APPROACHES FOR MODIFICATION OF FLOWER COLOUR – A REVIEW

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Abstract

Each flower is a soul blossoming in nature and it adds beauty to the environment with its attractive colours. The market value of flower is determined by the flower colour which is utmost important from the consumer point of view. Even though in nature, flowers have been bestowed with wide range of colours, mankind is still searching for more solid and novel ones. By altering the pigment biosynthetic pathway, metal ions and shape of surface cells, new colours could be developed in the flowers. In this review, various approaches like hybridization, mutation, polyploidization, genetic engineering, regulation of vacuolar pH, use of plant growth regulators and tinting used to modify flower colour have been discussed in detail with appropriate reviews.

Key words: Genetic engineering, Mutation, Pigment biosynthesis, Plant growth regulators, Tinting.

1. Introduction

The Earth laughs through flowers and they are considered as the best creatures of God. Botanically, a flower is the reproductive structure of seed bearing plants which comprises of calyx, corolla, androecium and gynoecium. The flowers add beauty to the environment with its attractive colours. Flower colour is an important agricultural trait that determines the market value. Apart from that, it plays a key role in pollination by attracting insects with their beautiful colours. The flower compounds are also found to act as intermediary for other compounds. For example, violaxanthin which is a carotenoid pigment act as the precursor for abscissic acid production. They also protect the tissues from photo-oxidative damage. These flower colours can be used as an effective tool in horticultural therapy for curing mental ailments. Although most of the flowers have been already blessed with wide spectra of colours, some species could not produce specific colours either due to absence of particular gene responsible for flower colour or environmental interactions. This can be achieved by understanding and applying the mechanisms practically in approaches employed for alteration of flower colour.

2. Scope for flower colour modification

As some of the flower crop varieties with desirable agronomic and consumer characteristics are lacking attractive colours, their demand is getting decreased in the market. Thus, by modifying the flower colour in such varieties, better market price can be expected. It has now become a trend to develop a colour that is naturally not occurring in a particular crop. For example, Chinese rose and chrysanthemum are lacking blue whereas, peony and cyclamen are lacking yellow colour naturally. The demand for flower colour changes with season to season and even year to year. So, there is a need to be in phase with the current trend to get better returns out of the produce.

3. Objective of this review

This review is mainly focused to clearly understand the biosynthetic pathway of different flower pigments and also the various mechanisms and approaches that could aid to alter the flower colour.

4. Flower pigments and their biosynthesis

Flower pigments are the compounds that are responsible for flower colour. They are the plant compounds that are perceived to produce colour. The colour pattern on the petals is primarily due to the accumulation of pigments. The major flower pigments include flavonoids, carotenoids and betalains. Of these, the flavonoids constitute a broader classification which consists of anthocyanins, metalloxanthins, chalcones and aurones. Carotenoids are of two types *viz.*, carotenes and xanthophylls. The taxonomically restricted betalain group is classified into red coloured betacyanins and yellow coloured betaxanthins. Despite, the final colour of the flower is determined by many factors such as pigment structure, type and its concentration, co-pigments, metal ion type and its concentration, vacuolar pH and shape of surface cells. An increase in the number of hydroxyl groups on the B-ring of the basic anthocyanidin structure imparts a blue colour to the anthocyanins, while methylation of the 3' or 5'-hydroxyl group results in slight reddening of petals. The anthocyanin types *viz.*, pelargonidin, cyanidin and delphinidin tend to develop orange to red, red to magenta and magenta to purple, respectively. Co-pigments like isoflavonones, flavonols and flavans influences the flower colour by reacting with the major pigments. The metal ions change the vacuolar pH and alter the colour of the flower. Generally, lower pH values produce red coloured anthocyanidins whereas, the higher pH induces blue colour in flowers. The rate of reflection varies with the incoming light based on the cell shape and modifies the flower colour. To effectively modify the flower colour, there is a need to understand the biosynthetic pathways of the pigments *viz.*, flavanoids, carotenoids and betalains which are given below.

