TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Review Article

Turk J Agric For
(2023) 47: 273-287
© TÜBİTAK
doi:10.55730/1300-011X.3086

The molecular regulators, pathways, and environmental impacts of white flowers

Sagheer AHMAD[1,2]*, Zaibun NISA[3], Muhammad Zeeshan MUNIR[4], Muhammad IMRAN[5], Shaista NOSHEEN[6], Kai ZHAO[1,2]

[1]College of Life Sciences, Fujian Normal University, Fuzhou, China
[2]Key Laboratory of National Forestry and Grassland Administration for Orchid Conservation and Utilization at College of Landscape Architecture, Fujian Agriculture and Forestry University, Fuzhou, China
[3]Cotton Research Institute, Multan, Punjab, Pakistan
[4]School of Environment and Energy, Peking University Shenzhen Graduate School, Shenzhen, China
[5]Department of Crop Science and Technology, College of Agriculture, South China Agricultural University, Guangzhou, P. R. China
[6]Colin Ratledge Center for Microbial Lipids, School of Agriculture Engineering and Food Science, Shandong University of Technology, Zibo, China

Received: 24.01.2023 • **Accepted/Published Online:** 09.03.2022 • **Final Version:** 02.06.2023

Abstract: White flowers are an important element of natural beauty, although they are thought of colorless. Anthocyanins are the major compounds responsible for a variety of pigments in flowers. White flowers surely lack pigmented anthocyanins and other compounds, but there are regulatory mechanisms that hinder the deposition of color pigments in white flowers. Moreover, there are also some compounds that deposit to instigate white appearance and the genetic regulators control the biosynthesis and deposition of such compounds. Experts have shown that fluctuation in the equilibrium of *FLS* and *DFR* genes determines the degree of accumulation of anthocyanins and flavonols in the flowers, leading to different color patterns. The gene suppression techniques to suppress certain flavonoid pathway genes, such as CHS, or the insertion of DNA transposable elements into CHS produce white flower color in a number of species. Although pigments find wide application in the everyday life, white color does possess special importance in nature. Land plants use white flowers for reproductive viability during cold early spring to conserve energy and reduce nutrient costs, particularly in low density pollinations. Moreover, white fruit skins deposit specific amounts of anthocyanins and phenols with significant health benefits. This review, thus, deciphers the regulatory mechanisms for the absence of pigments in the white flowers and the deposition of special compounds causing white appearance and the benefits of white color in nature.

Keywords: Anthocyanins, eco-environment, volatiles, white color, reproduction

1. Introduction

Visual floral traits, especially flower color, are the crucial elements of plants to shape their interaction with the environment (Roguz et al., 2020). An enormous flower color variation exists in plants and a diverse range of factors are involved in flower color polymorphism. However, the diversity of flower color among modern lineages has only been studied in a few plant groups. Flower color plays a role in plant–environment interaction, affects plant-pollination interaction patterns, and drives species viability in extreme conditions (Roguz et al., 2020). White flower color possesses significant reproductive, environmental, and nutritional value. White flowers have been used in ceremonial occasions as a symbol of purity and innocence. White flowers also make a fine blend with pigmented flowers in floral arrangements. Therefore, there is a huge commercial demand for white beauty, especially in Japan the white chrysanthemum and carnations are in demand throughout the year for ceremonial purposes (Totsuka et al.,, 2018).

Flower color is regulated by three main groups of pigments, including carotenoids, betalains, and flavonoids. Flavonoids are among the widely distributed metabolites in plants; especially, flavonols and anthocyanins are the most important flavonoids that determine flower colors, including white, red, yellow, and purple (Morita et al., 2014; Tanaka et al., 2008). Therefore, the flavonoid biosynthesis pathway is the most studied pathway in color regulation in plants (Quattrocchio et al., 2006). The genetic regulators of pigmented-anthocyanin biosynthesis have been studied well in a number of species, such as *Arabidopsis thaliana*, petunia (*Petunia hybrida*), grape (*Vitis vinifera*),

* Correspondence: zhaokai@fjnu.edu.cn

snapdragon (*Antirrhinum majus*), and maize (*Zea mays*), thereby becoming important targets of molecular breeding (Dixonet al., 2013; Dixon and Pasinetti 2010; Petroni and Tonelli 2011; Quattrocchioet al., 2006). However, not all flavonoids cause vibrant flower colors. At least 400 flavonoids are not anthocyanins, most of which are pale yellow, white or colorless.

Variations in flavonoid biosynthesis pathway genes, either natural or induced, cause differential accumulation of phytochemicals in the petals, leading to color polymorphism. Therefore, appearance of light or white color may result from individual gene activity in the flavonoid biosynthesis pathway. For example, chalcone synthase (CHS) is a pivotal enzyme responsible for the biosynthesis of flavonoids. It catalyzes the naringenin chalcone formation (Martin, 1993). The expression of *CHS* gene was drastically decreased in the white flowers buds of morning glories (*Ipomoea purpurea*) (Fukada-Tanaka et al., 1997). Moreover, fluctuation in the equilibrium of *DFR* and *FLS* genes determines the accumulation of anthocyanins and flavonols in the white and red flower species (Luo et al., 2016). Heterologous *FLS* expression in transgenic tobacco promotes flavonol biosynthesis and blocks anthocyanin accumulation, leading to white flowers (Luoet al., 2016). Analysis of flavonol and anthocyanin in 7 species with red and white flowers (rose (*Rosa indica*), peach (*Prunus persica*), carnation, azalea (*Rhododendron*), camellia (*Camellia japonica*) and petunia) showed a significantly higher anthocyanin levels in red flowers as compared to white ones. Overexpression of *DFR* gene in tobacco (*Nicotiana tabacum*) causes the predominant flavonol accumulation that produces pure white flowers. Linkage of anthocyanidins to one or more sugars, provides different colors in living cells. These pigments can take many forms in the solution based on the solvent and the pH. A solution pH range of 3–6 supports the linkage of colored pigments with copigments, such as carotenoids, colorless flavonols or metals. The anthocyanins can also attach to aromatic acyl groups or organic acids. In fact, most of the flavonoids are altered by the linkage of covalent bonds to glycosyl, acyl or methyl groups. These modifications stabilize the molecules, add pigments and finally change hue. Acylation is considered to be helpful in the stacking of these pigments (Miller et al., 2011). Therefore, anthocyanin accumulation is key to flower appearance in light and deep colors.

White flowers also affect pollination success of plants and express environmental adaptations to allow successful reproduction. This review summarizes the molecular framework of white flower color formation and the impact of white flowers on the reproductive success of plants.

2. Anthocyanin biosynthesis-step-breakdown causes white flowers

Anthocyanin biosynthesis can be divided into two steps: an early biosynthesis and a late biosynthesis step (Figure 1). While many color morphs are generated through early biosynthesis steps (Tanaka and Ohmiya, 2008), the diversions are also observed leading to while phenotypes. White *P. hybrida* line W43 flowers accumulate glucosides of caffeic acid and 4-coumaric acid and are able to make anthocyanins from exogenous supply of naringenin (Mol et al., 1983). This suggests that W43 has a blockage in the biosynthesis step preceding the formation of naringenin. Contrarily, the red W43 cultivar allows the anthocyanin production in certain parts of flower. In the white sectors of flower, *CHS* activity does not occur (Mol et al., 1983). The white flowers of *D. caryophyllus* contain kaempferol as a major pigment, while the red flowers contain pelargonidin (Forkmann and Dangelmayr, 1980). After the administration of dihydroquercetin to red petals, the naturally formed pelargonidin is augmented by enough amount of cyanidin. However, naringenin and dihydroquercetin did not affect white petals and anthocyanin production was not observed, showing a genetic block after the formation of dihydroflavonol (Forkmann and Dangelmayr, 1980).

Studies on the genetic regulation of anthocyanin pathway in some plants showed that the lack of anthocyanin was caused by the absence of chalcone synthase activity (Hrazdina and Weeden, 1986). Mutants with white flowers show an altered structural gene encoding *CHS*, resulting in a reduced level or absence of *CHS* (Rall and Hemleben, 1984). White mutant of *P. hybrida* showed the blockage of anthocyanin synthesis at the *CHS* step (Molet al., 1983). However, in some other species, the lack of anthocyanin expression was caused by the deficiency in the activity of glucosyltransferase, phenylalanine lyase (PAL), and CHS (Dooner, 1985).

