commercial mutant cultivars has been produced in tulip (cv. ‘Faraday‘) by De Mol 1949. It expressed an altered flower colour and resulted from irradiation of cv. ‘Fantasy‘in 1936. In recent decades, mutation induction has been used for the development of 'sport-families' in several ornamental crops, for example in *Alstroemeria, Dahlia, Dendranthema, Dianthus, Euphorbia* and *Streptocarpus*. Flower colour is one of the most prominent features of ornamentals and there are numerous reports on alterations arising after mutagenic treatment. Evaluation of the literature shows, that 55% of the records concerne changes in flower colour. As compared to the knowledge on the molecular basis and genetics of flower colour available today, practical mutation breeding has been undertaken from an empirical point of view.

The majority of reports is purely descriptive and devoid of analytical data on mutated genes. But profound experience of breeders and evaluation of derivation of sports allow the selection of promising starting genotypes, from which most of the colours known in the species of question can be derived. For example in chrysanthemums, mutagenic treatment of pink flowering genotypes renders the chance to obtain a wide spectrum of flower colours as bronze, brown, yellow, white, red and orange. On the other side, yellow flowering genotypes normally do not give rise to flower colour mutants. In several ornamental crops, so-called sport families solely diverging in flower colour have been derived from selected genotypes. They allow the production of an assortment of flower colours under identical environmental conditions. Most prominent examples for the use of such sport families are found in chrysanthemums, carnations and roses.

Mutation breeding in India:

Beside IARI, New Delhi, BARC, Mumbai, TNAU, Coimbatore, NBRI, Lucknow and other Mutation breeding work is going on. The success story of mutation breeding in ornamentals in India is impressive. Alone in Chrysanthemum 46 mutants are commercially released. (Source: Current Science, Vol.89, No. 2, 25th July 2005). Recently released Chrysanthemum variety from IARI through mutation induced by gamma rays; Pusa anmol, Pusa centenary, Pusa kesari and Pusa arunodaya.

Genetic Engineering

It is manipulation of plant genome through recombinant DNA technology to alter plant characteristics. Genetic engineering can be used to create genetically altogether a new plant of desired nature. It is possible to introduce genes from quite unrelated organisms like bacteria, fungi, yeasts into the plants to modify their traits. Novel plants with desirable characters created through genetic engineering methods are called "Transgenic plants". Creation of transgenic plant utilises the genetic engineering technology through tissue culture methods.

Colour modification in ornamental plants are by following mechanisms:

- By the over expression of structural genes to colour intensification
- By the use of sense or antisense enzyme construct
- By suppressing the expression of certain gene of the phenyl propanoid pathway that is Flavonoid biosynthesis pathway
- By blocking the anthocyanin synthesis at different stage
- By inhibiting the production of key biosynthetic enzyme and
- By adding an enzyme of a particular biosynthetic step to modify its pathway

Table 3: Examples of flower colour modifications by regulating flavonoid biosynthesis

Plant species	Original colors	Gene sources	Methods	Produced flower colors	References
Rosa hybrida	Red to pale cyanic	Viola sp. *F3'5'H*	Over expression	Bluish	17
Petunia hybrid	Blue	*Mazus pumilum CHS*	Dominat negative	Pale blue	12
Torenia hybrida	Blue	Endogenous *ANS*	RNAi	White to pale blue	21
Cyclamen persicum	Purple	Endogenous	F3'5'H Antisense	Red to pink	3
Gentiana sp	. Blue	Endogenous	CHS Antisense	White	23
Gentiana sp	Blue F3'5'H	Endogenous	RNAi	Lilac to pale blue	22
Petunia hybrid	Red	*Lotus japonicus PKR*	Over expression	Variegated red	24

Genes affecting the pattern of petal pigmentation

The cellular expression of the biosynthetic genes in uniformly pigmented petal tissue affect the pattern of petal pigmentation. However, within the petal, production of pigment may be patterned. Some patterns are seen in natural isolates and other patterns arise through mutation. Patterns may arise by loss of function of a regulatory gene that has a pattern to its area of activity within the flower. In this case, pigmentation will be lost in those areas with an absolute requirement for the transcription factor but not in those areas in which the regulator is not active or in which it can be substituted for by another factor. A pattern mutation may also arise in a biosynthetic gene itself if the mutation is in a regulatory region of the gene (the promoter) such that it interferes with the interaction between a regulatory gene product and the biosynthetic gene (for example, by modification of a cis-acting protein binding motif in a biosynthetic gene promoter[2]. In this respect, pattern mutants are common in the CHS *(Nivea)* and DFR *(Pa//ide)* genes expressed in Antirrhinum flowers but are very uncommon in the CHS (C2) and DFR *(Al)* genes expressed in maize aleurone, despite the presence of mutagenic transposon insertions in all of these genes.