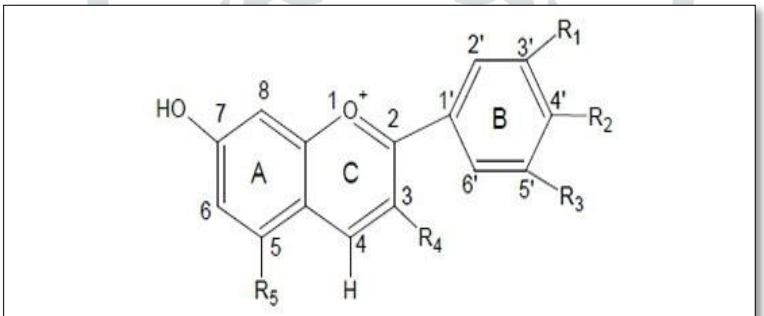
4.1 Flavonoid biosynthetic pathway:

Flavonoids are phenylpropanoid compounds that are generally water soluble and stored in the vacuole. The basic flavonoid structure is a 15-carbon (C_{15}) nucleus composed of two aromatic rings (A and B rings) joined by a three-carbon unit (which usually forms a third ring called C-ring). The various classes of flavonoids are determined by the degree of oxidation of the C-ring, while the individual aglycone compounds within each class are determined by the extent of hydroxylation, or other substitution of the A- and B-rings. Figure 1 indicates the pictorial representation of basic structure of anthocyanin which is further classified as pelargonidin, cyanidin, peonidin, delphinidin, malvidin and petunidin based on hydroxylation and methylation.

The first dedicated step to flavonoid biosynthesis is the formation of tetrahydroxy chalcones by the condensation of 4-coumaroyl CoA with three molecules of malonyl CoA in the presence of the enzyme chalcone synthase (CHS). This undergoes isomerization on reaction with chalcone isomerase (CHI) resulting in naringenin. Naringenin is converted into eriodictyol and pentahydroxy flavonone by the enzymes flavonoid 3-hydroxylase (F3'H) and flavonoid 3,5-hydroxylase (F3'5'H), respectively. These flavonones on further reaction with flavonone 3-hydroxylase (F3H) results in the production of dihydroflavonols like dihydroquercetin (DHQ), dihydrokaempferol (DHK) and dihydromyrcetin (DHM). Reduction of dihydroflavonols at the 4th position, catalysed by the dihydroxyflavonol 4-reductase (DFR) yields the corresponding leucoanthocyanidins. These leucoanthocyanidins are the direct precursors of anthocyanidins and generally do not accumulate in the plant cells. Leucoanthocyanidins react with anthocyanidin synthase (ANS) to produce the anthocyanidins. Anthocyanidins are unstable under normal physiological conditions and are stabilized to corresponding anthocyanins by the addition of sugar residues at 3 and/or 5 positions. The flavonoid biosynthesis is pictorially represented in the Fig. 2.

4.2 Carotenoid biosynthetic pathway

Carotenoids are a ubiquitous group of plant pigments differing greatly from flavonoids in structure and compartmentation. They are hydrophobic, lipid soluble pigments with a structure normally based on a 40-carbon chain derived from the general isoprenoid pathway. Their major role is to protect photosynthetic tissues from photo-oxidation. Two groups of carotenoids associated with flower colour are the hydrocarbon carotenes and their oxygenated derivatives, the xanthophylls which produce orange and yellow colours, respectively. In flowers, carotenoids are synthesized and stored in the chromoplasts, specialized plastids differentiated from chloroplasts or non-photosynthetic plastids.



Anthocyanins	R_1	R_2	R_3	R_4	R_5
Pelargonidin 3-*O*-glucoside	H	OH	H	Glucoside	OH
Cyanidin 3-*O*-glucoside	OH	OH	H	Glucoside	OH
Delphinidin 3-*O*-glucoside	OH	OH	OH	Glucoside	OH
Peonidin 3-*O*-glucoside	OCH_3	OH	H	Glucoside	OH
Malvidin 3-*O*-glucoside	OCH_3	OH	OCH_3	Glucoside	OH
Malvidin 3,5-*O*-diglucoside	OCH_3	OH	OCH_3	Glucoside	Glucoside
Cyanidin 3-*O*-rutinoside	OH	OH	H	Rutinoside	OH

