REVIEW

Understanding floral biology for CRISPR-based modification of color and fragrance in horticultural plants [version 1; peer review: awaiting peer review]

Zulqurnain Khan1, Asim Razzaq1, Tahmina Sattar1, Aftab Ahmed2, Sultan Habibullah Khan2, Muhammad Zubair Ghouri2

1Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan, Punjab, 60000, Pakistan
2CAS-AFS, University of Agriculture, Faisalabad, Punjab, 38040, Pakistan

Abstract
The global market of a total 42.4 billion US dollars of ornamental plants owes its worth to the color and fragrance associated with these plants. A lot of work has been done to understand the physiological, biochemical, genetic, and environmental factors responsible for the development of color and fragrance in flowers. The aim of these studies about the floral biology was to achieve the desired characters by manipulating all these factors to fulfill the changing demand of the global market. While, modifying flower color and fragrance, scientists moved from the traditional breeding methods to the comparatively modern genetic engineering techniques, yet limitations in results always remained a big issue for researchers. However, genome editing tools look promising to achieve the desired level of efficiency to modify the color and fragrance in the flowers. This review gives insight into the significance of floral characters, molecular factors responsible for these characters and applications of modern genome editing technologies for desirable modification.

Keywords
Genome Editing, Flower Color, Frangrance, Genetic Modification, Plant Transformation

This article is included in the Plant Science gateway.
1. Introduction

Esthetics and the appreciation of beauty are among the basic instincts of human beings, and ornamental plants are the purest form of natural esthetics. Humans have been breeding these plants for thousands of years in order to develop desired characteristics. Besides aesthetics, they have many economic, environmental and health benefits (Hall & Dickson, 2011; Hall & Hodges, 2011). Cut flowers, lawn grasses, palms, bedding plants, shrubs, and trees are some of the main categories of these ornamental plants. Among them, 4000 non-woody species have been commercialized so far (Pompeii, De Brito, Otoni, & Guerra, 2007). We believe it is justified to say that all value and mystique of ornamentals are directly or indirectly associated with their color and fragrance.

Flower color could be regarded as the most important feature of flowering plants (Zhao & Tao, 2015). Besides esthetics, specific flower color is also responsible for entomophily (Brouillard & Cheminat, 1988; Davies, Albert, & Schwinn, 2012). Furthermore, flower color has a major impact on the commercial value and consumer selection (Berghage & Wolnick, 2000). Flower color is determined by three classes of pigments: flavonoids, carotenoids, and betalains (Grotewold, 2006; Tanaka, Katsumoto, Brugliera, & Mason, 2005). It can be classified on the basis of either color depth or coloration (Nakayama, Tanikawa, Morita, & Ban, 2012). Carotenoids give a range of orange and yellow colors (Gutterson, 1995). Besides providing color, they have also an important role in photosynthesis, prevention from free radicals and entomophily (Bradshaw Jr & Schemske, 2003; Fang et al., 2008; Jo et al., 2016; Ruban et al., 2007).

The fragrance is a collective, complex and unpredictable trait of flower (Cherri-Martin, Jullien, Heizmann, & Baudino, 2007), produced by the products of several biosynthetic pathways within the plant. These products are low molecular weight volatiles (Croteau & Karp, 1991). More than 1700 such volatiles have been identified by the scientists (Ishizaka, 2007), giving characteristic orange, pink, purple, red, violet and blue colors to the flowers of different plant species (Forkmann, 1991; Gutterson, 1995). Around 1000 such pigments have been identified so far (Yoshida, Mori, & Kondo, 2009). Besides having a function in coloration, anthocyanin has also been found playing an antioxidative role by protecting the plant from free radicals produced as a result of various stresses (Chalker-Scott, 1999; Harborne & Williams, 2000; He et al., 2011; Steyn, Wand, Holcroft, & Jacobs, 2002; Teppabut, Oyama, Kondo, & Yoshida, 2018). Anthocyanidins (anthocyanin without sugars) have three main classes: pelargonidin (orange to brick red), cyanidin (red to pink) and delphinidin (purple to blue) (Hanumappa et al., 2007; Yoshida et al., 2009). Moreover, the intensity of color is also related to the concentration of anthocyanins (Tanaka, Sasaki, & Ohmiya, 2008).

Phenylpropanoids arise from phenylalanine and its synthesis is catalyzed by an enzyme called phenylalanine ammonia-lyase (PAL) (Dudareva & Pichersky, 2006c; Vainstein et al., 2001). It consists of number of secondary metabolites and those secondary metabolites which are volatiles, have a specific chemical configuration (Dudareva & Pichersky, 2006c). While benzenoids arise from trans-cinnamic acid through a side branch of general phenylpropanoid pathway (Boatright et al., 2004). Phenylpropanoids and benzenoids also originate from shikimic acid through shikimate pathway (Baldermann, Yang, Sakai, Fleischmann, & Watanabe, 2009). Besides having a role in fragrance, this class is also involved in plant defense (Dixon et al., 2002). A lot of studies have been performed to study the regulation of benzenoids pathway in various flowering plants (Boatright et al., 2004; Schuurink et al., 2006; van Schie, Haring, & Schuurink, 2006).