Generating blue flowers

Most blue flowers contain aromatically acylated delphinidin derivatives. Rose, chrysanthemum and carnation make up over 50% of the world cut flower market but only accumulate pelargonidin and cyanidin derivatives that are not modified with aromatic acyl groups. Thus they have become targets for attempts at engineering the synthesis of delphinidin derivatives with the hope of eventually generating blue flowers. The absorbance of anthocyanin shifts towards longer wavelengths (blue) by about 10 nm with each hydroxylation of the B ring and by 4 nm following an aromatic acylation[10]. As described previously, the key enzyme in the biosynthesis of delphinidin is F3’5’H. *F3’5’H* genes from petunia and lisianthus have been shown to direct production of a blue hue in flowers of petunia and tobacco[14].

Over-expression of a petunia *F3'5'H* gene under the control of a constitutive promoter in a pelargonidin producing carnation variety produces petals in which delphinidin derivatives contribute to about 70% of total anthocyanins. However, there was only a slight colour change toward blue. In order to increase the content of delphinidin derived anthocyanins it was necessary to avoid competition for substrates between two key endogenous enzymes of the anthocyanin pathway (DFR and F3'H) and the introduced enzyme (F3'5'H). To achieve this white carnation cultivars were selected that were specifically deficient in the *DFR* gene. Expression of petunia F3'5'H (under the control of a promoter region from the snapdragon *CHS* gene) and Petunia *DFR* genes in one such DFR mutant resulted in exclusive accumulation of delphinidin derivatives and significant colour change toward blue (Florigene Moondust, the first genetically modified floricultural crop to be sold in the world). Expression of a pansy *F3'5'H* gene (under the control of a promoter region from the snapdragon *CHS* gene) and a petunia *DFR-A* gene (under the control of its own promoter and terminator regions) resulted in transgenic plants which also exclusively accumulated delphinidin but at a higher concentration. These flowers had a dark violet colour (Florigene Moonshadow). Florigene Moonvista, Florigene Moonshade, Florigene Moonlite and Florigene Moonaqua are standard carnations that have been developed using the same strategy and are currently sold in North America, Australia and Japan are being trialled[25]. Petunia has a cytochrome *b5* that specifically transfers electrons to F3'5'H by which petunia can efficiently synthesize 3',5'-hydroxylated flavonoids. Expression of a petunia F3'5'H (*Hf1*) gene along with a petunia cytochrome *b5* gene in a carnation cultivar producing cyanidin derivatives resulted in efficient production of delphinidin based anthocyanins and subsequent colour change petals. In this case, it was not necessary to use

a carnation line mutant in DFR to achieve exclusive accumulation of delphinidin.

Roses are the most important flowers in today's global flower market and have been the center of attraction for consumers and breeders for hundreds of years. Modern roses (*Rosa hybrida*) have resulted from extensive hybridization of wild rose species and have various flower colours, except those in the violet to blue range[28,15]. Delphinidin production is a major breakthrough in the achievement of blue roses[15]. The expression of viola *F3'5'H* genes produced delphinidin[5]. The overexpression of a viola *F3'5'H* gene resulted in the efficient accumulation of delphinidin and colour changes with a novel blue hue in a rose cultivars. Furthermore, the down-regulation of the rose *DFR* gene and overexpression of the iris *DFR* gene, as well as the overexpression of the viola *F3'5'H* gene, resulted in more efficient and exclusive delphinidin production and a bluer flower colour. Suppression of the endogenous *DFR* gene was achieved by RNAi[23].

Aida et al.[1] observed that the flowers of torenia harbouring an antisense *DFR* gene were bluer than those harbouring an antisense *CHS* gene because incomplete down-regulation of DFR lead to an accumulation of flavones and the resulting copigmentation effect with the remaining anthocyanins shifted the flower colour towards blue.

Genetic Modification for Red Flowers

Accumulation of pelargonidin based anthocyanins in flowers confers an intense red or orange colour, which is very desirable commercially. Attempts have therefore been made to obtain such colours by genetic engineering. In petunia down regulation of the *F3'H* gene in conjunction with over-expression of rose *DFR* yielded transgenic petunia whose flowers accumulated pelargonidin based anthocyanins. Down regulation of *F3'5'H* and *F3'H* genes and over-expression of pelargonium DFR in blue torenia (which normally produces delphinidin based anthocyanins) resulted in transgenics with pink flowers accumulating anthocyanins based on pelargonidin. Some cut flower species such as gentian and iris do not accumulate pelargonidin derived pigments and thus lack flowers with an intense red colour. Genetic modification methods should be an alternative solution to achieving red flower colours. Though down-regulation of the *F3'H* and *F3'5'H* genes in combination with over expression of a correctly identified *DFR* gene should generate gentian flowers producing pelargonidin based pigments. Sophisticated optimization of gene expression or pathway engineering may be necessary to accumulate a large amount of pelargonidin, and intense red flower colour. Furthermore, *Agrobacterium* mediated transformation of gentian occurs at a low efficiency and the promoter of the 35S cauliflower mosaic virus, a commonly used constitutive promoter, is prone silencing by methylation in gentian[19].

