Genetic improvement of flower colour

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✓ Introduction

Flower colour can attract pollinators and protect floral organs. Furthermore, people enjoy these colours in daily life. For ornamental plants, flower colour 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 colours, colours 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 colour improvements has always been an important goal for breeders.

Researchers have found that the development of flower colour is related to petal tissue structure, pigment distribution and its types; it can be regulated through environmental factors and genetic engineering. Flower colours are of paramount importance in the ecology of plants and in their ability to attract pollinators and seed dispersing organisms. Florigene's Moonseries of genetically engineered carnations, 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 colours of flowers.

✓ Role of colour

- Attraction of pollinators
- Function in photosynthesis
- In human health as antioxidants and precursors of vitamin A
- Seed dispersal
- Protecting tissue against photo oxidative damage
- Resistant to biotic and abiotic stress
- Symbiotic plant-microbe interaction
- Act as intermediary for other compounds

✓ Why we need modification in colour?

- ➢ Modification in flower colour of a variety with desirable agronomic or consumer characteristics

Ex: A white carnation from preferable red-flowering variety

- ➢ A flower colour not occurring naturally in a particular crop

Ex: Blue colour in rose, carnation, orchids

- Change in trend for colour season to season, year to year
- High price for Novel colour.

Ex: The price for a single blue rose is about $22 to $33

✓ Major pigment in plants

❑ Betalains

Betalains are water-soluble, nitrogencontaining compounds synthesized from tyrosine by the condensation of betalamic acid, 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. 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.

❑ Carotenoids

In the plastids, where carotenoid biosynthesis takes place, IPP is synthesized through the plastid-specific DOXP (1-deoxyxylulose 5- phosphate) pathway. 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. 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 colourless phytoene.

❑ Anthocyanins

Anthocyanins are water-soluble pigments that occur in almost all vascular plants. The anthocyanin pigments are responsible for the majority of the orange, red, purple, and blue colours of flowers. Anthocyanins are derived from a branch of the flavonoid pathway 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 isomerise (CHI), an enzyme originally believed to have a structure unique to the plant kingdom, but which was also recently found in fungi and prokaryotes.

✓ Genes involved in pigment synthesis

1. Structural (enzyme) genes
2. Regulatory genes

- **Structural gene**

It is a gene that codes for any RNA or protein product other than a regulatory protein.

Enzyme	Gene	Species
CHS	*Chs*	Antirrhinum, Chrysanthemum, Orchid, Rosa, Dianthus
CHI	*Chi*	Antirrhinum, Petunia, Eustoma, Dianthus
F3H	*F3h*	Antirrhinum, Calistephus, Chrysanthemum, Dianthus, Orchid
F3'H	*F3'h*	Antirrhinum, Dianthus, Petunia
F3'5'H	*F3'5'h*	Calistephus, Eustoma, Petunia
FLS	*Fls*	Petunia, Rosa
FNS	*FnsII*	Antirrhinum, Gerbera
DFR	*Dfr*	Antirrhinum, Calistephus, Gerbera, Orchid, Dianthus, Petunia
ANS	*Ans*	Antirrhinum, Calistephus, Petunia
GT	*3Gt*	Antirrhinum, Gentiana
GTS	*Gts*	Petunia

- **Regulatory Gene**

It is now demonstrated 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. 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.

- **Other Factor affecting the flower colour**

Anthocyanins determine predominantly the pigmentation in flowers; however, the final visible colour 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 coloured pigments. Most flavones and flavonols are colourless; they appear to provide 'body' to white, cream and ivory-coloured flowers.

- **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 colour. In general, decrease in pH causes a reddening, and increase in pH causes a blueing effect.

- **Cell shape**

Accumulation of anthocyanin pigments is also affected by the shape of the cells. 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.

- **Colour modification done by**

- Over expression of structural genes.
- Use of sense or antisense enzyme constructs.
- Inhibit production of key biosynthetic enzyme.
- Add an enzyme of a particular biosynthetic step.

✓ Genetic Improvement of Flower Colour

Genetic Improvement: involves changing the plant’s genetic makeup. It is the science of applying genetics and plant breeding principle as well as biotechnology to improve plants for human use.

- Making deliberate crosses between two parents
- Hybridization
- Mutation
- Polyploidy

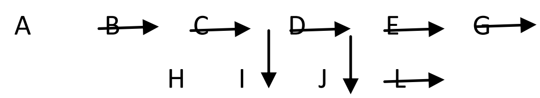
- Genetic Engineering of flower colour

- **Hybridization**

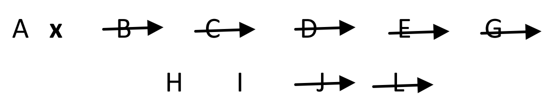
Hybridization is the process of crossing two genetically different individuals to result in a third individual with a different, often preferred, set of traits. Plants of the same species cross easily and produce fertile progeny. Wide crosses are difficult to make and generally produce sterile progeny because of chromosome-pairing difficulties during meiosis

- **Mutation**

Mutation is a sudden heritable change in a characteristics of an organism. Many different genes are involved in controlling the synthesis of the pigments. In a multi-step process



If a single enzyme is not present and early step in the synthetic pathway will not happen.