Flower color variations are generally resulted from the differences in either regulatory or structural genes involving flavonoid biosynthesis pathway (Nakatsuka et al., 2005). For example, blockage in the early steps of flavonoid biosynthesis due to mutation causes the white flower formation due to accumulation of colorless pigments; while the mutation blocking the late steps instigates the formation of colored flowers due to accumulation of particular anthocyanins. Moreover, the white flower phenotypes vary from white to ivory depending upon the blockage of respective steps of flavonoid biosynthesis. For instance, the accumulation of colorless flavonoids in the ivory flowers of *D. caryophyllus* (Mato et al., 2000; Stich et al., 1992), *A. majus* (Martin et al., 1985; Martin et al., 1991), *E. grandiflorum* (Davieset al., 1993), and *Pharbitis nil* (Hoshinoet al., 1997; Saitoet al., 1994) is caused by

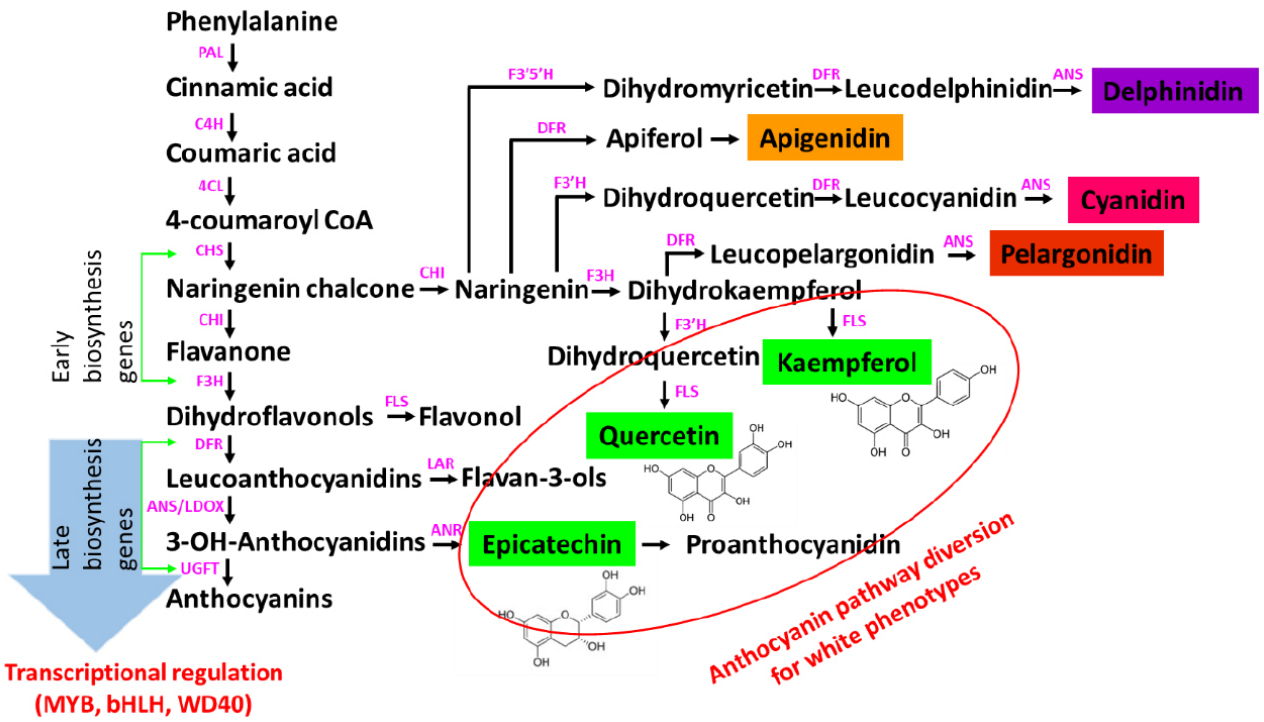


Figure 1. The modified anthocyanin pathway leading to white flower color (PAL, phenylalanine ammonialyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; FLS, Flavonol synthase; DFR, dihydroflavonol 4-reductase; LAR, leucoanthocyanidin reductase; ANS, anthocyanidin synthase; LDOX, leucoanthocyanidin dioxygenase; ANR, Anthocyanidin reductase; UGFT, UDP-glucoside: flavonoid glucosyltransferase; F3'5'H, flavonoid 3′,5′-hydroxylase).

mutations in the genes encoding *DFR*, *ANS*, or *F3H* during late steps of flavonoid biosynthesis. Contrarily, the pure white flowers accumulate organic acids instead of flavonoids (Mulder-Krieger and Verpoorte, 2012), which occurs due to an *f* mutation in *Matthiola incana* (Hemleben et al., 2004; Rall and Hemleben, 1984) and a *niv* mutation in *A. majus* (Spribille and Forkmann, 1982). Both mutations affect *CHS*. Mutants of flavonoid biosynthesis regulatory factors also trigger white flower phenotypes. For example, the *g* mutant in *M. incana* and *an1* (Spelt et al., 2002) and *an2* (Quattrocchio et al., 1999) mutants in *P. hybrida* (Ramsayet al., 2003). A defect in the *A* gene of carnation causes white color phenotype (Geissman and Mehlquist 1947; Mehlquist and Geissman 1947). The *A* gene involves anthocyanin synthesis pathway, regulating *chalcone isomerase* (*CHI*) (Itoh et al., 2002; Stichet al., 1992). Defective *F3H* (*flavonone 3-hydroxylase*) expression also causes white phenotype. *F3H* plays a role in catalysis of naringenin to dihydroflavonol before the *DFR* step (Mato et al., 2000). Therefore, mutation in carnation *F3H* and *DFR* can generate white phenotype. Four kaempferol glycosides accumulate in white carnation petals (Iwashina et al., 2010), suggesting the production of kaempferol instead of anthocyanins. Therefore, the genes encoding early biosynthesis enzymes of upstream flavonoid biosynthesis pathway up to *F3H* shall be active, but the genes downstream of *DFR* might be repressed. Transcription factors, such as MYB, bHLH and WD40 regulate the expression of late genes (Hichri et al., 2011). Transient expression of two bHLH genes (*MYC-146* and *GL3*) in the white petals of *M. incana* mutants complemented the anthocyanin deficiency by activating the biosynthesis of anthocyanins (Ramsay et al., 2003). MYB genes controlling the downstream pathway elements are exclusively linked to the convergent evolutionary transitions leading to white flowers (Larter et al., 2018). In the white *Iochroma loxense* flowers, the pigment loss involves R3MYB repressor that restricts the downstream gene expression (Gates et al., 2018).

3. Mutations causing white phenotype

Mutations either natural or spontaneous drive the process of evolution in the living organisms. Flower color mutation widely occurs in nature and a number of plants with

beautiful flowers have been produced through natural variability. Flower color mutations have been studied in species like, morning glory (Hoshino et al., 1997; Iida et al., 2004), petunia (Clegg and Durbin, 2000; Gerats et al., 1990), and snapdragon (Hudson et al., 2008; Martinet al., 1991). The flower color can be genetically modified by transforming chimeric or endogenous *chs* constructs into plants. The scientists have tried to produce white flowers in medicinal plants, such as *E. purpurea* (Wang and To, 2004) and ornamental plants, such as *E. grandiflorum* (Deroles et al., 1998), *D. grandiflora* (Courtney-Gutterson et al., 1994), *Torenia fournieri* (Aida et al., 2000), and *T. hybrida* (Suzuki et al., 2000) by using *chs* constructs. The resultant white transformants have been successfully achieved in *T. hybrida*, *E. grandiflorum*, and *D. grandiflora*. Use of petunia *CHS* construct to cosuppress tobacco *CHS* results in white flowers (Wang et al., 2006). Introducing additional *chs* genes into *Arabidopsis* and petunia causes the cosuppression or posttranscriptional gene silencing (PTGS) of *chs* (Depicker and Van Montagu, 1997; Metzlaff et al., 2000). CHS is the key enzyme in the biosynthetic pathway of anthocyanin and, therefore, its silencing can be easily observed by the loss of pigmentation in petals. Posttranscriptional silencing of two different *CHS* genes simultaneously causes pure while flowers in the octoploid dahlia (*Dahlia variabilis*) (Ohno et al., 2011).

Genetic engineering techniques have been used to modify flower color. Mostly, the flower color changes are successfully achieved by suppression technology, including RNAi (RNA interference), antisense and cosuppression (Davies 2008; Tanaka et al., 2005). The suppression of *CHS* gene, that encodes a key enzyme catalyzing the first step of flavonoid biosynthesis pathway, induces white flowers in petunia and torenia (Nakamura et al., 2006; Napoli et al., 1990; Suzuki et al., 2000; Tsuda et al., 2004; Van der Krol et al., 1990). Using antisense method, the *CHS* gene was suppressed in gentian plants to produce white flowers (Nishihara et al., 2006). By RNAi, the suppression of *ANS* or *CHS* was made to produce white gentian flowers (Nakatsuka et al., 2008). An *F3H* knockdown by RNAi in blue-flowered torenia produces white flowers (Ono et al., 2006). In addition, mutations were also observed in *F3'H* and *F3'5'H* genes.

Transposable elements have been shown to affect gene expression in different organisms (Huang et al., 2012; Rebollo et al., 2010). These elements have an established role in plant diversity and evolution (Oliver et al., 2013). Insertion of transposable elements often causes epigenetic changes, like DNA methylation that leads to strong gene silencing (Rebollo et al., 2010; Uchiyama et al., 2009). Retrotransposons are ubiquitous components of plant genomes (Grandbastien and Casacuberta, 2012) and the transposon insertions have been documented both in coding and noncoding regions. Insertion of an *En*/*Spm*-like transposon (*Ttf1*) into the *TfMYB1* caused mutated torenia phenotype due to suppression of flavonoid biosynthesis pathway genes, such as *ANS, CHS, DFR, F3H,* and *UGFT* (Nishijima et al., 2013). *TORE1* is a typical LTR-type retrotransposon, called 560-bp LTR bearing a target site duplication (TSD) of 5 bps, a PPT (polypurine tract), and PBS (primer binding site), encoding a partial gag-pol protein (Nishihara et al., 2014). It may be an autonomously derived nonautonomous element. In torenia (*Torenia fournieri* Lind.), the *TORE1* insertion completely suppressed the expression of F3H (Nishihara et al., 2014), causing white coloration. The expression analysis showed that the absence of *F3H* (flavanone 3-hydroxylase) in the petals was possibly caused by the insertion of *TORE1* (a novel LTR-type (long terminal repeat) retrotransposable element) into the 5'-upstream region of *F3H* gene in white torenia (Nishihara et al., 2014). This insertion significantly suppressed the promoter activity of *F3H*, causing its absence in the white petals. For cross-checking, insertion of *GtF3H*, a foreign *F3H* cDNA, restored the pink-pigmentation, justifying the *F3H* deficiency as the sole cause of white color appearance in torenia flowers due to reduced anthocyanin levels in the petals. *Hin*d III-aided partial digestion induced by *de novo* methylation has been documented in the transgenic pea, showing very strong transgene cosuppression after the viral infection (Jones et al., 1998). Such an epigenetic change not only disrupts *F3H* promoter but also reduces its activity. The white morning glory flowers are produced by inserting a DNA transposable element, related to *Tpn1*, into *CHS* intron (Hoshino et al., 2009). The variegated morning glory flower is obtained by inserting *DFR* (dihydroflavonol 4-reductase) with *Tpn1* transposable element (Inagaki et al., 1994). Red petunia flower is the result of a mutation of *F3'5'H* caused by a transposon insertion (Matsubara et al., 2005). Mutation driven pigment variations have also been studied in carnations (*D. caryophyllus*) (Itoh et al., 2002; Momose et al., 2013; Nishizaki et al., 2011).