Terpenes are from the largest class of plant volatiles, mainly consist of monoterpenes (linalool, Caryophyllene, limonene, farnesene, myrcene etc.) and are produced by the terpenoid pathway from a five-carbon compound called isopentenyl diphosphate, whose biosynthesis is catalyzed by various mono- and sesquiterpene synthases (Flowers & Dudareva, 2006; Trapp & Croteau, 2001). Terpenes also have a role in insect attraction and plant defense mechanism (Dewick, 2002).
Fatty acid derivatives form the second largest class of plant volatiles and are derived from membrane lipids through lipogenase pathway with the involvement of lipogenase enzymes (Gutiérrez, 2009). The shikimate pathway provides precursors for the biosynthesis of primary metabolites such as aromatic amino acids and folic acid. The shikimate pathway is a metabolic bridge between primary and secondary metabolism with regard to the regulation of AAA biosynthesis (Tzin & Galili, 2010). The regulation of the shikimate pathway has been studied extensively (Maeda & Dudareva, 2012; Tzin, Galili, & Aharoni, 2001).

Floral fragrance enhances the esthetic and commercial value of ornamental plants (Dudareva & Pichersky, 2006a; Noman et al., 2017; Pichersky, Noel, & Dudareva, 2006). It is an important trait for flowers to attract bees and other pollinators for the successful pollination (Dudareva & Pichersky, 2000; Pellmyr & Thien, 1986; Piechulla & Pott, 2003; Tholl, 2006; Vainstein et al., 2001). It also has a role in providing insect resistance and pathogen protection to flower (Dudareva, Klemplien, Muhlemann, & Kaplan, 2013; Dudareva & Pichersky, 2008). Modifying the floral fragrance of an ornamental will surely help to enhance its value (Pompelli et al., 2007).

Interestingly, the floral fragrance does not remain the same throughout the season or even throughout the day. Circadian rhythm has been found to control this trait for example, petunia and gentian were found to be more fragrant in the night (Verdonk et al., 2005). There are various studies mentioning the impact of circadian rhythm on fragrance (Helsper, Davies, Bouwmeester, Krol, & van Kampen, 1998; Hendel-Rahmanim, Masci, Vainstein, & Weiss, 2007; Kolosova, Gorenstein, Kish, & Dudareva, 2001; Simkin et al., 2004). Availability of light, production of certain hormones, climatic

![Figure 1a. Pathways of anthocyanin.](image-url)
factors, developmental stage of the flower and time of activity of pollinator are the major factors affecting this rhythmic emission of fragrance (Cna’Ani et al., 2015; Dudareva et al., 2000; Dudareva & Pichersky, 2006b; Jakobsen & Olsen, 1994). Floral color and fragrance are the most important factors of prime esthetic value to attract the customers and industry. Modification in the color and fragrance may have huge impact in scientific and economic terms.

2. Synthesis and regulation of flower color and fragrance
2.1 Genes regulating flower color
Structural and regulatory genes of flower color determine the color type and its intensity (Nakatsuka, Nishihara, Mishiba, & Yamamura, 2005). Many genes related to pigment biosynthesis in ornamental plants have been discovered so far.
Genes encoding 3GTs in snapdragon were characterized (Martin, Prescott, Mackay, Bartlett, & Vrijlandt, 1991). The role of the AmAS1 gene in yellow flower color in snapdragon was confirmed in 2000 (Nakayama et al., 2000). Moreover, genes related to floral pigments were found in various other ornamentals like lily and morning glory flowers (Yamagishi, 2013; Zufall & Rausher, 2003). A chromoplast-specific, carotenoid-associated gene (OgCHRC) was characterized in oncidium Gower Ramsey (Chiou, Wu, & Yeh, 2008). UDP-glucose: anthocyanin 5-O-glucosyltransferase (5GT), having a role in gentiodelphin accumulation in petals conferring blue color, was characterized from blue gentian (Nakatsuka et al., 2008c). The chalcone synthase (CHS) gene was found in in tree peony (Zhou, Wang, & Peng, 2011). Anthocyanin methyltransferase (AMT), and glutathione S-transferase (GST) were found to have a role in the anthocyanin biosynthesis pathway in carnations (Tanaka, 2012). Dual colors in petunia petals were related to the post-transcriptional gene silencing of CHS genes (Yamagishi, 2013). Similarly, VwF3’5’H, VwDFR and VwANS genes were found to play role in flower color patterns in pansy petals (Li, Wang, Sun, & Shang, 2014). The F3H, DFR, ANS and 3GT genes play a role in purple–red pigmentation were found in in tree peony (Shi et al., 2015). In the ornamental tree peony, PsDFR and PsANS were found to be related to anthocyanin biosynthesis (Zhao, Tang, Hao, & Tao, 2015). The impact of floral color and inflorescence genes interaction on the creation of floral color and type in ornamental sunflower was discovered in 2016 (Cvejić, Jocić, & Mladenović, 2016). The flavonol synthase gene (FLS) and Fh3GT1 were found to have a role in anthocyanin biosynthesis in the tree peony (Sun et al., 2017; Zhao, Wei, Liu, & Tao, 2016). Genes related to color intensity in peony were found (Gao, Yang, Liu, Yang, & Hu, 2016). The role of RrFLS, RrDFR and RrF3’5’H genes in petal color of rose was found in 2018 (Li et al., 2018). CpurFLS1 and CpurFLS2 were isolated from cyclamen and have a role in flavonol synthesis (Akita, Kitamura, Mikami, & Ishizaka, 2018).