Figure 1 Basic structure of Anthocyanin

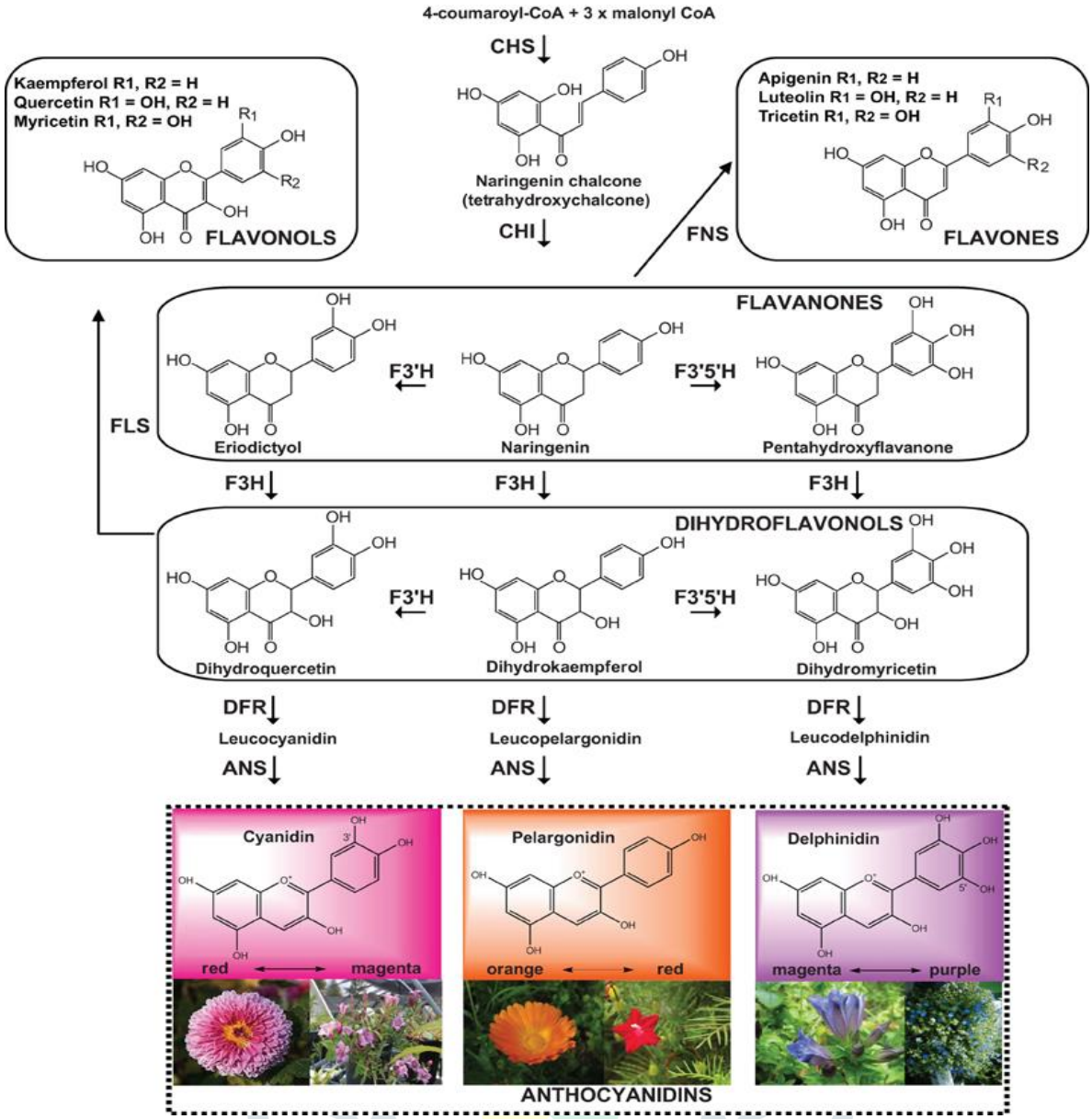


Figure 2 Flavonoid biosynthetic pathway

The first committed step in carotenoid biosynthesis is the formation of phytoene from two molecules of geranyl geranyl pyrophosphate. This two-step reaction occurs via the intermediate prephytoene pyrophosphate and is catalysed by the enzyme phytoene synthase, which is associated with the stroma of the plastid. The colourless phytoene is converted into coloured carotenoids by a series of membrane-bound enzymes which is shown in the Fig. 3.

