A Review of Genetic Improvement of Flower Colour

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ABSTRACT

Flower color contributes mainly to the market value of an ornamental plant, and coloration of plant. Its development and regulation are influenced by many internal and external factors. Therefore, understanding the mechanism of color development and its regulation provides an important theoretical basis and premise for the cultivation and improvement of new color varieties of flower plants. Flowers are determined by three classes of pigments: flavonoids, carotenoids and betalains. Flavonoids and carotenoids are widespread; however, betalains can be found only in plants of several genera in the order Caryophyllales, which belongs to one small group of angiosperms. Among these pigments, flavonoids (mainly anthocyanins) are the most common flower pigments contributing to a range of colors from yellow to orange to red to purple. Flower colour is determined other factors such as vacuolar pH, co-pigmentation and metal ion complexion.

Knowledge of flower coloration at the genetics, biochemical and molecular level has made it possible to developed novel colour. The expression of genes transferred across genera is not always predictable and so requires considerable trial to arrive at stable phenotype of commercial interest. Since commercial floriculture is becoming important from the export angle. Here we summarize the Genetics and biochemistry of flower colour.

Keywords: anthocyanin, betalain, carotenoid, flavonoid, flower color, pigmentation

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INTRODUCTION

Flower color can attract pollinators and protect floral organs. Furthermore, people enjoy these colors in daily life. For ornamental plants, flower color is an important quality determinant that not only affects the ornamental merit of a plant but also directly influences its commercial value. Although there is a wide range of natural flower colors, colors are limited in some important ornamental plants. For example, Chinese rose and chrysanthemum lack blue and herbaceous peony and cyclamen lack yellow. Therefore, making flower color improvements has always been an important goal for breeders [40]. Over the years, much research has been conducted on the development and regulation of ornamental plant color. Researchers have found that the development of flower color is related to petal tissue structure, pigment distribution and its types; it can be regulated through environmental factors and genetic engineering.

Flower colors are of paramount importance in the ecology of plants and in their ability to attract pollinators and seed dispersing organisms [17]. In addition, plants also play an important aesthetic function by providing flowers with a broad spectrum of colors. Not surprisingly, ornamentals were among the first plants to be hybridized to alter specific color traits, and fruit and flower color have contributed to elucidating fundamental genetic principles. Today, the market for ornamental plants and cut flowers is rapidly expanding and totals over $70 billion in annual sales [3]. 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 colors [34, 35]. Florigene's Moonseries of genetically engineered carnations (http://www.florigene.com/), marketed in the United States, Australia, Canada, Japan, and some European countries, provide the first genetically engineered commercial flowers. Three types of chemically distinct pigments, betalains, carotenoids, and anthocyanins are responsible for the colors of flowers [10].
FEW ROLE OF FLOWER COLOUR
- Attraction of pollinators
- Function in photosynthesis
- In human health as antioxidants and precursors of vitamin A
- Seed dispersal
- Protecting tissue against photooxidative damage
- Resistant to biotic and abiotic stress
- Symbiotic plant-microbe interaction
- Act as intermediary for other compounds