**Figure 1c. Pathways of phenylpropanoid.**
2.2 Transcriptional factors associated with flower color

Various transcriptional factors associated with the pigments biosynthesis and regulation has been identified in different ornamentals. Members of the \textit{R2R3-MYB} gene family were identified as the key activators of the flavonoid pathway and were isolated from various ornamentals (Davies \textit{et al.}, 2012; Morita, Saitoh, Hoshino, Nitasaka, & Iida, 2006; Naing \& Kim, 2018; Yamagishi, Shimoyamada, Nakatsuka, & Masuda, 2010). The \textit{R2R3-MYB} transcription family was found to control pigmentation pattern in petunias (Albert \textit{et al.}, 2011). Likewise, \textit{MYB} and bHLH transcription factors were found to be related to anthocyanin pigmentation in various ornamental flowers (Nakatsuka \textit{et al.}, 2008a; Schwinn \textit{et al.}, 2014). 50 differentially expressed transcription factors involved in flavonoid biosynthesis in the tree peony were discovered in 2015 (Shi \textit{et al.}, 2015).

So, there is a long list of discovered transcriptional factors involved in flower color formation and regulation process not only in ornamentals but also in other crops (Hichri \textit{et al.}, 2011).

2.3 Other factors

Besides genomics and proteomics, several physical and chemical factors like metal ions, vacuolar pH, temperature, biotic and abiotic stresses, and hormones have been found having a great impact on the color formation process in petals (Chandler & Tanaka, 2007; Lai, Yamagishi, & Suzuki, 2011; Takeda, 2006; Tatsuzawa, Tanikawa, & Nakayama, 2017; Tsuma \textit{et al.}, 2014; Weiss, 2000; Winkel-Shirley, 2002). All of these factors must be kept in mind while manipulating the flower color otherwise desired results could not be achieved, as occurred in case of failure of the attempt to create blue carnation in spite of successful transfer of \textit{F35H} gene due to inappropriate vacuolar pH (Holton \textit{et al.}, 1993).
2.4 Genes involved in flower fragrance

Various genes related to the volatile production have been discovered in various ornamental species like in snapdragon these genes were identified: S-adenosyl-L-methionine:benzoic acid carboxyl methyl transferase (Dudareva et al., 2000), S-adenosyl-L-methionine: salicylic acid carboxyl methyl transferase (Negre, Kolosova, Knoll, Kish, & Dudareva, 2002), myrcene synthase (Dudareva et al., 2003), and terpene synthases (Nagegowda, Gutensonh, Wilkerson, & Dudareva, 2008).

While in roses: orcinol O-methyl transferase (Lavid et al., 2002), germacrene D synthase (Guterman et al., 2002), OOMT1 and OOMT2 (Guterman et al., 2002), geraniol/citronellol acetyl transferase (Shalit et al., 2003), RhAAT1 (Shalit et al., 2003), OOMT1 and OOMT2 (Wu et al., 2003), and phloroglucinol O-methyltransferase (POMT) (Wu et al., 2004), and RhPAAS (Farhi et al., 2010).

In petunia: S-adenosyl-L-methionine: benzoic acid/salicylic acid carboxyl methyl transferase (Negre et al., 2003), benzoyl-coenzyme A:benzyl alcohol/phenylethanol benzoyl transferase (Boatright et al., 2004), phenylacetaldehyde synthase (PAAS) (Kaminaga et al., 2006b), and PhCM1 (Colquhoun et al., 2010b).

In Clarkia breweri: (S)-linalool synthase (Dudareva, Cseke, Blanc, & Pichersky, 1996), S-adenosyl-L-methionine (SAM):iso eugenol O-methyltransferase (IEMT) (Wang & Pichersky, 1998), acetyl-coenzyme A:benzyl alcohol acetyltransferase (Dudareva, D’auria, Nam, Raguso, & Pichersky, 1998), S-adenosyl-L-methionine: salicylic acid carboxyl methyl transferase (Ross, Nam, D’Auria, & Pichersky, 1999), benzoyl-coenzyme A:benzyl alcohol benzoyl transferase (D’Aura, Chen, & Pichersky, 2002).

Finally, in Hedychium spp., two terpene synthase genes were found (HcTPS7 and HcTPS8) (Yue, Yu, & Fan, 2014).

2.5 Involvement of transcriptional factors:

Besides structural genes, transcriptional factors’ role in fragrance biosynthesis has been also studied (Colquhoun & Clark, 2011). A few such transcriptional factors have been discovered in ornamentals like MYB transcriptional factors in roses (Yan et al., 2011). ODORANT1, EMISSION OF BENZENOIDS II (EOBII), ODO1 from petunia (Spitzer-Rimon et al., 2012; Spitzer-Rimon et al., 2010; Van Moerkercke, Galván-Ampudia, Verdonk, Haring, & Schuurink, 2012; Verdonk et al., 2005) and ODORANT1 from lilies (Yoshida, Oyama-Okubo, & Yamagishi, 2018).

3. Methods to improve flower color and fragrance

A detailed account of successful attempts of flower color and fragrance modification in ornamental plants using various techniques has been provided in the Table 1.