4.3 Betalain biosynthetic pathway

Betalains are the nitrogenous vacuolar pigments providing yellow, orange, red and violet colours. They not only occur in plants but also in some fungi such as *Amanita muscaria*. Their importance as colour pigments in plants is however limited as they are restricted to the order Caryophyllales. Betalains contain betalamic acid as the chromophore. Betalamic acid itself is a yellow betalain pigment and accumulates in some species. However, it is commonly conjugated with cyclo-3, 4-dihydroxy phenylalanine (cyclo-DOPA) to form the red-violet betacyanins or with amino acids or amine side chains other than cyclo-DOPA to form yellow betaxanthins. Betacyanins and betaxanthins are derived from the amino acid tyrosine via the intermediates L-DOPA and betalamic acid as shown in Fig. 4.

5. Approaches for flower colour modification

Understanding the pigment biosynthetic pathway would be helpful to know the function of the various enzymes responsible for the end product. Identifying and isolating the genes responsible for particular enzymes could aid in further colour modifications. Various approaches like hybridization, mutation, polyploidization, genetic engineering, regulation of vacuolar pH, use of plant growth regulators and tinting can be adopted to modify the flower colour by targeting the particular step or particular enzyme in the biosynthetic process and are discussed with suitable literatures.

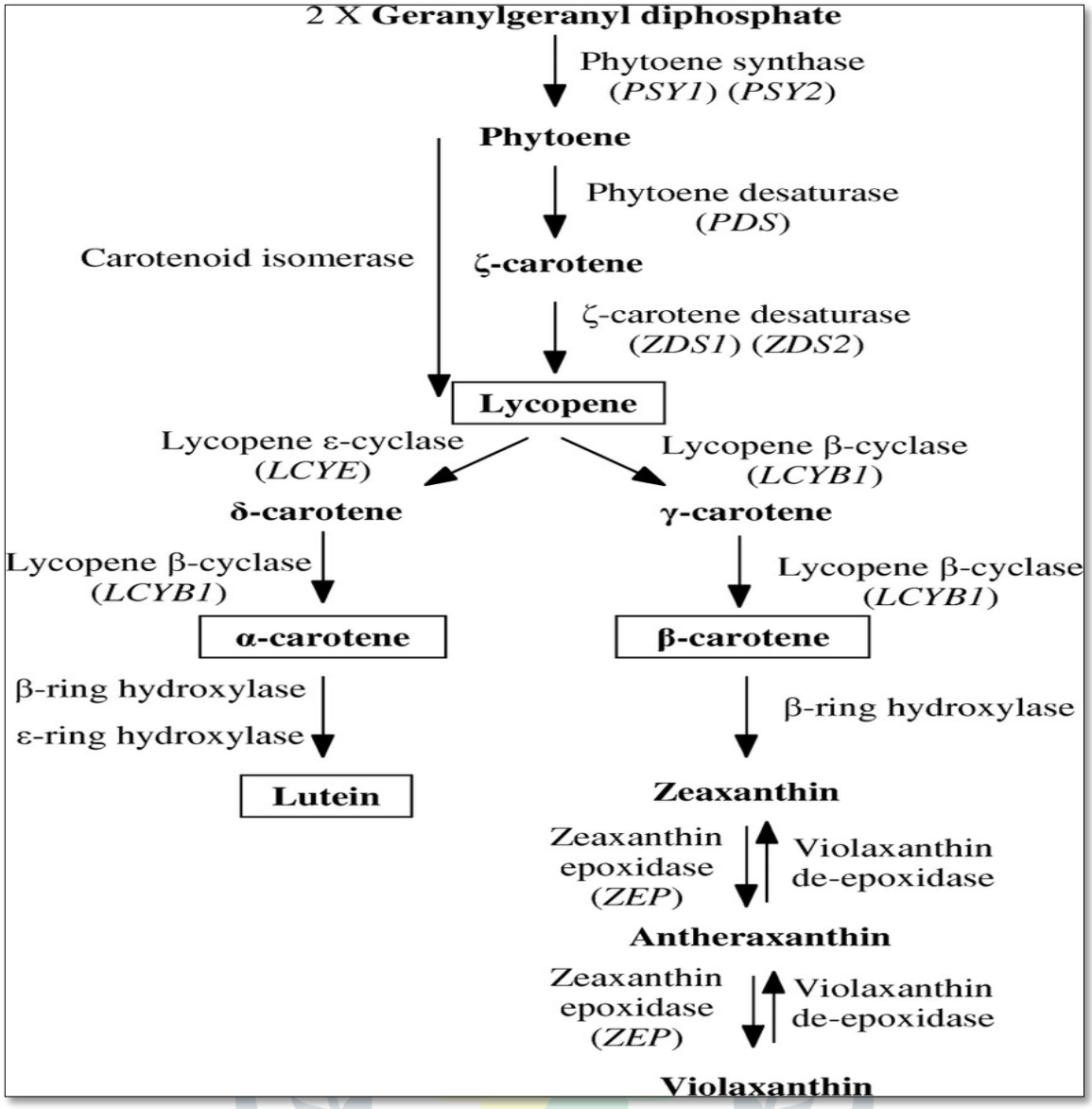


Figure 3 Carotenoid biosynthesis

5.1 Hybridization