MAJOR PIGMENT IN PLANTS
BETALAINS

Biosynthesis
Betalains are water-soluble, nitrogen containing compounds synthesized from tyrosine by the condensation of betalamic acid (Figure 1), a central intermediate in the formation of all betalains, with a derivative of dihydroxyphenylalanine (DOPA). This reaction results in the formation of the red to violet betacyanins, such as those found in red beets or in the flowers of portulaca. The condensation of betalamic acid with an amino acid (e.g., Ser, Val, (e.g., 3-methoxytyramine) results in the formation of the yellow to orange betaxanthins. Betacyanins and betaxanthins can be further classified into several subclasses, based on the chemical characteristics of the betalamic acid conjugate [31, 32]. Recent advances in the separation and analysis of betalains, which are unstable under the acidic conditions normally used for Nuclear Magnetic Resonance (NMR) spectra analyses, are likely to shed additional light on the existence of novel conjugates [30]. As is common for many other phytochemicals, light and hormones have a dramatic effect on the accumulation of betalains [25]. The conversion of tyrosine to DOPA (Figure 1) is carried out by a tyrosinase type phenoloxidase [29], a group of coppercontaining bifunctional enzymes involved in the hydroxylation of phenols to o-diphenols. In addition to participating in the formation of the betalamic acid core, the tyrosinase enzyme also oxidizes DOPA to dopaquinone, contributing to the biosynthesis of cyclo-DOPA, which conjugates with betalamic acid to form the chromophore of all betacyanins, betanidin [32]. The formation of betalamic acid from DOPA requires the extradiol cleavage of the 4, 5 bond carried out by a DOPA dioxygenase, first identified in the basidiomycete fly agaric (Amanita muscaria) [35]. The plant enzyme was subsequently cloned by a subtractive cDNA approach using Portulaca grandiflora isogenic lines with different color phenotypes [4]. The plant enzyme exhibits no obvious sequence or structural similarity with the fungal enzymes. Moreover, the plant enzyme displays regiospecific extradiol 4,5-dioxygenase [4], in contrast to the 2,3- and 4,5 dioxygenase activity of the Amanita muscaria enzyme [14]. The 4,5-seco-DOPA is subsequently recyclized, a step likely to occur spontaneously [31]. This different activity of the plant and fungal enzymes permits Amanita muscaria to accumulate muscaflavin, in addition to betalain, in the cuticle of the cap. The introduction of the DOPA dioxygenase from Amanita muscaria into Portulaca grandiflora petals by particle bombardment resulted in the accumulation of various betalains, and also of muscaflavin, a pigment normally not found in plants [22], which is synthesized by the extradiol ring cleavage of the 2,3 bond followed by recyclization into the 6-atom, N-containing ring muscaflavin. The next step in the biosynthesis of betalains involves the formation of an aldimine link between betalamic acid and cyclo-DOPA (to make betanidin) or an amino acid derivative (to make betaxanthin) (Figure 1). No enzyme capable of carrying out the aldimine reaction has yet been identified, opening the possibility that this step occurs spontaneously in vivo [32]. It remains unclear how the spontaneous condensation of betalamic acid with various different DOPA or amino acid derivatives results in the specific patterns of betalains consistently obtained in the same plant.
**CAROTENOID Biosynthesis**

As is the case for other isoprenoids, isopentenyl diphosphate (IPP) provides the five-carbon building block for carotenoids. In the plastids, where carotenoid biosynthesis takes place, IPP is synthesized through the plastid-specific DOXP (1-deoxyxylulose 5-phosphate) pathway [18]. The first committed step in the carotenoid pathway is catalyzed by phytoene synthase (PSY), resulting in the condensation of two C20 geranylgeranyl diphosphate (GGPP) molecules to form phytoene (Figure 2). Four desaturation reactions, two each catalyzed by the membrane-associated phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), result in the formation of the pink lycopene from the colorless phytoene (Figure 2). In addition to the desaturases, the formation of lycopene (trans configuration) requires the action of the carotenoid isomerase (CRTISO) enzyme, cloned from the tangerine tomato mutant [16], which is responsible for converting poly-cis-lycopene (prolycopene) to lycopene. A single enzyme, phytoene desaturase (CRTI), carries out all four desaturation and isomerization reactions in bacteria and fungi. Although plant desaturases have no homology to CRTI, CRTISO does [16, 24]. The cyclization of lycopene represents a branch point in the pathway, and two products can be formed depending on the position of the double bond on the cyclohexane ring. On one hand, lycopene β-cyclase, for which there are two forms in tomato, one specific to green tissues (LCY-B) and the other to chromoplasts (CYC-B), first produces γ-carotene containing one β-ring (Figure 2), which is subsequently converted to β-carotene by the same enzyme. On the other hand, lycopene ε-cyclase (LCY-E) produces δ-carotene. The formation of α-carotene, the precursor for lutein, involves formation of a β-ring on δ-carotene by lycopene β cyclase (Hirschberg, 2001). The α- and β-carotenoids are the precursors for the xanthophylls, which are oxygenated carotenoids generated by β- and ε-ringspecific hydroxylases. β-carotene is converted to zeaxanthin by the carotenoid β-ring hydroxylases (HYD-B), encoding a nonheme diiron enzyme for which there are two genes in *Arabidopsis* (Tian et al. 2003). The hydroxylation of the ε-ring is carried out by the carotenoid ε-ring hydroxylase (HYD-E), a cytochrome P450 enzyme, CYP97C1, encoded by the *Arabidopsis* LUT1 locus. In addition to displaying activity toward the ε-ring, LUT1 can also hydroxylate the β-ring [36].
Hydroxylation of the β-ring of α-carotene is also mediated by a P450 enzyme (E. Wurtzel, personal communication). Lutein is the main carotenoid present in the petals of marigold, and the broad range of colors that characterize marigold flowers is due to the very different levels of this xanthophyll. Indeed, marigold varieties with very light flower color (e.g., French Vanilla) have a reduced expression of all the carotenoid biosynthetic genes, suggesting a regulatory mutation, rather than a defect in a single biosynthetic enzyme [20]. Interestingly, however, the varieties with reduced xanthophyll accumulation in the petals display normal levels of carotenoids in the leaves, strengthening the notion that the “primary” role of carotenoids is independently regulated from their function as secondary metabolites. The formation of ketocarotenoids, such as, for example, astaxanthin, requires the addition of keto groups in each β-ring of zeaxanthin. The initial engineering of astaxanthin in tobacco flowers was accomplished by the expression of the CrtO gene, encoding a β-carotene ketolase, from the algae Haematococcus pluvialis [19]. Subsequently, the AdKeto enzyme was identified from Adonis aestivalis (summer pheasant’s eye, Ranunculaceae), which is capable of desaturating the 3,4 positions of the β-ring followed by the 4-hydroxylation and the final keto-enol tautomerization, resulting in the formation of the blood-red pigment astaxanthin, abundantly present in the petals of this plant [5]. The identification of AdKeto creates novel opportunities for the metabolic engineering of the commercially important ketocarotenoids from the abundant pools of β-carotenes present in many plants, offering alternatives to current approaches to manipulating the pathway involving the introduction of the 4,4'-oxygenase and 3,3'-hydroxylase from marine bacteria into plants [26].