Table 1. Examples of flower color and fragrance modification in ornamental plants using various techniques.

| Sr. No. | Technique         | Flower    | Trait modified | Gene       | Reference                               |
|---------|-------------------|-----------|----------------|------------|-----------------------------------------|
| 1       | Antisense Suppression | Petunia   | Color          | CHS        | (Van der Krol et al., 1988)            |
|         |                   | Chrysanthemum | Color          | CHS        | (Hanumappa et al., 2007)               |
|         |                   | G. hybridia. | Color          | CHS        | (Elomaa et al., 1993)                  |
|         |                   | Lisianthus  | Color          | FLS        | (Nielsen et al., 2002)                 |
|         |                   | Cyclamen   | Color          | F35’H      | (Boase et al., 2010)                   |
|         |                   | Nierembergia sp | Color          | F35’H      | (Ueyama et al., 2006)                  |
|         |                   | cv. Eilat   | Color          | F3H        | (Zuker et al., 2002)                   |
|         |                   | Torenia    | Color          | DFR        | (Aida et al., 2000a)                   |
| 2       | Sense Suppression  | Petunia    | Color          | CHS, CHS & DFR | (Napoli et al., 1990)               |
|         |                   | Torenia    | Color          | CHS & DFR  | (Van der Krol et al., 1990)            |
|         |                   | Chrysanthemum | Color          | CHS        | (Courtney-Gutterson et al., 1994)      |
3.1 Conventional breeding

Breeding tools have been used to bring modifications in the flower color of ornamentals; for example, interspecific hybridization has been used to modify the flower color in *Ornithogalum* spp. (Griesbach, Meyer, & Koopowitz, 1993).

Some of the random mutation tools have also been applied to achieve flower color modification in ornamentals such as the application of various sugars (sucrose, mannose, glucose) to peonies affecting flower color and anthocyanin biosynthesis (Zhang et al., 2015); white–purple color variant violets were obtained by colchicine treatment (Seneviratne & Wijesundara, 2007); magnesium treatment increased anthocyanin accumulation in four different ornamentals (Nissim-Levi, Ovadia, Forer, & Oren-Shamir, 2007; Venkatachalam & Jayabalan, 1994) used gamma radiations to realise mutations in zinnia flowers.

After a long era of ignorance of fragrance traits, finally, progress was made on selecting ornamental varieties, not for agronomic or color traits but for fragrance traits; for example, in 2013, an effort was made to enhance the benzenoid diversity in carnations by crossing it with wild dianthus (*Dianthus hungaricus*) (Kishimoto et al., 2013). The resultant hybrid carnations were found to have a relatively diverse fragrant compounds’ profile than their parents.

A relatively new mutation tool, ion beam radiation, has been also used to change the flower color by deposition of high energy through ion beams (heavy charged particles); inducing epigenetic changes (as suggested by: (Nakayama et al., 2012)) or creating double-strand breaks in DNA (Hoglund, 2000) with a low probability of DNA repairing (Goodhead, 1995). It has been induced successfully to induce flower color mutants in ornamentals like geraniums (Yu et al., 2016),

| Sr. No. | Technique | Flower | Trait modified | Gene | Reference |
|--------|-----------|--------|----------------|------|-----------|
| 3      | Introduction of non-native gene | Petunia | Color | A1 CHR | (Meyer et al., 1987; Davies et al., 1998) |
|        |           | Osteospermum | Color | DFR | (Seitz et al., 2007) |
|        |           | Rose | Color | F3’5’H & DFR A3’5’OMT | (Katsumoto et al. 2007; Nakamura et al., 2015) |
|        |           | Torenia | Color | DFR | (Nakamura et al., 2010) |
|        |           | Petunia | Fragrance | S-linalool synthase alcohol acetyltransferase | (Lücker et al., 2001; Guterman et al., 2006) |
|        |           | Carnation | Fragrance | S-linalool synthase | (Lavy et al., 2002) |
|        |           | Lisianthus | Fragrance | benzyl alcohol acetyl transferase | (Aranovich et al., 2007) |
| 4      | RNAi      | Torenia | Color | CHS anthocyanidin synthase | (Fukusaki et al., 2004a; Nakamura et al., 2006) |
|        |           | Gentian | Color | 5/3’AT & F3’5’H CHS, ANS & F3’5’H | (Nakatsuka et al., 2008b, 2010) |
|        |           | Petunia | Fragrance | PhMYB4 PhBSMT Arogenate Dehydratase1 PhcFAT | (Underwood et al., 2005; Dexter et al., 2007; Colquhoun et al., 2010a; Maeda et al., 2010) |
|        |           | Petunia hybrida | Fragrance | ODO1 PhPAAS | (Verdonk et al., 2005; Kaminaga et al., 2006a) |
| 5      | Genome editing | Torenia | Color | F3H | (Nishihara et al., 2018) |
|        |           | Japanese Morning Glory | Color | Carotenoid cleavage dioxygenase 4, dihydroflavonol-4-reductase-B | (Watanabe et al., 2017, 2018) |
|        |           | Petunia hybrida | Color | F3H | (Subburaj et al., 2018) |
Torenia spp. (Miyazaki et al., 2006), chrysanthemum (Matsumura et al., 2010), and cyclamen (Ishizaka, Kondo, & Kameari, 2012; Kondo et al., 2009). This technique has also been used in combination with other techniques like tissue culture (Matsumura et al., 2010) or by pretreatment of the sample with potential mutants (Hase, Okamura, Takeshita, Narumi, & Tanaka, 2010).