The process of crossing inbred parental lines to produce a F_1 hybrid with desirable characteristics is called hybridization. An interspecific *Epidendrum* hybrid was developed by crossing *E. radicans* and *E. xanthinum*. The selected line (NRCO-*Epidendrum* cross/2005-01) was characterized bigger than both parents with bright saffron orange colour (RHS44A) acquired from female parent and the shape and lobular characteristics attributed by male parent. Further, the F_1 progeny of the selected line flowered with different shades *viz.*, red-orange, orange-yellow and yellow (Devadas et al., 2010). Magdalita and Pimentel (2013) developed seven hybrids with unique and new flower traits namely, *Hibiscus rosa-sinensis* 'Domini M. Torevillas', 'Cynthia A. Villar', 'Marilyn D. Maranon', 'Maria Rosario O. Montejo', 'Arlene B. Arcillas', 'Connie S. Angeles' and 'Sylvia P. Lina' which were collectively called as "Women in Public Service Series II". Thus, flower colour modification is achieved through hybridization by either co-dominance, genetic interaction, transgressive segregation or introgression of desired gene from the wild type.

5.2 Mutation

Mutation is the sudden heritable change in the phenotype of an individual. It may be spontaneous or induced, macro or micro level. The physical or chemical agents which enhance the mutation frequency are commonly called as mutagens. Physical mutagens include ionizing radiations like α, β, γ, X-rays and non-ionizing radiations like UV and infrared rays. The chemical mutagens consists of ethyl methyl sulphonate, dimethyl sulphonate, bromouracil, etc.,. Mutation is the best method to create variability among the ornamental plants. Out of the total mutants developed, approximately 55 per cent were related with flower colour modification. Several mutants like Abhisarika, Madhosh and Twinkle in rose, Shoha and Tambari in gladiolus, Raktima, Agnisikha and Alankar in chrysanthemum had been developed so far. Mutation is the easiest and economical way for modifying flower colour.