**Figure 2:** Schematic representation of the biosynthetic pathway of some major carotenoid pigments. The names of the compounds are indicated. GGPP corresponds to geranylgeranyl diphosphate. The enzyme names, in black boxes, are PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; CYC-B, chromoplastic form of lycopene β-cyclase; LCY-E, lycopene ε-cyclase; HYD-B, carotenoid β-ring hydroxylases; HYD-E, carotenoid ε-ring hydroxylase.
ANTHOCYANINS

Biosynthesis

Anthocyanins are water-soluble pigments that occur in almost all vascular plants [7]. The anthocyanin pigments are responsible for the majority of the orange, red, purple, and blue colors of flowers. Anthocyanins are derived from a branch of the flavonoid pathway (Figure 3) for which chalcone synthase (CHS) provides the first committed step by condensing one molecule of the C-ring, resulting in the formation of flavanones, is carried out by chalcone isomerase (CHI), an enzyme originally believed to have a structure unique to the plant kingdom, but which was also recently found in fungi and prokaryotes [1, 6, 9]. In some bacteria, CHI-like enzymes contribute to the degradation of flavonoids by taking advantage of the reversible nature of the isomerization, which permits CHI to also convert flavanones to the corresponding chalcones (Herles et al. 2004). Flavanones (e.g., naringenin) provide a central branch point in the flavonoid pathway and can serve as substrates for enzymes that introduce –OH groups at the 3 prime and 5 prime positions of the B-ring (e.g., F3'H and F3'5'H), or for the hydroxylation of the C-ring by flavanone 3-hydroxylase (F3'H), a soluble dioxygenase. Dihydroflavonol 4-reductase (DFR) provides one entry step to the biosynthesis of anthocyanin, and depending on the plant species, it can utilize as a substrate any one or all three of the possible dihydroflavonols, dihydromyricetin, dihydrokaempferol, or dihydroquercetin, resulting in the formation of the corresponding leucoanthocyanidins, providing structure to the anthocyanin biosynthetic grid (Figure 3). In some plant species an activity that has sometimes been referred to as flavanone 4-reductase (FNR) reduces naringenin to the corresponding flavan 4-ol (e.g., apiferol). However, recent studies in maize suggest that DFR and FNR correspond to the same enzyme [10]. The resulting 3-deoxy flavonoids, whose distribution is limited to some bryophytes, a few grasses (e.g., maize and sorghum), and the flowers of the Gesneriaceae (e.g., sinningia), can form 3-deoxyanthocyanin pigments [28], in contrast to the broadly distributed 3-hydroxyanthocyanins. The leucoanthocyanidins are converted into the corresponding anthocyanidins by the action of a leucoanthocyanidin dioxygenase/anthocyanidin synthase (LDOX/ANS). More than 17 different anthocyanidins have been described [12], and the major three are shown in Figure 3. Anthocyanidins also serve as substrates for anthocyanidin anthocyanidin reductases (e.g., BANYLUS from Arabidopsis), key enzymes in the formation of proanthocyanidins [39]. The next step in the anthocyanin pathway is catalyzed by ANS. The structure of the Arabidopsis ANS enzyme has been solved [38]. ANS, similar to F3H, flavone synthase 1 (FNSI), and flavonol synthase (FLS), is a member of the nonheme ferrous and 2-oxoglutarate (2OG)-dependent family of oxygenases, sufficient for the conversion of the leucoanthocyanidin (e.g., leucocyanidin) to the corresponding anthocyanidin (e.g., cyanidin) [23]. Anthocyanidins, most often represented as the flavylium cation (red), can adopt multiple forms in solution in an equilibrium that primarily depends on the pH and the solvent. In aqueous solutions at pH of 3–6, conditions similar to those present in plant cells, the flavylium cation can be covalently hydrated at position 2, resulting in the corresponding colorless carbinol pseudobases [12]. The colored flavylium ion is stabilized in the cell by inter- or intramolecular copigmentation [12]. Intermolecular copigmentation involves the interaction of anthocyanins with other noncolored flavonoids (e.g., flavonols), phenylpropanoids, carotenoids, or metals (e.g., Mg2+ or Al3+) [7, 8, 12]. Noncolored flavonoids provide “depth” to many white or cream flowers. In intramolecular copigmentation, the anthocyanin chromophores are covalently modified by organic acids, other flavonoids, or aromaticacyl groups. These modifications, together with the stacking of planar anthocyanins, add protection from nucleophilic water addition and result in increased anthocyanin pigmentation and hue changes.
GENES INVOLVED IN PIGMENT SYNTHESIS