The insertion of solo, partial or full transposable elements affects the color shift. Deep examination of *F3H* promoter's 5'-upstream region of white torenia genome showed the insertion of a solo-LTR in place of full length *TORE1*, suggesting that *TORE1*- and solo *LTR*-inserted *F3H* are nonallelic. The inter-LTR recombination within *TORE1* may constitute solo LTR in certain cells (Vitte and Panaud, 2003). White torenia flowers sometimes show faint pink-recovered sections in the petals. However, insertion of a solo-LTR is comparatively less effective to reduce the promoter activity as compared to the full insertion of *TORE1* (Nishihara et al., 2014). Therefore, solo LTR can contribute partial recovery of phenotypes.

The mutations mainly restricted the activities of flavonoid B-ring hydroxylation, revealed by the presence of epigenin as the only flavone and accumulation of pelargonidin derivatives rather than delphinidin ones. Such mutations have also been observed in some crops. A deletion of single-base in *F3'H* and *FLS* causes grey pubescence color and magenta flower color, respectively, in soybean (Takahashi et al., 2007). Insertion of a 4-bp mutation in 3GGT (3-*O*-glucoside-*O*-glucosyltransferase) causes dusky colored flowers in morning glory (Morita et al., 2005). In three species of morning glory, the insertion of a single T, and transposable element Tip201 in *F3'H* causes reddish flowers (Hoshino et al., 2003). In the white torenia, a deletion of 12-bp in exon 3 of *F3'H* did not drive a frameshift, rather caused the absence of 4 amino acid residues. The deleted amino acid residues can partially overlap with a binding pocket (A/G-G-X-D/E-T-T/S) containing threonine, which is an oxygen-binding motif contained by cytochrome P450 monooxygenase, used in catalysis (Durst and Nelson, 1995). The absence of this motif is supposed to inhibit the enzymatic activity of F3'H. Moreover, the white torenia also shows a shorter maximum absorption peak (333 nm) of UV as compared with 346 nm of violet torenia.

4. White color affects mating system

Flower color may cause difference in the mating system due to differential visitation rates and pollinator species (Mu et al., 2011). Moreover, flower color also affects the time of visitation. A study explores that in the early spring, when few pollinators are available, white gentian (*Gentiana leucomelaena*) flowers always bloom earlier than blue gentian flowers (Muet al., 2011). The polymorphism of flower color is associated with the diversity of pollinator species (Figure 2a). For example, birds mainly prefer pink and red flowers; bees prefer yellow, purple, and blue flowers; while moths nocturnally like white flowers (Rausher, 2008; Wilson et al., 2004). This specification justifies that flower color polymorphism can be linked with different pollinators as well as different mating systems (Figure 2b). Pale and white flowers of *Delphinium nelsonii* receive less natural pollinators as compared to blue flowers, although the flowers of both colors give equal seed production after artificial pollination (Waser and Price, 1981). In the southern California, the populations of *Camissonia cheiranthifolia* produce large colorful flowers with enough amount of nectar, making them strongly self-incompatible. Contrarily, the small and plain flowers are mostly self-compatible and possess contrasting rates of pollinator visitation (Raven, 1969). Such studies suggest that colorful flowers are more capable of cross-fertilization as compared to colorless and plain flowers. The species visited by bees often possess purple or blue flowers with short and wide corolla tubes, wide limbs, inserted stigmas, and concentrated nectar in small amounts. However, the flowers pollinated by moths are usually white and fragrant, bearing long tubes that are often open at night (Rausher, 2008). Therefore, flower color can also be correlated with rewarding/attractive traits, such as pollen productivity, flower size, life span, nectar volume and concentration, and diurnal cycles.

Light spectrum is another factor the flower visitors use to differentiate flower color. Bees have good color vision, especially the yellow, blue and ultraviolet spectra; whereas flies prefer yellow or white flowers (Briscoe and Chittka, 2001). Therefore, bees prefer to visit blue flowers compared to white flowers (Ackermann and Weigend, 2006; Thomson and Wilson, 2008). Because honeybees possess higher pollination efficiency compared to flies, blue flowers show greater reproductive success than white flowers. Similarly, white *D. nelsonii* flowers produce less seeds than blue flowers because bumblebees visit blue flowers more frequently than white ones (Waser and Price, 1981). Moreover, bumblebees and hummingbirds also prefer blue flowers due to their high quality nectar as compared to white flowers (Waser and Price, 1981), as some flies or bees distinguish flowers based on pollen or the quality/quantity of nectar (Nepi et al., 2009; Smith and Cobey, 1994). Flowers pollinated by beetles are usually white or dull color, possibly because the visual sense of beetles is poor. Moth-pollinated flowers are also white or yellow, distinguishing the flowers to attract the active moths in dwindling light.

The difference in the mating systems for white and blue flowers have ecological significance. At an extremely low pollinator density, the production of white flowers could be advantageous. Plants that flower in early spring face extreme temperature and low pollination rate at alpine areas (Kudo, 1993), thus favoring self-pollination. Similarly, emergence of white flowers is an effective strategy for reproductive viability that not only conserves energy but also reduces nutrient costs, particularly in cold early spring with low density of pollination (Charlesworth, 2006). Contrarily, cross-pollinated colorful flowers maintain a high genetic diversity (Mu et al., 2011).

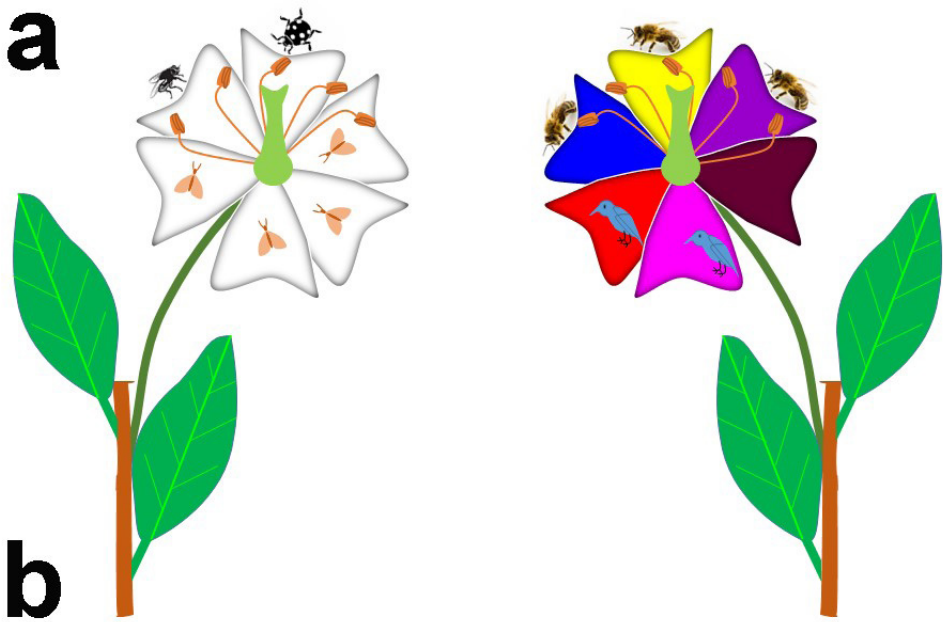
Seed dispersal has an effect on flower color. The wind-pollinated flowers are rarely colorful (Friedman and Barrett, 2008). Fruits with wind-dispersed seeds are rarely brightly colored. Pollinating animals visit the flowers based on colors. Colors are helpful in plant species selection, ripeness, flower location, nectar and pollen location inside the flower (Miller et al., 2011). Therefore, flower pigmentation plays a significant role in the success of pollination.

For specific color morphs, the variation in flower color is often related to pollinator selection (Gigord et al., 2001;

Meléndez-Ackerman et al., 1997; Stanton 1987; Waser and Price, 1981). *Parrya nudicaulis* is self-compatible, but in the Russian Far-East, it mostly depends on pollinators for reproduction (Tikhmenev 1981). This species produces white to deep violet flowers (Dick et al., 2011). However, this species was not detected with any significant visitation preference for flower color (Fulkerson et al., 2012). The authors further specified that the sample size was not enough to confirm the absence of flower color preference in this species. Indirect color selection may be a stronger selective pressure in conditions where visitation rates are extremely low (Whittall and Carlson, 2009).

Flower-color polymorphism is common in insect-pollinated temperate herbs, bearing blue, pink or purple anthocyanin pigments (Warren and Mackenzie, 2001). Variation in anthocyanin contents is more common than changes in other pigments, and in many cases, single mutations may give rise to anthocyanin-less (white) morphs (Richards, 1997). Several studies have shown that pollinators discriminate between color morphs (Levin, 1969; Mogford, 1974). However, some wind-pollinated grasses such as *Poa trivialis* and *Holcus lonatus* possess pink/white-color morphs (Hubbard 1968), suggesting that pollinator preference cannot be fully explained.

Diversity of flower colors affect pollinator attraction and is supposed to be molded through pollinator-mediated selection (Trunschke et al., 2021). Pollinators apply significant selective pressure on flower color and determine the evolution of color signaling through pollination efficiency and preferential visitation (Chittka and Menzel, 1992; Chittka and Raine, 2006; MENZEL and Shmida, 1993). Fluctuation in flower perception and preferences by pollinators likely causes variable pollination success and visitation among color phenotypes (Campbell et al., 1997; Waser and Price, 1981).