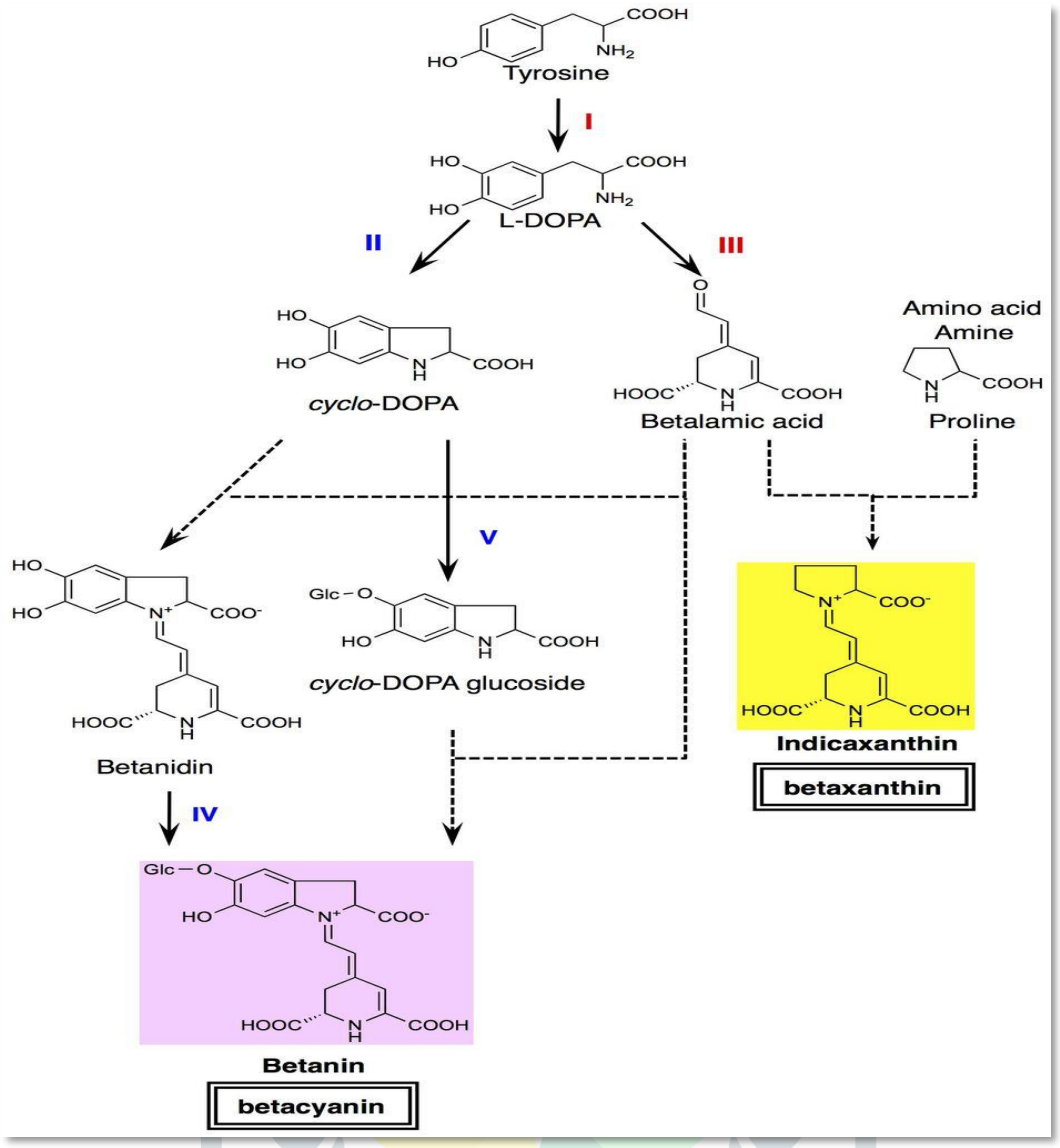


Figure 4 Betalain biosynthesis

Hase et al. (2010) stated that 8 days old petunia seedlings pre-treated with 3 per cent sucrose followed by 8 Gy of 320 MeV carbon ion beam irradiation (1.52, 1.20 and 1.26 %) increased frequency of flower colour mutants as compared to non-pretreated group (0.56, 0.58 and 0.47 %). It is thought that the sucrose pretreatment increased the radiation sensitivity of particular genes or chromosomes involved in pigment biosynthesis by altering the packaging status of chromatin.

Jimba is a white flowering type and its budsport exhibited a faintly yellow colour. This budsport on subsequent irradiation with carbon ions obtained IB-1 lines with pale yellow flowers and IB-2 lines with deep yellow flowers. Moreover, the number of *CmCCD4a* (Chrysanthemum carotenoid cleavage dioxygenase 4a) homologs decreased by irradiation resulting in increasing level of carotenoid. It has been found that the *CmCCD4a* inhibited the production of carotenoid pigment and by the suppression of the gene, yellow flowers were achieved in the cultivar (Ohmiya et al., 2012).