Regulatory Gene

The final concentrations of anthocyanins/flavonoids in plant cells are not only determined by expression levels of enzymes involved in flavonoid biosynthetic pathway, it is now demonstrated very clearly that some regulatory genes are also involved in controlling the transcription level of the flavonoid biosynthesis genes in some plants examined including maize, snapdragon, *Petunia, Arabidopsis* and tomato. In general, these regulatory genes in the flavonoid biosynthesis pathway are specific transcription factors [21]. These DNA binding proteins interact with promoter regions of the target genes and regulate the initiation rate of mRNA synthesis. These regulatory genes relevant to flavonoid biosynthesis can be divided into 2 classes:

- TF with MYB domain
- TF with MYC/bHLH motif

An additional third class of WD40 proteins may also be important and universal, although the mechanism is not known. In various plant species, the tissue-specific expression pattern of the structural genes in the flavonoid biosynthetic pathway is controlled by the combination of regulatory genes from these two classes of transcription factors. Some transcription factors have been expressed ectopically in various transgenic plant species such as *Petunia*, tobacco and tomato, indicating the functional conservation of these regulatory genes among different plant species [27].
Other Factor affecting the flower colour
Anthocyanins determine predominantly the pigmentation in flowers; however, the final visible color of a flower is also affected by other factors.

Copigments
Flavonols and flavones are two common copigments. Copigments are often associated with anthocyanins, and thus stabilize the colored pigments. Most flavones and flavonols are colorless; they appear to provide ‘body’ to white, cream and ivory-colored flowers. The enzyme flavonol synthase (FLS) introduces a double bond between position 2 and position 3 of the C-ring in the flavonoid skeleton and converts dihydroflavonols (dihydrokaempferol, dihydroquercetin, dihydromyricetin) into flavonols (kaempferol, quercetin, myricetin, respectively). The genes encoding FLS have been cloned from various plants. In several plants such as *Petroselinum*, *Chrysanthemum*, *Dahlia* and *Gerbera* can also be converted into flavones by the enzyme flavone synthase (FNS) [8, 28, 34].

Vacuolar pH
It is well known that the pH value of the vacuole is acidic (around pH 5.5), and this weakly acidic condition is critical to stabilize anthocyanins. Any small changes of pH may have visible effects on flower color. In general, decrease in pH causes a reddening, and increase in pH causes a bluing effect. How a plant cell regulates the vacuolar pH is not clear, even though some genes influencing pH value have been identified [21, 37].

Cell shape
Accumulation of anthocyanin pigments is also affected by the shape of the cells [21]. For example, epidermal cells in petals of wild type snapdragon are conical, which confers higher light absorption and as a velvet sheen; a mutant with fainter colour was found a flattening of these epidermal cells. In comparison with other factors, the mechanism controlling the cell shape is unclear, and manipulating the cell shape by molecular approach is not reported yet [21].

CONCLUSION
Classical breeding methods have been extensively used to develop cultivars with flowers varying in both the colour and its intensity. Spectral difference in flower colour is mainly determined by the ratio of different classes of pigments and other factors such as vacuolar pH, co-pigmentation and metal ion complexation. Knowledge of flower colouration at the biochemical and molecular level has made it possible to developed novel colour. Genetic engineering overcomes almost all the limitations of traditional breeding approaches. The expression of genes transferred across genera is not always predictable and so requires considerable trial to arrive at stable phenotype of commercial interest.

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REFERENCES