Characteristics	White Flowers	Colored Flowers
Blooming	Early blooming	Late blooming
Pollinator species	Moths, flies, beetles	Birds, bees
Pollination types	Mostly self pollinated	Mostly cross pollinated
Nectar	Small amount of nectar	Large amount of nectar
Reproductive structures	Long tubes, usually nocturnal	Short and wide corolla tubes, wide limbs, inserted stigmas, usually diurnal
Seeds	Less seeds	More seeds

Figure 2. Effect of flower color on plant-pollinator interaction. a) comparison of white and colored morphs on pollinator visitation preference and b) comparison of pollination and reproduction related characteristics affected by white and colored flowers.

5. The ornamental and food crops with white flowers

White phenotypes have been observed in a number of ornamental and food crops (Table 1), such as dahlia (Ohno et al., 2013; Ohno et al., 2011), gentian flowers (Nakatsuka et al., 2008), petunia (Zenoni et al., 2011), arctic mustard flowers (*Parrya nudicaulis*) (Dick et al., 2011), *M. incana* (Dressel and Hemleben 2009), mock strawberry (*Duchesnea indica*) (Debes et al., 2011), strawberry (*Fragaria* × *ananassa*) (Li et al., 2013; Salvatierra et al., 2013; Saud et al., 2009), and pomegranate (*Punica granatum*) (Ben-Simhon et al., 2015).

The ornamental plants with white flowers make a beautiful arrangement along with colored flowers. The white tissues of *Dendrobium sonia* orchid showed the repressed expression of *dihydroflavonol 4-reductase* (*DFR*) (Piluk and Ratanasut, 2012). Downregulation of *F3′5′H* gene causes strong activity of *FLS* and a weak activity of *F3′H*, resulting in white flowers in *Nierembergia* sp. (Ueyama et al., 2006). The white chrysanthemum flowers accumulate colorless flavonols and flavones (Chen et al., 2012). In chrysanthemum petals, the degradation of carotenoids by *CCD4* (carotenoid cleavage dioxygenase) results in white flower; while the suppression of *CCD4* can turn petals from white to yellow (Ohmiya et al., 2006). In carnation, the downregulation of flavonone 3-hydroxylase results in pale or white flowers, while the suppression of anthocyanidin synthase gene of blue torenia through RNAi results in white flowers (Tanaka and Ohmiya, 2008). Glycosylation determines the presence of noncolor or color phenotype in white-centered petunia. The white parts contain quercetin with 7-O and 3′-O glucosides, while colored parts do not have these molecules (Saito et al., 2007).

The Japanese gentian flowers are of naturally blue color, but the breeding of white flowers is achieved by using spontaneous mutants. Nakatsuka et al. compared white flower cultivar to a blue one (Nakatsuka et al., 2005). They found that the levels of flavone in white cultivar were one-half the levels in blue cultivars, and the anthocyanins were absent in the white cultivar. They further found that out of 10 flavonoid biosynthesis pathway genes, white flower cultivars either lacked or showed a decreased expression of *ANS*, *CHS*, *F3H* (*flavanone 3-hydroxylase*), (*F3′,5′H*) *flavonoid 3′,5′-hydroxylase*, *DFR* (*dihydroflavonol 4-reductase*), *3GT* (*UDP-glucose: flavonoid 3-glucosyltransferase*), and *5AT* (*anthocyanin 5-aromatic acyltransferase*).

The white soybeans contain a DNA rearrangement bringing small tandem repeats in a gene as compared to purple soybeans. This W1 gene locus is required to form delphinidin-3-glucoside (Zabala and Vodkin, 2007). In pink-flowered strawberry, cyanidin 3-*O*-glucoside is the primary anthocyanin for pink color (Xue et al., 2016). In *Prunus mume*, cyanidin 3,5-*O*-diglucoside, peonidin 3-*O*-glucoside, and cyanidin 3-*O*-glucoside were detected in pink flowers, but no such secondary metabolites were observed in white petals (Ma et al., 2018). Similarly, red, purple, and orange flowers of *Lycoris longitude* contained anthocyanins, which were absent in white flowers (He et al., 2011). Moreover, specific anthocyanins, such as petunidin 3- *O*-glucoside, *O*-syringic acid and pelargonidin 3- *O*-ß-D-glucoside, accumulate in pink tea (*Camellia sinensis* L.) flowers, but absent in white flowers (Zhou et al., 2020). A significantly high *FLS* level and a significantly low *DFR* level were observed in the white tea flowers (Zhou et al., 2020).

White phenotype can also be caused by the accumulation of flavonoid intermediated upstream of *LDOX* gene. For instance, in white pomegranate fruit tissues, flavonoids were accumulated in considerable amounts corresponding to the upstream stages of *LDOX* function. The accumulated flavonoids included kaempferol derivatives, quercetin derivatives, catechin and its derivatives, suggesting the blockage of flavonoid pathway at *LDOX* stage in white pomegranate (Ben-Simhon et al., 2015).

Studies indicate that white *Nicotiana sylvestris* flowers lack anthocyanin regulators which activate *ANS* and *DFR* in the flavonoid biosynthesis pathway, causing lower contents of pelargonidin and cyanidin (McCarthy et al., 2017). Introducing apple *ANR* genes into tobacco causes the loss of anthocyanins through the inhibition of *DFR* and *CHI* genes (Han et al., 2012). Overexpression of *MdANR2b* produces pale pink or white flowers in the transgenic lines and the lines with overexpressing *MdANR2b-4* produce pure white flowers which contain the increased level of epicatechin (Han et al., 2012). *ANR* affects the anthocyanin synthesis by competing with the activity of *UGFT* (*UDP-glucose:flavonoid 3-O-glucosyltransferase*) which converts anthocyanidin to anthocyanin (Bogs et al., 2005). *BANYULS* (*BAN*) gene encodes *ANR*, and the ectopic *BAN* expression in tobacco can significantly inhibit anthocyanin biosynthesis, causing the production of white flowers (Xie et al., 2003). Wild *Nicotiana tabacum* produces pink petals; however, a white flower mutant was deficient of DFR (Kazama et al., 2013).

6. Economic values of white flowers

White flowers have long-range environmental and health benefits with their significant effects on plant-pollinator interaction, environmental-reproductive interaction, and deposition of phytochemicals, besides ceremonial efficacy. People prefer white paper flowers with medium diameter and small numbers (1–13 buds) (Pongoh and Paat, 2022). White and yellow chrysanthemums are the most loved flowers by the consumers as they believe that light colors create a sense of hope and social energy as well as stimulate mental activities (Wijayani et al., 2017). According to an estimate, the plants with white flowers receive higher amounts of herbivory than do pigmented flowers (Vaidya et al., 2018). Herbivores show reduced performance on anthocyanin-dominant pigmented morphs than white anthocyanin-

Table 1. Overview of the white color induction using anthocyanin pathway genes in different crops.

Species Name	Candidate Gene	Function	References
Ipomoea purpurea	*CHS*	The expression of *CHS* gene is drastically decreased in the white flowers buds of *I. purpurea*	(Fukada-Tanaka et al., 1997)
Ipomoea purpurea	DNA transposable element	The white flowers are produced by inserting a DNA transposable element, related to *Tpn1*, into *CHS* intron	(Hoshino et al., 2009)
Gentiana leucomelaena	*CHS*/*ANS*	By RNAi, *ANS*, or *CHS* is suppressed to produce white flowers	(Nakatsuka et al., 2008)
Torenia fournieri Lind.	*CHS*	The suppression of *CHS* gene, that encodes a key enzyme catalyzing the first step of flavonoid biosynthesis pathway, induces white flowers	(Nakamura et al., 2006; Napoli et al., 1990; Suzuki et al., 2000; Tsuda et al., 2004; Van der Krol et al., 1990)
Torenia fournieri Lind.	*F3H*	Absence of *F3H* is caused by the insertion of a retrotransposable element *TORE1*	(Nishihara et al., 2014)
Torenia fournieri Lind.	*F3H*	An *F3H* knockdown by RNAi in blue-flowered *T. fournieri* Lind. produces white flowers	(Ono et al., 2006)
Petunia hybrida	*CHS*	Use of petunia *CHS* construct to cosuppress tobacco *CHS* results in white flowers	(Wang et al., 2006)
Dahlia variabilis	*CHS*	Posttranscriptional silencing of two different *CHS* genes simultaneously causes pure while flowers in the octoploid *D. variabilis*	(Ohno et al., 2011)
Dianthus caryophyllus	*F3H*/*DFR*	Mutation in carnation *F3H* and *DFR* can generate white phenotype	(Iwashina et al., 2010)
Iochroma loxense	R3MYB	In the white *I. loxense* flowers, the pigment loss involves R3MYB repressor that restricts the downstream gene expression	(Gates et al., 2018)
Dendrobium sonia	*DFR*	The white tissues of *D. sonia* orchid show the repressed expression of DFR	(Piluk and Ratanasut, 2012)
Chrysanthemum morifolium	*CCD4*	The degradation of carotenoids by *CCD4* results in white flower	(Chenet al., 2012)
Nicotiana tabacum	*FLS*	Heterologous *FLS* expression in transgenic tobacco promotes flavonol biosynthesis and the blocks anthocyanin accumulation	(Luoet al., 2016)
Nicotiana tabacum	*DFR*	Overexpression of *DFR* gene in tobacco causes the predominant flavonol accumulation that produces pure white flowers	(Luo et al., 2016)
Nicotiana tabacum	*ANR*	Introducing apple *ANR* genes into tobacco causes the loss of anthocyanins through the inhibition of *DFR* and *CHI* genes	(Han et al., 2012)
Nicotiana tabacum	*BAN*	*BAN* gene encodes *ANR*, and the ectopic *BAN* expression in tobacco can significantly inhibit anthocyanin biosynthesis, causing the production of white flowers	(Xie et al., 2003)

recessive morphs of radish (Irwin et al., 2003). The white flesh peach genotypes in southern Italy are characterized by an excellent flavor and a persistent aroma, which is highly appreciated by consumers (Sortino et al., 2020).The white eggplant is an effective source of lowering hyperglycemia and it also contains high levels of mineral contents, such as sodium and potassium (Samir El-Dashlouty et al., 2016). Studies on berries show that the anthocyanin percentage varies from 92% to 95% for colored berries to 31% for white berries; while the red guava has more phenolic contents and antioxidant activity than white one (González-Aguilar et al., 2008). This suggests that white color imparts a clear difference in the deposition of phytochemicals in food or ornamental crops.