Yamaguchi (2013) investigated the characteristics of ion beams as mutagens for flower colour modification in chrysanthemum cv. 'Taihei' and found that 5 Gy of 320 MeV carbon ion beams resulted in comparatively higher mutation frequency (16.3 %) followed by 5 Gy of 220 MeV carbon ion beam (14.5 %) and 15 Gy of 100 MeV Helium ion beam (12.3 %). Because the ion beam rays are effective in inducing point mutation and novel colour mutants because of their higher linear energy transfer.

The efficiency of gamma ray was worked out by Madhu Bala and Singh (2015) in generating mutation populations of *Rosa hybrida* L. cv. Raktima and it was reported that *in vitro* mutagenesis using 40 and 55 Gy gamma rays exhibited two mutants, RK-1 with 7.48 % flower colour variation and RK-2 with 8.51 % flower colour variation, respectively.

5.3 Polyploidization

Polyploidy is the condition in which the organism is having more than two monoploid sets of chromosomes. It is of two types *viz.*, euploidy and aneuploidy. Euploidy is the condition in which numerical change in the entire genome takes place and it is classified into auto and allopolyploidy. Aneuploidy is the condition which involves change in chromosome number with one or few chromosomes of the genome. Aneuploidy is again divided into hyperploidy (trisomy, tetrasomy) and hypoploidy (monosomy, nullisomy). As the cell size, number, thickness of leaves increase with ploidy level, the pigment concentration may

also increase with increasing ploidy level resulting in intensification of a particular colour. In the flavonol biosynthetic pathway of *Petunia* 'Mitchell', polyploidy has a differential effect increasing the relative concentration of the major metabolite Quercetin-3-sophoroside ($Q3^2$) and decreasing the relative concentration of the minor metabolite Quercetin-3,7-diglucoside (Q3,7) (Griesbach and Kamo, 1996).

5.4 Genetic engineering

Genetic engineering is the manipulation of plant genome through recombinant DNA technology to alter plant characteristics. It can also be defined as the controlled introduction of specific DNA segments into recipient plant genome. Gene transfer can be done by various methods like *Agrobacterium* mediated gene transfer, particle bombardment, glass bead method, ultrasonication, electroporation and shock wave method. Colour modification could be achieved through genetic engineering by either over expression of structural genes, inhibition of key biosynthetic enzyme, addition of an enzyme in a particular biosynthetic step or use of sense or antisense enzyme construct. Blue carnation and blue rose are the milestones in the ornamental genetic engineering as they were commercialized globally.

Tsuda et al. (2004) modified the flower colour in some commercial varieties of *Petunia hybrida* by metabolic engineering and reported that the complete suppression of *F3H* and *DFR* genes in Surfinia Purple yielded pale flowers while, the down-regulation of *F3'5'H* gene in Surfinia Purple altered the flower colour similar to Surfinia Hot Pink. Further, suppression of *F3'5'H, AR-AT* and *FLS* genes in Surfinia Purple Mini and Surfinia Purple redirected the metabolic pathway from malvidin to cyanidin. For the generation of orange petunias, *F3'H* gene was down-regulated and rose *DFR* gene was over-expressed. They also indicated that over-expression of *FLS* and *FNS* genes increased the flavonol and flavone contents in petals, respectively.

Noda et al. (2013) studied the modification of anthocyanin biosynthetic pathway in chrysanthemum to produce delphinidin based anthocyanins instead of cyanidin based anthocyanidins by metabolic engineering and resulted that *Chrysanthemum F3H* promoter driven *ADH* translational enhancer fused with *Campanula F3'5'H* (1408-9 line) efficiently induced delphindin production in chrysanthemum ray florets, leading to high accumulation of delphinidin.