Not only the desired gene but also many other genes get interchanged during hybridization, so backcrosses must be made to somehow get close to the desired level of the trait (Hammond, 2004). We can just either knock-out a gene through these methods or interchange them but, unlike genome editing tools, we cannot introduce the desired gene into the target organism, so we are limited by the genome of our target organism and dependent on the genes only naturally present in parent plants (Nishihara & Nakatsuka, 2010; Tanaka et al., 2005). Secondly, unlike genome editing techniques, all of these mutation-inducing methods are non-precise, random and have not much science involved in them. Some problems with being dependent on the mutation breeding to modify plants were identified (Shibata, 2008).

Ion beam radiation although yielded some successful results and could be termed as “new infatuation” for the mutation breeders. But compromising randomness over precision would not be a good choice. Some precision in this method was attempted by using it in accordance with pre-discovered genomic data (Tanaka, 2012), to make it look more “scientific” but it still remains a hit and trial method in which one performing it is not sure that either the target gene or trait would be targeted or not.

3.2 Antisense gene technique

Reintroducing genes in antisense or sense orientation to induce genetic transformation is one of the oldest and outdated techniques in genetic engineering of ornamentals. In antisense suppression, an antisense RNA (having sequence complementary to the target RNA) blocks the target RNA by binding to it via base pairing, thus resulting in the knock-down of that particular gene (extensively reviewed by: Green, Pines, & Inouye, 1986). It all started in 1988 with antisense CHS gene producing mutant (white) color in petunias (Van der Krol et al., 1988). Later on, it was extended to many other ornamentals, targeting various genes involved in floral pigment biosynthesis and regulation like CHS (Elomaa et al., 1993; Hanumappa et al., 2007), FLS (Nielsen et al., 2002), F3’5’H (Boase et al., 2010; Ueyama et al., 2006), F3H (Zuker et al., 2002), and DFR (Aida et al., 2000a).

While, in sense/co-suppression, transgenes, aimed at overexpressing a particular gene, tend to knock down the targeted endogenous gene. The exact phenomenon of its working is still unknown; however, many possibilities exist, like epistatic interaction or RNA–duplex DNA interaction (as discussed by: Van der Krol et al., 1990) and its effectivity is dependent on many other factors like transgene sequence and promoter (Que, Wang, English, & Jorgensen, 1997). This technique was discovered accidentally in 1990 when CHS was tried to overexpress to enhance anthocyanin biosynthesis and instead it blocked it (Napol, Lemieux, & Jorgensen, 1990). Since then it was implied in other ornamentals like petunia (Van der Krol et al., 1990), Torenia spp. (Aida et al., 2000b; Suzuki et al., 2000), chrysanthemum (Courtney-Gutterson et al., 1994), and others (reviewed by: Gutterson, 1995).

We have found only one modification being reported so far in which antisense suppression of flavanone 3-hydroxylase gene in Carnation resulted in the modification in fragrance due to the production of benzoic acid (Zuker et al., 2002).

3.3 Transgenic or cisgenic approach

Genome modification techniques were rendered useful to overcome the genetic limitation of the plants not allowing having the colors out of a specific range (Chandler & Sanchez, 2012). We were able to introduce novel genes related to floral pigment biosynthesis and regulation, naturally not present in that ornamental. The first example of the color modification through this method was the generation of the color mutant in petunia through activating pelargonidin biosynthesis in it by transfer of AI gene from maize into it (Meyer, Heidmann, Forkmann, & Saedler, 1987). Similarly, pelargonidin accumulation in Osteospermum spp. was achieved by the introduction of the DFR gene (Seitz et al., 2007). The most famous example is the creation of blue roses. Rose does not have a naturally blue color due to the lack of delphinidin biosynthesis. Scientists downregulated the native DFR gene of rose and took the F3’5’H from pansy and the DFR gene from iris and transferred it to rose resulting in blue roses (Katsumoto et al., 2007). Further, a yellow-colored petunia was generated by introducing the CHR gene into it (Davies, Bloor, Spiller, & Derolets, 1998). Further, pink-colored Torenia spp. and magenta-colored roses have been achieved by the introduction of DFR and anthocyanin 3’,5’-O-methyltransferase (A3’5’OMT) genes into them, respectively (Nakamura, Fukuchi-Mizutani, Miyazaki, Suzuki, & Tanaka, 2006).

The first case of fragrance modification using gene transfer was the production of S-linalyl-b-D-glucopyranoside in petunia after the transfer of the linalool synthase gene from Clarkia breweri into it (Lücker et al., 2001). Similar gene
transfer from *Clarkia breweri* into carnation resulted in the production of trans-linalool oxide in the transgenic carnation (Lavy *et al.*, 2002). However, fragrance modification remained undetectable for humans.

Rose alcohol acetyltransferase (*RhaAT*) gene transfer to petunia resulted in the accumulation of phenyl ethyl acetate and benzyl acetate in transgenic petunia (*Guterman et al.*, 2006). Similarly, the transfer of *Clarkia breweri* benzyl alcohol acetyltransferase (*BEAT*) into lisianthus resulted in the enhancement of benzyl acetate in the transgenic plant (*Aranovich et al.*, 2007).

Introduction of production of Anthocyanin Pigment1 (*Pap1*) from arabidopsis in *P. hybrida* and rose resulted in enhancement of phenylpropanoid/benzenoid and terpenoid compounds in the transgenic petunia and rose and thus modification was detectable by the human nose (*Zvi et al.*, 2008, 2012). Genetic transformation tools have enabled scientists to perform cross-species gene transfer like a petunia plant was transformed with feedback-insensitive bacterial DAHPsF 3-deoxy-di-arabino-heptulosonate 7-phosphate synthase enzyme (*AroG*) gene which resulted in the enhanced formation of phenylalanine, tyrosine, and tryptophan in petunia and thus enhancing its fragrance (*Oliva et al.*, 2015).