Effect of regulatory genes on flower pigmentation:

An increase in anthocyanin levels has been achieved by over expression of genes encoding such transcription factors. For example, the maize *Lc* allele gene (bHLH) under the control of an essentially constitutive (CaMV35S) promoter led to an increase in the amount of anthocyanins in tobacco flowers. Expression of the same genes also resulted in increased anthocyanins levels in floral and vegetative tissues, including the leaves of transgenic petunias. These leaves were purple due to accumulation of anthocyanins, and may represent a novel ornamental plant of commercial value[4].

Other factors affecting flower colouration

Co-pigment

Flavonols and flavones are common co-pigments that stabilize and lend bluing to anthocyanins by forming complexes with them[10]. Flavonols are derived from dihydroflavonols by the activity of flavonol synthase (FLS). The genes encoding FLS have been cloned from various plants[18]. Flavones are synthesized from flavanones by flavone synthase (FNS). Interestingly there are two kinds of FNS, a dioxygenase type (FNSI) and a cytochrome P-450 type (FNSII). The FNSII gene has been cloned from torenia,

snapdragon, gerbera and perilla. A parsley FNSI gene has also been cloned.

Vacuolar pH

Vacuolar pH, that is most often maintained as weakly acidic, is critical to anthocyanin stability and colour. Although higher (neutral) pH generally yields bluer flower colours, anthocyanins are less stable at higher pH and must be stabilized with 4 more than one glycosyl and aromatic acyl group. Genetic control of petal vacuolar pH is known in petunia and morning glory. The only structural gene shown to regulate vacuolar pH to date encodes a Na^+/H^+ antiporter (Purple) in morning glory[8]. The gene is highly expressed just before flower opening, which elevates pH from 6.5 to 7.5 and changes the colour from purple to blue. Homologues have been isolated from petunia, torenia and Nierembergia but their function in vivo is unclear. Higher vacuolar pH has been shown to be specific to epidermal cells where anthocyanins accumulate.

CONCLUSION

Flower colour is mainly determined by the ratio of different pigments and other factors such as vascular pH, co-pigments and metal ions. Knowledge at the biochemical and molecular level has made it possible to develop novel colour which are otherwise absent in nature. Genetic engineering is providing a valuable means of expanding the floriculture gene pool. Generally, genetically modified (GM) flowers are more acceptable to consumers than GM food crops; therefore many floricultural plants are at advanced stages of development.

REFERENCES

1. Aida, R., Yoshida, K., Kondo, T., Kishimoto, S. and Sibata, M., Copigmentation gives bluer flowers on transgenic torenia plants with the antisense dihydroflavonol-4-redutase gene. *Plant Sci*, **160:** 49-56 (2000).
2. Almeida, J., Carpenter, R., Robins, T.P., Martin, C., and Coen, E.S., Genetic interactions underlying flower color patterns in Antirrhinum majus. *Genes Dev*, **3:** 1756-1767 (1989).
3. Boase, M.R., Lewis, D.H., Davies, K.M., Marshall, G.B., Patel, D., Schwinn, K.E. and Deroles, S.C., Isolation and antisense suppression of flavonoid 3', 5'-hydroxylase modifies flower pigments and colour in cyclamen. *BMC Plant Biol*, **10:** 107 (2010).
4. Bradley, J.M., Davies, K.M., Deroles, S.C., Bloor, S.J. and Lewis, D.H., The maize Lc regulatory gene up-regulates the flavonoid biosynthetic pathway of Petunia. *Plant J.*, **13:** 381-392 (1998).
5. Brugliera, F., Tanaka, Y. and Mason, J., Flavonoid 3', 5'-hydroxylase gene sequences and uses therefor. *WO2004/020637* (2004).
6. Ewa Młodzinska, Survey of plant pigments: molecular and environmental determinants of plant colours. *Acta Biologica Cracoviensia Series Botanica,* **51(1):** 7-16 (2009).
7. Forkmann, G., Flavonoids as flower pigments: the formation of the natural spectrum and its extension by genetic engineering. *Plant Breed.,* **106:** 1-26 1991.
8. Fukada-Tanaka, S., Inagaki, Y., Yamaguchi, T., Saito, N. and Iida, S., Colouring-enhancing protein in blue petals. *Nature*, **407:** 581 (2000).
9. Gatta, M.K., Hammettb, K.R.W., Markhamc, K.R. and Murraya, B.G., Yellow pinks: interspecific hybridization between *Dianthus plumarius* and related species with yellow flowers. *Scientia Horticulturae*, **77:** 207-218 (1998).
10. Goto, T. and Kondo, T., Structure and molecular stacking of anthocyanins-flower color variation. *Angrew. Chem. Int. Ed. Engl*., **30:** 17-33 (1991).
11. Grotewold, E., The genetics and biochemistry of floral pigments. *Annu Rev Plant Biol*, **57:** 761-780 (2006).
12. Hanumappa, M., Choi, G., Ryu, S. and Choi, G., Modulation of flower colour by rationally designed dominant-negative chalcone synthase. *J Exp Bot*, **58:** 2471-2478 (2007).