- **Poluploidy**

Polyploid cells and organisms are those containing more than two paired (homologous) sets of chromosomes. Most species whose cells have nuclei (Eukaryotes) are diploid, meaning they have two sets of chromosomes—one set inherited from each parent. However polyploidy is found in some organisms and is especially common in plants.

- **Genetic Engineering**

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's genome using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms.

- **Why Genetic Engineering are important**
  - Limitations of conventional breeding for attaining the desirable traits
  - Development of organisms that express a “novel” trait: normally not found in the species

- **Colour modification**

- Over expression of structural genes
- Use of sense or antisense enzyme construct
- Inhibit production of key biosynthetic enzyme
- Add an enzyme of a particular biosynthetic step

❖ **Engineering for Blue Rose**

✓ *Rosa hybrida* lacks violet to blue flower.

✓ Due to absence of delphinidin-based anthocyanins

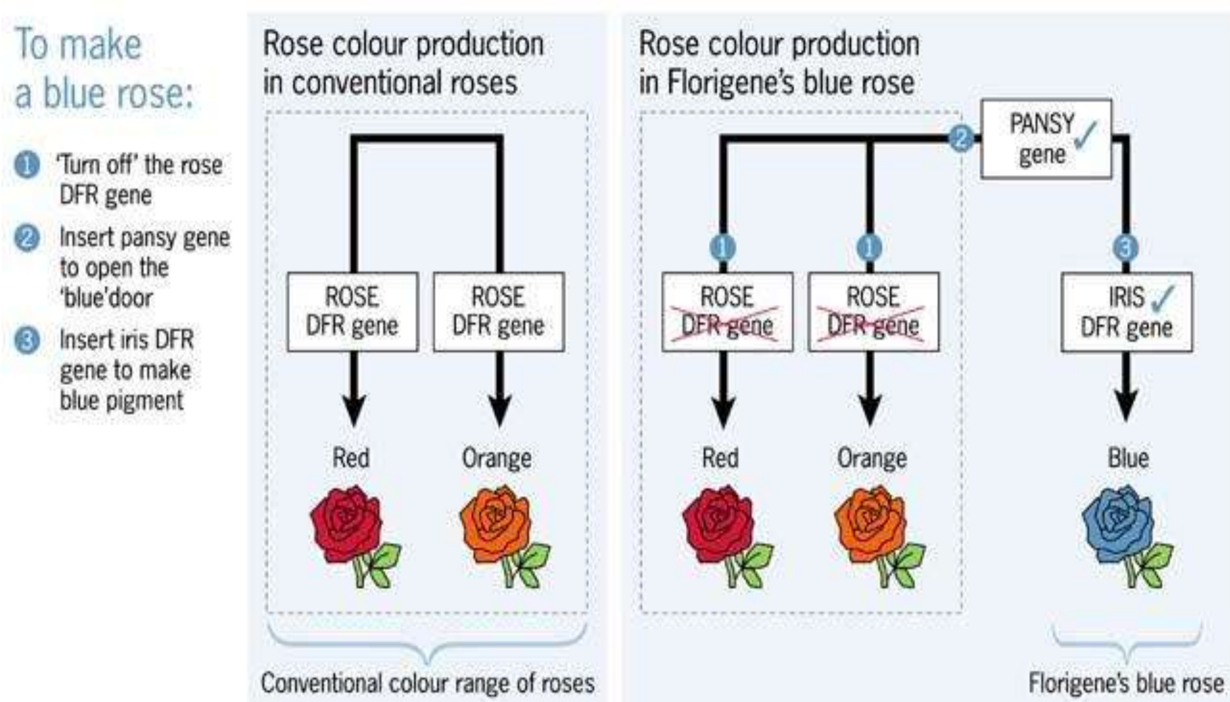
✓ Roses do not possess flavonoid 3',5'-hydoxylase (F3'5'H) For delphinidin biosynthesis

- **Process:**

➢ Down-regulation of the rose DFR gene and over-expression of the iris DFR gene by RNAi technique

➢ The over-expression of a F3'5'H – efficient accumulation of delphinidin and colour changes to blue.

➢ Efficient and exclusive delphinidin production and a bluer flower colour



- **Steps:**

1. Turn off the production of red pigment;

2. Open the 'door' to production of blue pigment; and then
3. Produce blue pigment.

✓ **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 overcome 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

✓ Future thrust:

- Species-specific genes in flavonoid biosynthetic pathway
- Changing flower pigmentation by modification of carotenoids and betalain biosynthetic pathway.
- Production of colour in scented flowers.
- Function, expression, regulation and interaction of the structural genes and regulatory genes
- Transport mechanism of pigments

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