7. Conclusions and future prospects

Flower and fruit color has always been an important commercial and aesthetic indicator of a crop's value. Pigments, such as anthocyanins impart multiple colors to fruits and flowers with great health benefits. Modifications in the flavonoid biosynthesis pathways induce white color phenotype. Flavonoids constitute major pigmented compounds in the flowering plants. In pomegranate and mock strawberry, the white color is supposed to be due to low expression of *LDOX*/*ANX* gene. Different flavonoid pathway genes and TFs are involved in the regulation of white phenotype, such as *CHS* (arctic mustard flowers and dahlia), TTG1-like (*M. incana*), *MYB* (strawberry and gentian flowers), or *bHLH* (dahlia).

White flower color has a significant role in the plant-pollinator interaction, and affects seed dispersal and self- and cross-pollination capabilities. Plants flowering in the early spring with extreme low temperature usually have low pollination density. Under such situations white flowers are the best choice to promote self-pollination, allowing reproductive viability that not only conserves energy but also reduces nutrient costs. Moreover, white color is also a commercial indicator affecting outer surface of fruits under environmental and storage conditions. White anthocyanins do possess their own health benefits, different from colored ones. White fruits contain health-promoting phytochemicals, such as allicin with antibacterial and antiviral properties. Moreover, white color vegetable and fruits possess nutrients that can lower cholesterol level and cure high blood pressure. The deposition of phytochemicals, including anthocyanins and phenols, is also affected by white color. Therefore, elucidation of the molecular mechanisms and the environmental impacts of white color could not only explain the color regulation in ornamental and food crops but also enhance our ability to appraise the influence of different anthocyanin compositions on the human aesthetic preference, diet, and protection from environmental cues (e.g., heat, radiation, and shelf life). The future food processing can be revolutionized to adjust the levels of phytochemicals in the food keeping in mind the vast range of health benefits provided by specific accumulation of anthocyanins and phenols. Moreover, mutation breeding techniques for white flower induction can be adopted for producing the ornamental cultivars and visible genetic markers.

Acknowledgment

Ethics approval and consent to participate

Not applicable.

Code availability

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are provided within this manuscript.

Competing interests

The authors declare that they have no competing interest.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

The work was supported by National Natural Science Foundation of China (No. 31901353).

Authors' contributions

Sagheer Ahmad: Conceptualization, writing-original draft; Zaib-Un-Nisa: Data curation; Muhammad Zeeshan Munir: Investigation; Muhammad Imran: Data curation; Shaista Nosheen: Visualization; Kai Zhao: Writing-reviewing and editing.

References

Ackermann M, Weigend M (2006). Nectar, floral morphology and pollination syndrome in Loasaceae subfam. Loasoideae (Cornales). Annals of Botany 98: 503-514. https://doi.org/10.1093/aob/mcl136

Aida R, Kishimoto S, Tanaka Y, Shibata M (2000). Modification of flower color in torenia (*Torenia fournieri* Lind.) by genetic transformation. Plant Science 153: 33-42. https://doi.org/10.1016/S0168-9452(99)00239-3

Ben-Simhon Z, Judeinstein S, Trainin T, Harel-Beja R, Bar-Ya'akov I et al. (2015). A" White" anthocyanin-less pomegranate (*Punica granatum* L.) caused by an insertion in the coding region of the leucoanthocyanidin dioxygenase (LDOX; ANS) gene. PLoS One 10:e0142777. https://doi.org/10.1371/journal.pone.0142777

Bogs J, Downey MO, Harvey JS, Ashton AR, Tanner GJ et al. (2005). Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. Plant physiology 139: 652-663. https://doi.org/10.1104/pp.105.064238

Briscoe AD, Chittka L (2001). The evolution of color vision in insects. Annual review of entomology 46: 471-510. https://doi.org/10.1146/annurev.ento.46.1.471

Campbell DR, Waser NM, Melendez-Ackerman EJ (1997). Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. The American Naturalist 149: 295-315. https://doi.org/10.1086/285991

Charlesworth D (2006). Evolution of plant breeding systems. Current Biology 16:R726-R735. https://doi.org/10.1016/j.cub.2006.07.068

Chen SM, Li CH, Zhu XR, Deng YM, Sun W et al. (2012). The identification of flavonoids and the expression of genes of anthocyanin biosynthesis in the chrysanthemum flowers. Biologia Plantarum 56: 458-464. https://doi.org/10.1007/s10535-012-0069-3

Chittka L, Menzel R (1992). The evolutionary adaptation of flower colours and the insect pollinators' colour vision. Journal of Comparative Physiology A 171:171-181. https://doi.org/10.1007/BF00188925

Chittka L, Raine NE (2006). Recognition of flowers by pollinators. Current opinion in plant biology 9: 428-435. https://doi.org/10.1016/j.pbi.2006.05.002

Clegg MT, Durbin ML (2000). Flower color variation: a model for the experimental study of evolution. Proceedings of the National Academy of Sciences 97: 7016-7023. https://doi.org/10.1073/pnas.97.13.7016

Courtney-Gutterson N, Napoli C, Lemieux C, Morgan A, Firoozabady E et al. (1994). Modification of flower color in florist's Chrysanthemum: production of a white–flowering variety through molecular genetics. Bio/technology 12: 268-271. https://doi.org/10.1038/nbt0394-268

Davies KM (2008) Modifying anthocyanin production in flowers. In: Anthocyanins. Springer, pp 49-80. https://doi.org/10.1007/978-0-387-77335-3_3

Davies KM, Bradley JM, Schwinn KE, Markham KR, Podivinsky E (1993). Flavonoid biosynthesis in flower petals of five lines of lisianthus (*Eustoma grandiflorum* Grise.). Plant Science 95: 67-77. https://doi.org/10.1016/0168-9452(93)90080-J

Debes MA, Arias ME, Grellet-Bournonville CF, Wulff AF, Martínez-Zamora MG et al. (2011). White-fruited *Duchesnea indica* (Rosaceae) is impaired in ANS gene expression. American Journal of Botany 98: 2077-2083. https://doi.org/10.3732/ajb.1000373

Depicker A, Van Montagu M (1997). Post-transcriptional gene silencing in plants. Current opinion in cell biology 9: 373-382. https://doi.org/10.1016/S0955-0674(97)80010-5

Deroles SC, Bradley JM, Schwinn KE, Markham KR, Bloor S et al. (1998). An antisense chalcone synthase cDNA leads to novel colour patterns in lisianthus (*Eustoma grandiflorum*) flowers. Molecular Breeding 4:59-66. https://doi.org/10.1023/A:1009621903402

Dick CA, Buenrostro J, Butler T, Carlson ML, Kliebenstein DJ et al. (2011). Arctic mustard flower color polymorphism controlled by petal-specific downregulation at the threshold of the anthocyanin biosynthetic pathway. PloS one 6:e18230. https://doi.org/10.1371/journal.pone.0018230

Dixon RA, Liu C, Jun JH (2013). Metabolic engineering of anthocyanins and condensed tannins in plants. Current Opinion in Biotechnology 24: 329-335. https://doi.org/10.1016/j.copbio.2012.07.004

Dixon RA, Pasinetti GM (2010). Flavonoids and isoflavonoids: from plant biology to agriculture and neuroscience. Plant Physiology 154: 453-457. https://doi.org/10.1104/pp.110.161430

Dooner HK (1985). Viviparous-1 mutation in maize conditions pleiotropic enzyme deficiencies in the aleurone. Plant physiology 77: 486-488. https://doi.org/10.1104/pp.77.2.486

Dressel A, Hemleben V (2009). Transparent Testa Glabra 1 (TTG1) and TTG1-like genes in *Matthiola incana* R. Br. and related Brassicaceae and mutation in the WD-40 motif. Plant Biology 11: 204-212. https://doi.org/10.1111/j.1438-8677.2008.00099.x

Durst F, Nelson DR (1995). Diversity and evolution of plant P450 and P450-reductases. Drug Metabolism and Drug Interaction 12: 189-206. https://doi.org/10.1515/DMDI.1995.12.3-4.189

Forkmann G, Dangelmayr B (1980). Genetic control of chalcone isomerase activity in flowers of *Dianthus caryophyllus*. Biochemical genetics 18: 519-527. https://doi.org/10.1007/BF00484399

Friedman J, Barrett SC (2008). A phylogenetic analysis of the evolution of wind pollination in the angiosperms. International Journal of Plant Sciences 169: 49-58. https://doi.org/10.1086/523365

Fukada-Tanaka S, Hoshino A, Hisatomi Y, Habu Y, Hasebe M et al. (1997). Identification of new chalcone synthase genes for flower pigmentation in the Japanese and common morning glories. Plant and cell physiology 38: 754-758. https://doi.org/10.1093/oxfordjournals.pcp.a029232