5.5 Regulation of vacuolar pH

Anthocyanin changes its colour depending on pH. In strong acidic conditions, it shows red; in neutral, purple and in alkaline, blue. But generally, the vacuolar pH of plant cells is weakly acidic and under such conditions, almost all anthocyanins are purple and very unstable. The following three mechanisms have been proposed for stable flower colour: metal chelation, vacuolar pH change and molecular associations. Yoshida et al. (2003) observed the correlation between the sepal colour variation and vacuolar pH of *Hydrangea macrophyllus* using micro-spectrophotometry and proton-selective microelectrode. They found that the average values for the vacuolar pH of blue (λ_{vismax}: 589 nm) and red cells (λ_{vismax}: 537 nm) were 4.1 and 3.3, respectively with vacuolar pH of blue cells being significantly higher.

5.6 Plant growth regulators

Plant growth regulators have different process to change and regulate the synthesis of anthocyanin and secondary metabolites. It directly or indirectly affects the flower colour by expression or suppression of related genes involved in flavonoid biosynthesis. Banon et al. (2002) investigated the influence of paclobutrazol on flower colorimetric values in *Dianthus caryophyllus* cv. Mondriaan and stated that red colour flowers of the cultivar turned to purple tone after drenching of 0.25 mg paclobutrazol during winter season with reduced chroma value (33.4) and hue angle (354.0).

The foliar spray of 2000 mg/L 'A 1699-DF' increased lightness and decreased hue value with less saturation which resulted in light pink coloured petals compared to dark red coloured petals of *Impatiens walleriana* 'Accent Cranberry'. Further, Geranium plants treated with foliar spray of 1000 mg/L A 1699-DF produced pale orange flowers with maximum lightness and minimum values of hue and saturation compared to control. 'A 1699-DF' containing Ca-prohexadione competes with 2-oxoglutaric acid dependent dioxygenase (flavonone 3-hydroxylase) resulting in decreased anthocyanin pigment synthesis (Cavins, 2006). Yaghoub et al. (2017) studied the modification of flower colour pigments with hormonal treatments and sucrose in *Tulipa gesneriana* 'Kingsblood' and reported that the highest total flavanoid content (2.912 mg/gfw) and anthocyanin content (2.406 mg/L) were found in the perianthes of tulips sprayed with 500 mg/L GA_3 without sucrose.

5.7 Tinting

Tinting is the process of artificial colouring of flowers. It is generally practiced in flowers with white colour or shades of white colour like carnation, tuberose, orchid, rose and chrysanthemum. In this technique, the flowers or flower spikes are dipped in dyes like eosin yellow, phenol red and other food colourants to produce brilliant coloured flowers. This is the simple and easy technique which can be adopted even by farmers to get more profit out of their produce. The spikes dipped in 1.5 % sunset yellow + carmosine orange red edible dye for 24 hrs immersion time resulted in highest overall acceptability of tuberose cvs. Mexican Single and Pearl Double (Safeena et al., 2016). The experiment to standardize the tinting techniques in China aster cv. Local White revealed that food dyes *viz*., Apple Green, Lemon Yellow and Orange Red at 4 % concentration expressed full bright coloured flowers with quick uptake of dyes in a short period of two hours duration (Ranchana et al., 2017).

6. Conclusion

Development of flower with novel colours had been a dream in floriculture industry as flower colour is the key element in consumer selection. It came into reality by the adoption of various techniques which mainly affects the vacuolar pH, metal ions and co-pigments responsible for flower colour variation. Colour change in flowers through hybridization is achieved through introgression of target gene from wild genotype and co-dominance. A wide spectrum of colour variation can be obtained by exploiting heavy ion beam radiation for mutation which has higher linear energy transfer and mutation efficiency. The concentration of pigments can be enhanced with increasing ploidy level which results in more intense flower colours.

Modification of flower colour is possible with genetic engineering by either suppression of endogenous gene, over-expression of target gene or combination of both. This approach has been found more beneficial due to extensive available information on pigment biosynthetic pathway. Regulation of metal ions in flower petals results in change of vacuolar pH which in turn influences the flower colour. Plant growth regulators alter the flower colour by affecting the enzymes involved in pigment biosynthesis. Tinting is the easy and economical way to modify the flower colour even by farmers, retailers and consumers. Further efforts should be taken to improve the phenotypic stability of colour-altered plants and also to access more information about carotenoid and betalain biosynthetic pathways which remained unclear.

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