As far as the method of gene transfer is related, no doubt we are no more limited by the genome of our target plant. However, the limitation is that we can perform just a gene transfer and in order to create a mutant in which we have to target a specific gene knock out, the only option will be genome editing tools, as RNAi and other PTGS tools just perform a knockdown (which too not up to 100% as discussed below). Specifically when it comes to fragrance modification using gene transfer we have several short comes. Floral fragrance production is complex phenomenon. Introduction of a gene (producing particular volatile) may not give significant results due to lack of specific substrate or enzyme necessary to catalyze its production in that transgenic plant or due to accumulation of that volatile in the undesirable location or due to no proper storage facility. Moreover, this kind of volatile production will also have to compete with the other volatiles which are essential for development and growth (As discussed by: (Oudareva & Pichersky, 2006c)).

Secondly, unlike as in case of genome editing techniques, the integration of transferred gene in the host genome is at a random location. This location has an impact on the expression of that gene due to the chromosomal position effect. Also, this randomly induced gene can disrupt the functioning of neighbor genes (as discussed by: (Son *et al.*, 2006)). These concerns have to be addressed while approving a GM variety, making the regulation process very lengthy. While in case of genome editing, we have targeted knock-ins in the host genome, thus eliminating these unwanted problems and reducing the time required for its regulation (*Pfeiffer et al.*, 2018).

### 3.4 RNAi

RNAi technology, whose idea came from (*Napoli *et al.*, 1990), bought a revolution in the whole scenario of not only floral color modification but also in the whole agriculture industry (*Yogindran & Rajam, 2015*). Scientists started preferring it over antisense and sense technology because of its potential and efficiency (*Nakamura *et al.*, 2006). RNAi is a post-transcriptional gene silencing technique in which double-stranded RNA gets cleaved into small interfering RNAs, which further degrade targeted mRNA (*Angaji *et al.*, 2010). It has been used to induce targeted color mutations in ornamentals. Like in *Torenia* spp. to produce white-colored flowers (*Fukusaki *et al.*, 2004b; *Nakamura *et al.*, 2006) or in gentian to produce white, blue and magenta-colored flowers (*Nakatsuka *et al.*, 2008b; *Nakatsuka *et al.*, 2010). *Lilaeas* spp. and petunia flower colors were also altered using RNAi (*Kamiishi *et al.*, 2012; *Keykha, Bagheri, Moshtaghi, Bahrami, & Sharifi, 2016*).

Talking about fragrance modification in ornamentals using RNAi, we have seen examples in petunia only. These too were mostly focused on reducing the expression of a certain volatile and most of them were just performed to verify the involvement of a certain gene in the formation of a particularly volatile. In petunia, the *BPBT* gene was suppressed using RNAi and resulted in the reduced production of benzyl benzoate in transgenic petunia (*Orlova *et al.*, 2006). In another experiment, RNAi suppression of the *PhMYB4* transcriptional factor in petunia resulted in the increased production of FVBP (floral volatile benzenoid/phenylpropanoid) compounds in transgenic petunia (*Colquhoun *et al.*, 2010a). In *P. hybrida*, downregulation of ODO1 transcriptional factor using RNAi resulted in the reduced production of benzoic acid (*Verdonk *et al.*, 2005). The knockdown of the *PhBSMT* gene resulted in the elimination of methyl benzoate in transgenic petunia (*Underwood *et al.*, 2005). In another experiment, the knockdown of the Arogenate Dehydratase1 (*ADT1*) gene resulted in the reduced level of shikimate and benzenoid in petunia (*Maeda *et al.*, 2010). RNAi-mediated silencing of the *PhPAAS* gene resulted in the termination of phenylacetaldehyde production (*Kaminaga *et al.*, 2006b). The *PhFAT* gene was suppressed using RNAi and it resulted in the elimination of isoegenol in the transgenic petunia plants (*Dexter *et al.*, 2007).

No doubt RNAi is a powerful tool to induce genome modifications, but it is a post-transcriptional genome silencer and it has been found difficult to restrict such tools only to the intended target (*Hanumappa *et al.*, 2007). RNAi is not efficient
for plants having multiple copies of the same gene in their genome; if we knock down one gene, other genes continue to express (Shikata & Ohme-Takagi, 2008). While in the case of genome editing tools, we can use multiplex genome editing to solve this problem efficiently. Just like the case of mutation tools in RNAi, we are limited by the genome of our targeted organism. Further, we can neither perform a 100% knock out using RNAi (Angaji et al., 2010) nor introduce or activate desired genes in our target (Unniyampurath, Pilankatta, & Krishnan, 2016), making it inferior to genome editing tools.

3.5 Genome editing tools

For ornamental modification, from using selective breeding to the usage of genetic engineering now we have entered the era of genome modification.