Fulkerson JR, Whittall JB, Carlson ML (2012). Reproductive ecology and severe pollen limitation in the polychromic tundra plant, *Parrya nudicaulis* (Brassicaceae). PLoS One 7: e32790. https://doi.org/10.1371/journal.pone.0032790

Gates DJ, Olson BJ, Clemente TE, Smith SD (2018). A novel R3 MYB transcriptional repressor associated with the loss of floral pigmentation in Iochroma. New Phytologist 217:1346-1356. https://doi.org/10.1111/nph.14830

Geissman T, Mehlquist GA (1947). Inheritance in the carnation, *Dianthus caryophyllus*. IV. The chemistry of flower color variation, I. Genetics 32: 410. https://doi.org/10.1093/genetics/32.4.410

Gerats A, Huits H, Vrijlandt E, Maraña C, Souer E et al. (1990). Molecular characterization of a nonautonomous transposable element (dTph1) of petunia. The Plant Cell 2: 1121-1128. https://doi.org/10.1105/tpc.2.11.1121

Gigord LD, Macnair MR, Smithson A (2001). Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soo. Proceedings of the National Academy of Sciences 98: 6253-6255. https://doi.org/10.1073/pnas.111162598

González-Aguilar G, Robles-Sánchez R, Martínez-Téllez M, Olivas G, Alvarez-Parrilla E et al. (2008). Bioactive compounds in fruits: health benefits and effect of storage conditions. Stewart Postharvest Review 4: 1-10. https://doi.org/10.2212/spr.2008.3.8

Grandbastien M, Casacuberta J (2012) Plant transposable elements. Impact on genome structure and function. Springer, Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-31842-9

Han Y, Vimolmangkang S, Soria-Guerra RE, Korban SS (2012). Introduction of apple ANR genes into tobacco inhibits expression of both CHI and DFR genes in flowers, leading to loss of anthocyanin. Journal of Experimental Botany 63: 2437-2447. https://doi.org/10.1093/jxb/err415

He Q, Shen Y, Wang M, Huang M, Yang R et al. (2011). Natural variation in petal color in *Lycoris longituba* revealed by anthocyanin components. PLoS One 6: e22098. https://doi.org/10.1371/journal.pone.0022098

Hemleben V, Dressel A, Epping B, Lukačin R, Martens S et al. (2004). Characterization and structural features of a chalcone synthase mutation in a white-flowering line of *Matthiola incana* R. Br.(Brassicaceae). Plant Molecular Biology 55: 455-465. https://doi.org/10.1007/s11103-004-1125-y

Hichri I, Barrieu F, Bogs J, Kappel C, Delrot S et al. (2011). Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. Journal of experimental botany 62: 2465-2483. https://doi.org/10.1093/jxb/erq442

Hoshino A, Abe Y, Saito N, Inagaki Y, Iida S (1997). The gene encoding flavanone 3-hydroxylase is expressed normally in the pale yellow flowers of the Japanese morning glory carrying the speckled mutation which produce neither flavonol nor anthocyanin but accumulate chalcone, aurone and flavanone. Plant and cell physiology 38: 970-974. https://doi.org/10.1093/oxfordjournals.pcp.a029260

Hoshino A, Morita Y, Choi JD, Saito N, Toki K et al. (2003). Spontaneous mutations of the flavonoid 3'-hydroxylase gene conferring reddish flowers in the three morning glory species. Plant and Cell Physiology 44: 990-1001. https://doi.org/10.1093/pcp/pcg143

Hoshino A, Park KI, Iida S (2009). Identification of r mutations conferring white flowers in the Japanese morning glory (*Ipomoea nil*). Journal of plant research 122: 215-222. https://doi.org/10.1007/s10265-008-0202-8

Hrazdina G, Weeden NF (1986). Enzymatic control of anthocyanin expression in the flowers of pea (*Pisum sativum*) mutants. Biochemical genetics 24: 309-317. https://doi.org/10.1007/BF00502797

Huang CR, Burns KH, Boeke JD (2012). Active transposition in genomes. Annual Review of Genetics 46: 651-675. https://doi.org/10.1146/annurev-genet-110711-155616

Hubbard CE (1968). Grasses. A guide to their structure, identification, uses, and distribution in the British Isles. Grasses A guide to their structure, identification, uses, and distribution in the British Isles.

Hudson A, Critchley J, Erasmus Y (2008). The genus Antirrhinum (snapdragon): a flowering plant model for evolution and development. CSH Protocols 2008: 1-7. https://doi.org/10.1101/pdb.emo100

Iida S, Morita Y, Choi JD, Park KI, Hoshino A (2004). Genetics and epigenetics in flower pigmentation associated with transposable elements in morning glories. Advances in biophysics 38: 141-159. https://doi.org/10.1016/S0065-227X(04)80136-9

Inagaki Y, Hisatomi Y, Suzuki T, Kasahara K, Iida S (1994). Isolation of a Suppressor-mutator/Enhancer-like transposable element, Tpn1, from Japanese morning glory bearing variegated flowers. The Plant Cell 6: 375-383. https://doi.org/10.1105/tpc.6.3.375

Irwin RE, Strauss SY, Storz S, Emerson A, Guibert G (2003). The role of herbivores in the maintenance of a flower color polymorphism in wild radish. Ecology 84: 1733-1743. https://doi.org/10.1890/0012-9658(2003)084[1733:TROHIT]2.0.CO;2

Itoh Y, Higeta D, Suzuki A, Yoshida H, Ozeki Y (2002). Excision of transposable elements from the chalcone isomerase and dihydroflavonol 4-reductase genes may contribute to the variegation of the yellow-flowered carnation (*Dianthus caryophyllus*). Plant and cell physiology 43: 578-585. https://doi.org/10.1093/pcp/pcf065

Iwashina T, Yamaguchi M, Nakayama M, Onozaki T, Yoshida H et al. (2010). Kaempferol glycosides in the flowers of carnation and their contribution to the creamy white flower color. Natural Product Communications 5: 1934578X1000501213.

https://doi.org/10.1177/1934578X1000501213

Jones AL, Thomas CL, Maule AJ (1998). De novo methylation and co-suppression induced by a cytoplasmically replicating plant RNA virus. EMBO J 17 (21): 6385-6393. https://doi.org/10.1093/emboj/17.21.6385

Kazama Y, Fujiwara MT, Takehisa H, Ohbu S, Saito H et al. (2013). Characterization of a heavy-ion induced white flower mutant of allotetraploid *Nicotiana tabacum*. Plant cell reports 32: 11-19. https://doi.org/10.1007/s00299-012-1336-7

Kudo G (1993). Relationship between flowering time and fruit set of the entomophilous alpine shrub, *Rhododendron aureum* (Ericaceae), inhabiting snow patches. American Journal of Botany 80: 1300-1304. https://doi.org/10.1002/j.1537-2197.1993.tb15368.x

Larter M, Dunbar-Wallis A, Berardi AE, Smith SD (2018). Convergent evolution at the pathway level: predictable regulatory changes during flower color transitions. Molecular Biology and Evolution 35: 2159-2169. https://doi.org/10.1093/molbev/msy117

Levin DA (1969). The effect of corolla color and outline on interspecific pollen flow in Phlox. Evolution 444-455. https://doi.org/10.2307/2406699

Li H, Mao W, Liu W, Dai H, Liu Y et al. (2013). Deep sequencing discovery of novel and conserved microRNAs in wild type and a white-flesh mutant strawberry. Planta 238:695-713. https://doi.org/10.1007/s00425-013-1917-x

Luo P, Ning G, Wang Z, Shen Y, Jin H et al. (2016). Disequilibrium of flavonol synthase and dihydroflavonol-4-reductase expression associated tightly to white vs. red color flower formation in plants. Frontiers in plant science 6: 1257. https://doi.org/10.3389/fpls.2015.01257

Ma KF, Zhang QX, Cheng TR, Yan XL, Pan HT et al. (2018). Substantial epigenetic variation causing flower color chimerism in the ornamental tree *Prunus mume* revealed by single base resolution methylome detection and transcriptome sequencing. International journal of molecular sciences 19: 2315. https://doi.org/10.3390/ijms19082315

Martin C (1993). Structure, function, and regulation of the chalcone synthase. International review of cytology 147:233-284. https://doi.org/10.1016/S0074-7696(08)60770-6

Martin C, Carpenter R, Sommer H, Saedler H, Coen ES (1985). Molecular analysis of instability in flower pigmentation of *Antirrhinum majus*, following isolation of the pallida locus by transposon tagging. The EMBO journal 4: 1625-1630. https://doi.org/10.1002/j.1460-2075.1985.tb03829.x

Martin C, Prescott A, Mackay S, Bartlett J, Vrijlandt E (1991). Control of anthocyanin biosynthesis in flowers of *Antirrhinum majus*. The Plant Journal 1:37-49. https://doi.org/10.1111/j.1365-313X.1991.00037.x

Mato M, Onozaki T, Ozeki Y, Higeta D, Itoh Y et al. (2000). Flavonoid biosynthesis in white-flowered Sim carnations (*Dianthus caryophyllus*). Scientia Horticulturae 84: 333-347. https://doi.org/10.1016/S0304-4238(99)00140-5

Matsubara K, Kodama H, Kokubun H, Watanabe H, Ando T (2005). Two novel transposable elements in a cytochrome P450 gene govern anthocyanin biosynthesis of commercial petunias. Gene 358: 121-126. https://doi.org/10.1016/j.gene.2005.05.031

McCarthy EW, Berardi AE, Smith SD, Litt A (2017). Related allopolyploids display distinct floral pigment profiles and transgressive pigments. American Journal of Botany 104: 92-101. https://doi.org/10.3732/ajb.1600350