13. Henry, R., Plant diversity and evolution: genotypic and phenotypic variation in higher plants. *CABI Publishing*, Cambridge (2005).
14. Holton, T.A. and Cornish, E.C., Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell*, 7: 1071-1083 (1995).
15. Holton, T.A. and Tanaka, Y., Blue roses a pigment of our imagination. *Trends Biotechnol*, **12:** 40-42 (1994).
16. Holton, T.A., Brugliera, F., Lester, D., Tanaka, Y., Hyland, C.D., Menting, J.G.T., Lu, C.Y., Farcy, E., Stevenson, T.W. and Cornish, E.C., Cloning and expression of cytochrome P450 genes controlling flower colour. *Nature*, **366:** 276-279 (1993).
17. Katsumoto, Y., Fukuchi-Mizutani, M., Fukui, Y., Brugliera, F., Holton, T.A., Karan, M., Nakamura, N., Yonekura-Sakakibara, K., Togami, J., Pigeaire, A., Tao, G., Nehra, N.S., Dyson, B.K., Tsuda, S., Ashikari, T., Kusumi, T., Mason, J.J. 2 and Tanaka, Y., Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiol*, **48(11):** 1589-1600 (2007).
18. Martens, S., Knott, J., Seitz, C., Janvari, L., Yu, S.N. and Forkmann, G., Impact of biochemical pre-studies on specific metabolic engineering strategies of flavonoid bisynthesis in plant tissues. *Biochem. Eng. J.*, **14:** 227-235 (2003).
19. Mishiba, K., Nishihara, M., Nakatsuka, T., Abe, Y., Hirano, H., Yokoi, T., Kikuchi, A. and Yamamura, S., Consistent transcriptional silencing of 35S-driven transgenes in gentian. *Plant J.*, **44:** 541-556 (2005).
20. Mol, J., Grotewold, E. and R. Koes, R., How genes paint flowers and seeds. *Trends Plant Sci.*, **3:** 212-217 (1998).
21. Nakamura, N., Fukuchi-Mizutani, M., Miyazaki, K., Suzuki, K. and Tanaka, Y., RNAi suppression of the anthocyanidin synthase gene in Torenia hybrida yields white flowers with higher frequency and better stability than antisense and sense suppression. *Plant Biotechnol*, **23:** 13-18 (2006).
22. Nakatsuka, T. and Nishihara, M., UDP-glucose: 3-deoxyanthocyanidin 5-O-glucosyltransferase from Sinningia cardinalis. *Planta*, **232:** 383-392 (2010).
23. Nishihara, M. and Nakatsuka, T., Genetic engineering of flavonoid pigments to modify flower color in floricultural plants. *Biotechnol Lett*, **33:** 433-441 (2011).
24. Shimada, N., Nakatsuka, T., Nishihara, M., Yamamura, S., Ayabe, S. and Aoki, T., Isolation and characterization of a cDNA encoding polyketide reductase in Lotus japonicus. *Plant Biotechnol*, **23:** 509-513 (2006).
25. Tanaka, Y., Brugliera, F. and Chandler, S. Recent progress of flower colour modification by Biotechnology. *Int. J. Mol. Sci.* **10:** 5350-5369 (2009).
26. Tanaka, Y., Katsumoto, Y., Brugliera, F. and Mason, J., Genetic engineering in floriculture. *Plant Cell Tiss. Org. Cult.* **80:** 1-24 (2005).
27. Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J. and Yamaguchi, S., Inhibition of shoot branching by new terpenoid plant hormones. *Nature*, **455:** 195-200 (2008).
28. Vries, D.P., Keulen, H.A. and Bruyn, J.W. Breeding research on rose pigments. 1. The occurrence of flavonoids and carotenoids in rose petals. *Euphytica*, **23**: 447-457 (1974).
29. Winkel-Shirley, B., Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol*, **126:** 485-493 (2001).