Although conventional breeding and genome engineering tools were being used for the improvement of crops and things were going on, there was an urge among the scientists to have a technology which can perform crop improvement with great precision. This dream came true in the form of genome editing tools. These tools comprising of zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated system (CRISPR) (Figure 2) can be harnessed not only to knock out a specific gene by creating a double stand break (DSB) (Haun et al., 2014; Shan, Zhang, Chen, Zhang, & Gao, 2015), but also to knock in a gene at a specific location in the target genome (Cai et al., 2009; J Li et al., 2016; Miki, Zhang, Zeng, Feng, & Zhu, 2018). Back in 2011, these genome editing methods were named as the method of the year by the nature journal (Baker, 2011). These genome editing tools are not limited to just perform knock-ins and knockouts by binding with an endonuclease domain only instead ZFN, TALE and Cas9 proteins can be paired with various other effector domains besides endonucleases to achieve the activation, repression or epigenome modification at the desired location (Khan, Khan, Mubarik, & Ahmad, 2018).

![Figure 2. Genome editing tools.](image-url)
The DSB created by these genome editing tools is repaired by the host endogenous repair machinery either through homology-directed repair (HDR) or non-homologous end joining (NHEJ). In HDR, DSB is repaired by copying the information from a template DNA which could be from the sister chromatid or we can artificially provide that template DNA and thus performing a targeted knock-in. While in the absence of a template DNA, DSB is repaired through non-homologous end joining (NHEJ). It produces INDELs (insertions, deletions) thus leading to frameshift mutations and ultimately knocking out gene at the target loci (Lieber, 2010; Puchta, 2004; Symington & Gautier, 2011; Wyman & Kanaar, 2006).

### 3.5.1 ZFN

ZFN (zinc finger nuclease) is the first to be created among the targeted genome editing tools back in 1994 (Kim & Chandrasegaran, 1994). It consists of a zinc finger protein domain bound with a FokI endonuclease domain (Durai et al., 2005). FokI endonuclease cleaves the site upon dimer formation due to the binding of ZF proteins with the specific target sequence thus creating a double stand break there (Weeks, Spalding, & Yang, 2016). One monomer of ZF protein is specific for three DNA bases. So an array of zinc finger proteins is made according to the target sequence (Sovová, Kerins, Demnerová, & Ovesná, 2016). Most of the time, this DSB is usually repaired through the NHEJ pathway (Mishra & Zhao, 2018). Platforms like Oligomerized Pool Engineering (OPEN) and Context Dependent Assembly (CODA) are available for the ZFN assembly (Sander et al., 2010; Zhang et al., 2010).

So far it has been used in crops like soybean (Curtin et al., 2011) and tobacco (Schneider et al., 2016; Wright et al., 2005).

This tool was useful but a bit costly and complex (in terms of designing) and also to an extent, its target sites were limited (Nemudryi, Valetdinova, Medvedev, & Zakian, 2014).

### 3.5.2 TALENS

Addressing some of the complexity and high cost associated with ZFN, TALEN was created (Joung & Sander, 2013). Just like ZFN it also consists of two components, one is TALE protein and the other is a FokI nuclease domain, which is fused with the TALE proteins (Christian et al., 2010; Weeks et al., 2016). TALE is basically the infectious protein injected by *Xanthomonas* spp. bacteria into its host plant and thus recruiting host genome for its own benefit (Boch & Bonas, 2010; Bonas, Stall, & Staskawicz, 1989; Scholze & Boch, 2011). They have a translocational domain for moving, binding domain for binding at the specific target site and a transcription activator domain for activating the expression of that target host gene (Khan, Khan, Mubarik, Sadia, & Ahmad, 2017).

TALE proteins consist of a repeat of 33–35 amino acids. This specific sequence of amino acid remains conserved in all TALE proteins except the amino acids at positions 12 and 13, known as repeat variable di-residues (RVDs), these amino acids are different in every TALE protein and each RVD specifically binds with one DNA base (Deng et al., 2012). HD, NG, NL, and NN specify for C, T, A and G/A respectively (Boch et al., 2009; Moscou & Bogdanove, 2009). Thus, every monomer of TALE protein binds with one specific nucleotide and TALENS can be assembled using this information. Designing of TALENS is less complex as compared to that of ZFNs (Petersen & Niemann, 2015). Modular assembly (T. Li et al., 2011) and Golden gate assembly (Cermak et al., 2011) are used most of the time by scientists for the designing and assembly of TALEs and TALEN. Furthermore, (Khan et al., 2017) had excellently reviewed the available tools and methods for the assembly and designing of TALEs and TALENS. TALEN has been successfully used to modify the genome of many crops like rice (T. Li, Liu, Spalding, Weeks, & Yang, 2012; Shan et al., 2015), potato (Clasen et al., 2016), tobacco (Mahfouz et al., 2011), barley (Wendt et al., 2013), wheat (Y. Wang et al., 2014), maize (Char et al., 2015), and tomato (Lor, Starker, Voytas, Weiss, & Olszewski, 2014).

### 3.5.3 CRISPR

Back in 2012, the invention of CRISPR appeared as a revolutionary tool in the field of genome editing (Jinek et al., 2012). It is a kind of immune system named CRISPR/Cas which is found widely among archaea and bacteria (Barrangou, 2013; Barrangou et al., 2007; Makarova et al., 2015). When a bacteriophage attacks bacterium, the bacterial Cas9 endonuclease protein cleaves the phage DNA into small fragments and integrates them in the CRISPR locus. Upon transcription, that locus forms CRISPR RNAs (crRNAs) which gets paired with its complementary trans-activating crRNA (tracrRNA). And this complex guides the Cas9 protein in identification and cleavage of invading foreign nucleic acid (Datsenko et al., 2012; Jinek et al., 2012; Van Der Oost, Westra, Jackson, & Wiedenheft, 2014).
The CRISPR tool used for genome editing has two components: Cas9 protein and a sgRNA (single guide RNA), which is a chimera of trRNA and crRNA. A sequence of 3–5 nucleotides known as protospacer adjacent motif (PAM) must be present downstream to target DNA for the binding of and cleavage by CRISPR complex at that location. In case of Streptococcus pyogenes-derived CRISPR-Cas system, this sequence is 5'-NGG. Cleavage is made by the Cas9 protein at three base pairs (bp) upstream to the PAM sequence (Jinek et al., 2012; Schaeffer & Nakata, 2015).