Mehlquist GA, Geissman T (1947). Inheritance in the carnation (*Dianthus caryophyllus*). III. Inheritance of flower color. Annals of the Missouri Botanical Garden 39-74. https://doi.org/10.2307/2394439

Meléndez-Ackerman E, Campbell DR, Waser NM (1997). Hummingbird behavior and mechanisms of selection on flower color in Ipomopsis. Ecology 78: 2532-2541. https://doi.org/10.1890/0012-9658(1997)078[2532:HBAMOS]2.0.CO;2

MENZEL R, Shmida A (1993). The ecology of flower colours and the natural colour vision of insect pollinators: the Israeli flora as a study case. Biological Reviews 68: 81-120. https://doi.org/10.1111/j.1469-185X.1993.tb00732.x

Metzlaff M, O'dell M, Hellens R, Flavell RB (2000). Developmentally and transgene regulated nuclear processing of primary transcripts of chalcone synthase A in petunia. The Plant Journal 23: 63-72. https://doi.org/10.1046/j.1365-313x.2000.00793.x

Miller R, Owens SJ, Rørslett B (2011). Plants and colour: flowers and pollination. Optics & Laser Technology 43: 282-294. https://doi.org/10.1016/j.optlastec.2008.12.018

Mogford D (1974). Flower colour polymorphism in *Cirsium palustre*. Heredity 33:257-263. https://doi.org/10.1038/hdy.1974.91

Mol J, Schram A, De Vlaming P, Gerats A, Kreuzaler F et al. (1983). Regulation of flavonoid gene expression in *Petunia hybrida*: description and partial characterization of a conditional mutant in chalcone synthase gene expression. Molecular and General Genetics MGG 192: 424-429. https://doi.org/10.1007/BF00392185

Momose M, Nakayama M, Itoh Y, Umemoto N, Toguri T et al. (2013). An active hAT transposable element causing bud mutation of carnation by insertion into the flavonoid 3'-hydroxylase gene. Molecular Genetics and Genomics 288: 175-184. https://doi.org/10.1007/s00438-013-0742-z

Morita Y, Hoshino A, Kikuchi Y, Okuhara H, Ono E et al. (2005). Japanese morning glory dusky mutants displaying reddish-brown or purplish-gray flowers are deficient in a novel glycosylation enzyme for anthocyanin biosynthesis, UDP-glucose:anthocyanidin 3-O-glucoside-2"-O-glucosyltransferase, due to 4-bp insertions in the gene. The Plant Journal 42: 353-363. https://doi.org/10.1111/j.1365-313X.2005.02383.x

Morita Y, Takagi K, Fukuchi MM, Ishiguro K, Tanaka Y et al. (2014). A chalcone isomerase-like protein enhances flavonoid production and flower pigmentation. The Plant Journal 78: 294-304. https://doi.org/10.1111/tpj.12469

Mu J, Li G, Niklas KJ, Sun S (2011). Difference in floral traits, pollination, and reproductive success between white and blue flowers of *Gentiana leucomelaena* (Gentianaceae) in an alpine meadow. Arctic, Antarctic, and Alpine Research 43: 410-416. https://doi.org/10.1657/1938-4246-43.3.410

Mulder-Krieger T, Verpoorte R (2012) Anthocyanins as flower pigments: feasibilities for flower colour modification. Springer Science & Business Media

Nakamura N, Fukuchi MM, Miyazaki K, Suzuki K, Tanaka Y (2006). 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 Biotechnology 23: 13-17. https://doi.org/10.5511/plantbiotechnology.23.13

Nakatsuka T, Haruta KS, Pitaksutheepong C, Abe Y, Kakizaki Y et al. (2008). Identification and characterization of R2R3-MYB and bHLH transcription factors regulating anthocyanin biosynthesis in gentian flowers. Plant and Cell Physiology 49: 1818-1829. https://doi.org/10.1093/pcp/pcn163

Nakatsuka T, Mishiba KI, Abe Y, Kubota A, Kakizaki Y et al. (2008). Flower color modification of gentian plants by RNAi-mediated gene silencing. Plant Biotechnology 25: 61-68. https://doi.org/10.5511/plantbiotechnology.25.61

Nakatsuka T, Nishihara M, Mishiba K, Yamamura S (2005). Two different mutations are involved in the formation of white-flowered gentian plants. Plant Science 169: 949-958. https://doi.org/10.1016/j.plantsci.2005.06.013

Napoli C, Lemieux C, Jorgensen R (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. The plant cell 2: 279-289. https://doi.org/10.2307/3869076

Nepi M, von Aderkas P, Wagner R, Mugnaini S, Coulter A et al. (2009). Nectar and pollination drops: how different are they? Annals of Botany 104: 205-219. https://doi.org/10.1093/aob/mcp124

Nishihara M, Nakatsuka T, Hosokawa K, Yokoi T, Abe Y et al. (2006). Dominant inheritance of white-flowered and herbicide-resistant traits in transgenic gentian plants. Plant biotechnology 23: 25-31. https://doi.org/10.5511/plantbiotechnology.23.25

Nishihara M, Yamada E, Saito M, Fujita K, Takahashi H et al. (2014). Molecular characterization of mutations in white-flowered torenia plants. BMC plant biology 14: 1-13. https://doi.org/10.1186/1471-2229-14-86

Nishijima T, Morita Y, Sasaki K, Nakayama M, Yamaguchi H et al. (2013). A torenia (*Torenia fournieri* Lind. ex Fourn.) Novel mutant 'Flecked' produces variegated flowers by insertion of a DNA transposon into an R2R3-MYB gene. Journal of Japanese Society for Horticultural Science 82: 39-50. https://doi.org/10.2503/jjshs1.82.39

Nishizaki Y, Matsuba Y, Okamoto E, Okamura M, Ozeki Y et al. (2011). Structure of the acyl-glucose-dependent anthocyanin 5-O-glucosyltransferase gene in carnations and its disruption by transposable elements in some varieties. Molecular Genetics and Genomics 286: 383-394. https://doi.org/10.1007/s00438-011-0655-7

Ohmiya A, Kishimoto S, Aida R, Yoshioka S, Sumitomo K (2006). Carotenoid cleavage dioxygenase (CmCCD4a) contributes to white color formation in chrysanthemum petals. Plant physiology 142: 1193-1201. https://doi.org/10.1104/pp.106.087130

Ohno S, Deguchi A, Hosokawa M, Tatsuzawa F (2013). A basic helix-loop-helix transcription factor DvIVS determines flower color intensity in cyanic dahlia cultivars. Planta 238: 331-343. https://doi.org/10.1007/s00425-013-1897-x

Ohno S, Hosokawa M, Kojima M, Kitamura Y, Hoshino A et al. (2011). Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia. Planta 234: 945-958. https://doi.org/10.1007/s00425-011-1456-2

Oliver KR, McComb JA, Greene WK (2013). Transposable elements: powerful contributors to angiosperm evolution and diversity. Genome biology and evolution 5: 1886-1901. https://doi.org/10.1093/gbe/evt141

Ono E, Fukuchi MM, Nakamura N, Fukui Y, Yonekura SK et al. (2006). Yellow flowers generated by expression of the aurone biosynthetic pathway. Proceedings of the National Academy of Sciences 103: 11075-11080. https://doi.org/10.1073/pnas.0604246103

Petroni K, Tonelli C (2011). Recent advances on the regulation of anthocyanin synthesis in reproductive organs. Plant Science 181: 219-229. https://doi.org/10.1016/j.plantsci.2011.05.009

Piluk P, Ratanasut K (2012) Expression profiles of the dihydroflavonol 4-reductase (DFR) gene in the sepals and petals of *Dendrobium Sonia* Earsakul. In: Proceedings of the 1st Mae Fah Luang University International Conference, pp 1-8

Pongoh J, Paat FJ (2022). The Diversity of Several Flower Color Types of the Zinnia Plant (*Zinnia elegans* Jacq.). Jurnal Agroekoteknologi Terapan 3: 108-115

Quattrocchio F, Baudry A, Lepiniec L, Grotewold E (2006) The regulation of flavonoid biosynthesis. In: The science of flavonoids. Springer, pp 97-122. https://doi.org/10.1007/978-0-387-28822-2_4

Quattrocchio F, Wing J, van der Woude K, Souer E, de Vetten N et al. (1999). Molecular analysis of the anthocyanin2 gene of petunia and its role in the evolution of flower color. The Plant Cell 11: 1433-1444. https://doi.org/10.1105/tpc.11.8.1433

Rall S, Hemleben V (1984). Characterization and expression of chalcone synthase in different genotypes of *Matthiola incana* R. Br. during flower development. Plant molecular biology 3:137-145. https://doi.org/10.1007/BF00016061

Ramsay NA, Walker AR, Mooney M, Gray JC (2003). Two basic-helix-loop-helix genes (MYC-146 and GL3) from Arabidopsis can activate anthocyanin biosynthesis in a white-flowered *Matthiola incana* mutant. Plant molecular biology 52: 679-688. https://doi.org/10.1023/A:1024852021124

Rausher MD (2008). Evolutionary transitions in floral color. International Journal of Plant Sciences 169: 7-21. https://doi.org/10.1086/523358

Raven PH (1969). revision of the genus Camissonia (Onagraceae). US Nat Mus Contrib US Nat Herb

Rebollo R, Horard B, Hubert B, Vieira C (2010). Jumping genes and epigenetics: Towards new species. Gene 454: 1-7. https://doi.org/10.1016/j.gene.2010.01.003

Richards AJ (1997) Plant breeding systems. Garland Science. https://doi.org/10.1007/978-1-4899-3043-9

Roguz K, Gallagher MK, Senden E, Bar-Lev Y, Lebel M et al. (2020). All the colors of the rainbow: Diversification of flower color and intraspecific color variation in the genus Iris. Frontiers in plant science 11:569811. https://doi.org/10.3389/fpls.2020.569811

Saito N, Cheng J, Ichimura M, Yokoi M, Abe Y et al. (1994). Flavonoids in the acyanic flowers of Pharbitis nil. Phytochemistry 35: 687-691. https://doi.org/10.1016/S0031-9422(00)90588-0

Saito R, Kuchitsu K, Ozeki Y, Nakayama M (2007). Spatiotemporal metabolic regulation of anthocyanin and related compounds during the development of marginal picotee petals in *Petunia hybrida* (Solanaceae). Journal of plant research 120: 563-568. https://doi.org/10.1007/s10265-007-0086-z

Salvatierra A, Pimentel P, Moya-León MA, Herrera R (2013). Increased accumulation of anthocyanins in *Fragaria chiloensis* fruits by transient suppression of FcMYB1 gene. Phytochemistry 90: 25-36. https://doi.org/10.1016/j.phytochem.2013.02.016

Samir El-Dashlouty M, Amin El-Sherif FE-Z, Abbas Shalaby M, FekrySerag El-Din M, El-Mageed El-Mosselhy S (2016). Round black, long white eggplant diets for clinical nutrition of fructose induced hypertensiona, hyperglycemia and hyperlipidemia. Alexandria Journal of Agricultural Sciences 61: 139-151. https://doi.org/10.21608/alexja.2016.244434

Saud G, Carbone F, Perrotta G, Figueroa CR, Moya M et al. (2009). Transcript profiling suggests transcriptional repression of the flavonoid pathway in the white-fruited Chilean strawberry, *Fragaria chiloensis* (L.) Mill. Genetic resources and crop evolution 56: 895-903. https://doi.org/10.1007/s10722-009-9487-2

Smith BH, Cobey S (1994). The olfactory memory of the honeybee *Apis mellifera*. II. Blocking between odorants in binary mixtures. The Journal of experimental biology 195: 91-108. https://doi.org/10.1242/jeb.195.1.91

Sortino G, Saletta F, Puccio S, Scuderi D, Allegra A et al. (2020). Extending the shelf life of white peach fruit with 1-methylcyclopropene and aloe arborescens edible coating. Agriculture 10: 151. https://doi.org/10.3390/agriculture10050151

Spelt C, Quattrocchio F, Mol J, Koes R (2002). ANTHOCYANIN1 of petunia controls pigment synthesis, vacuolar pH, and seed coat development by genetically distinct mechanisms. The Plant Cell 14: 2121-2135. https://doi.org/10.1105/tpc.003772

Spribille R, Forkmann G (1982). Genetic control of chalcone synthase activity in flowers of *Antirrhinum majus*. Phytochemistry 21: 2231-2234. https://doi.org/10.1016/0031-9422(82)85183-2

Stanton ML (1987). Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: I. Pollinator response to color morphs. American Journal of Botany 74: 178-187. https://doi.org/10.1002/j.1537-2197.1987.tb08595.x

Stich K, Eidenberger T, Wurst F, Forkmann G (1992). Enzymatic conversion of dihydroflavonols to flavan-3, 4-diols using flower extracts of *Dianthus caryophyllus* L.(carnation). Planta 187: 103-108. https://doi.org/10.1007/BF00201630

Suzuki KI, Xue HM, Tanaka Y, Fukui Y, Fukuchi MM et al. (2000). Flower color modifications of Torenia hybrida by cosuppression of anthocyanin biosynthesis genes. Molecular Breeding 6: 239-246. https://doi.org/10.1023/A:1009678514695

Takahashi R, Githiri SM, Hatayama K, Dubouzet EG, Shimada N et al. (2007). A single-base deletion in soybean flavonol synthase gene is associated with magenta flower color. Plant Molecular Biology 63: 125-135. https://doi.org/10.1007/s11103-006-9077-z

Tanaka Y, Katsumoto Y, Brugliera F, Mason J (2005). Genetic engineering in floriculture. Plant cell, tissue and organ culture 80: 1-24. https://doi.org/10.1007/s11240-004-0739-8

Tanaka Y, Ohmiya A (2008). Seeing is believing: engineering anthocyanin and carotenoid biosynthetic pathways. Current opinion in biotechnology 19: 190-197. https://doi.org/10.1016/j.copbio.2008.02.015

Tanaka Y, Sasaki N, Ohmiya A (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The Plant Journal 54: 733-749. https://doi.org/10.1111/j.1365-313X.2008.03447.x

Thomson JD, Wilson P (2008). Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. International Journal of Plant Sciences 169: 23-38. https://doi.org/10.1086/523361

Tikhmenev E (1981). Pollen viability and pollination in arctic plants. Soviet Journal of Ecology 12: 271-276

Totsuka A, Okamoto E, Miyahara T, Kouno T, Cano EA et al. (2018). Repressed expression of a gene for a basic helix-loop-helix protein causes a white flower phenotype in carnation. Breeding science 68: 139-143. https://doi.org/10.1270/jsbbs.17072

Trunschke J, Lunau K, Pyke GH, Ren ZX, Wang H (2021). Flower color evolution and the evidence of pollinator-mediated selection. Frontiers in plant science 12: 617851. https://doi.org/10.3389/fpls.2021.617851

Tsuda S, Fukui Y, Nakamura N, Katsumoto Y, Yonekura-Sakakibara K et al. (2004). Flower color modification of *Petunia hybrida* commercial varieties by metabolic engineering. Plant Biotechnology 21: 377-386. https://doi.org/10.5511/plantbiotechnology.21.377

Uchiyama T, Fujino K, Ogawa T, Wakatsuki A, Kishima Y et al. (2009). Stable transcription activities dependent on an orientation of Tam3 transposon insertions into *Antirrhinum* and yeast promoters occur only within chromatin. Plant Physiol 151: 1557-1569. https://doi.org/10.1104/pp.109.142356

Ueyama Y, Katsumoto Y, Fukui Y, Fukuchi MM, Ohkawa H et al. (2006). Molecular characterization of the flavonoid biosynthetic pathway and flower color modification of Nierembergia sp. Plant biotechnology 23: 19-24. https://doi.org/10.5511/plantbiotechnology.23.19

Vaidya P, McDurmon A, Mattoon E, Keefe M, Carley L et al. (2018). Ecological causes and consequences of flower color polymorphism in a self-pollinating plant (*Boechera stricta*). New Phytologist 218: 380-392. https://doi.org/10.1111/nph.14998

Van der Krol AR, Mur LA, Beld M, Mol J, Stuitje AR (1990). Flavonoid genes in petunia: addition of a limited number of gene copies may lead to a suppression of gene expression. The Plant Cell 2: 291-299. https://doi.org/10.1105/tpc.2.4.291

Vitte C, Panaud O (2003). Formation of solo-LTRs through unequal homologous recombination counterbalances amplifications of LTR retrotransposons in rice *Oryza sativa* L. Molecular Biology and Evolution 20: 528:540. https://doi.org/10.1093/molbev/msg055

Wang CK, Chen PY, Wang HM, To KY (2006). Cosuppression of tobacco chalcone synthase using Petunia chalcone synthase construct results in white flowers. Botanical Studies 47: 71-82

Wang HM, To KY (2004). Agrobacterium-mediated transformation in the high-value medicinal plant *Echinacea purpurea*. Plant Science 166: 1087-1096. https://doi.org/10.1016/j.plantsci.2003.12.035

Warren J, Mackenzie S (2001). Why are all colour combinations not equally represented as flower-colour polymorphisms? New Phytologist 151: 237-241. https://doi.org/10.1046/j.1469-8137.2001.00159.x

Waser NM, Price MV (1981). Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. Evolution 376-390. https://doi.org/10.1111/j.1558-5646.1981.tb04896.x

Whittall J, Carlson M (2009). Plant defense: a pre-adaptation for pollinator shifts. New Phytologist 5-8. https://doi.org/10.1111/j.1469-8137.2009.02796.x

Wijayani A, Muafi M, Sukwadi R (2017). Market actor's response towards flower colours in d etermining the economic value of Chrysanthemum flowers. Journal of Business and Retail Management Research 12: 69-75. https://doi.org/10.24052/JBRMR/V12IS01/MARTFCIDTEVOCF

Wilson P, Castellanos MC, Hogue JN, Thomson JD, Armbruster WS (2004). A multivariate search for pollination syndromes among penstemons. Oikos 104: 345-361. https://doi.org/10.1111/j.0030-1299.2004.12819.x

Xie DY, Sharma SB, Paiva NL, Ferreira D, Dixon RA (2003). Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. Science 299: 396-399. https://doi.org/10.1126/science.1078540

Xue L, Wang Z, Zhang W, Li Y, Wang J et al. (2016). Flower pigment inheritance and anthocyanin characterization of hybrids from pink-flowered and white-flowered strawberry. Scientia Horticulturae 200: 143-150. https://doi.org/10.1016/j.scienta.2016.01.020

Zabala G, Vodkin LO (2007). A rearrangement resulting in small tandem repeats in the F3′ 5′ H gene of white flower genotypes is associated with the soybean W1 locus. Crop Science 47: 113-124. https://doi.org/10.2135/cropsci2006.12.0838tpg

Zenoni S, D'Agostino N, Tornielli GB, Quattrocchio F, Chiusano ML et al. (2011). Revealing impaired pathways in the an11 mutant by high-throughput characterization of *Petunia axillaris* and *Petunia inflata* transcriptomes. The Plant Journal 68: 11-27. https://doi.org/10.1111/j.1365-313X.2011.04661.x

Zhou C, Mei X, Rothenberg DON, Yang Z, Zhang W et al. (2020). Metabolome and transcriptome analysis reveals putative genes involved in anthocyanin accumulation and coloration in white and pink tea (*Camellia sinensis*) flower. Molecules 25: 190. https://doi.org/10.3390/molecules25010190