The construct for CRISPR-Cas can be delivered via conventional gene transfer methods. However, the scientists prefer to use the transient gene expression. Because, firstly, stable gene transfer methods are laborious one and secondly, in these methods the trans-gene, coding for our synthetic nuclease, will get integrated into the host genome. No doubt it will segregate in the progeny, and we will select only those plants which do not express that trans-gene but it makes whole process so laborious. Instead, we can use transient gene expression, as we have the aim of creating knock out at desired location, once it has been created than we do not need the expression of that gene. We can even perform genome editing via CRISPR without even using a trans-gene; sgRNA–Cas9 ribonucleoprotein complexes are delivered directly to the target organisms where they perform cleavage at the target location and then get degraded by the enzymatic machinery of that organism (As reviewed by: (Pfeiffer, Quetier, & Ricroch, 2018)). This trans-gene free method has been used recently in maize (Svitashev, Schwartz, Lenderts, Young, & Cigan, 2016), and bread wheat (Liang et al., 2017).

CRISPR-Cas9 mediated genome editing has been used in many crops so far like tobacco (Li et al., 2013; Nekrasov, Staskawicz, Weigel, Jones, & Kamoun, 2013), wheat (Wang et al., 2014), tomato (Brooks, Nekrasov, Lippman, & Van Eck, 2014); Ino, Nishizawa-Yokoi, Endo, Mikami, & Toki. 2015; Pan et al., 2016; Shimatani et al., 2017), rice (Feng et al., 2013; Wang et al., 2016), and maize (Char et al., 2017; Feng et al., 2016, 2018; Shi et al., 2017).

No doubt, CRISPR has much improved the overall efficiency of genetic manipulation experiments but still off targets remained a great hurdle for achieving the ideal level of results. Different variants of Cas proteins like Cas12 (Zetsche et al., 2015) and Cas13 (Abudayyeh et al., 2016) have been utilized to overcome this problem. A detailed comparison of these variants with other genome editing tools have been provided in the Table 2.

Prime editing and base editing are relatively a fresh arrival in the toolkit of genome editing to tackle the off targets issue. Base editing is a technique involving irreversibly changing a single base in the whole genome. It can be very useful for manipulating single base mutation related properties or diseases, without producing unwanted double stand breaks in the DNA (Komor, Kim, Packer, Zuris, & Liu, 2016) converted Cytosine (C) into Thymine (T) by first using cytidine deaminase to convert C into Uracil (U). Consequent changing in the complementary strand through DNA repair mechanism resulted in the final conversion of G:U into A:T.

There are some reports of off targeting caused by Cytosine based base editing (Jin et al., 2019; Zhou et al., 2019). However, to further increase the efficiency of base editing, various amendments including addition of Uracil N-glycosylase (UGI), Gam protein (Komor et al., 2017) and POBEC3A (eA3A) (Gehrke et al., 2018) have been made so far. Also, to increase the target sites, various variants of Cas proteins have been used with base editing machinery (Hua, Tao, & Zhu, 2019; Wang et al., 2020). The unintended modification in RNA by base editing complex was also reported, which can be reduced by using deaminases (Zhou et al., 2019).

Base editing has been used for genome manipulation in various plants including wheat (Li et al., 2018; Zhang et al., 2019; Zong et al., 2018; Zong et al., 2017), rice (Hua, Tao, Yuan, Wang, & Zhu, 2018; Jingying Li, Sun, Du, Zhao, & Xia, 2017; Lu & Zhu, 2017; Yan et al., 2018; Zong et al., 2017), arabidopsis (Chen et al., 2017), tomato (Shimatani et al., 2017), maize (Zong et al., 2017) and potato (Zong et al., 2018). However, we still wait for the first ever report for genome editing inamentals for fragrance and color using base editing.

The newest member in the family of genome editing tools is prime editing. It was reported back in 2019 (Anzalone et al., 2019). It not only overcomes the off-targeting issue of base editing but also it can perform all 12 kinds of base-to-base conversions, including four transition mutations and eight transversion mutations (Anzalone et al., 2019). The complex consists of a prime editing guide RNA (peg-RNA), a reverse transcriptase domain (RT domain) and a Cas9 protein. The Cas9 in it does not produces a double strand break at the target areas instead it produces a nick in a single strand, thus avoiding the complex aftermath of double strand breaks. The peg-RNA contains the sequence needed to be introduced at the target place. Upon attachment of prime editing complex at targeted sequence, Cas 9 produces a nick in the PAM containing DNA strand and the primer binding site (PBS) of peg-RNA hybridizes with it and RT produces the desired DNA strand using the sequence provided by peg-RNA (Anzalone et al., 2019).
| Features                  | ZF(N)s | TALE(N)s | CRISPR  | RNAi | Antisense/Sense | Argonate | Reference                      |
|---------------------------|--------|----------|---------|------|----------------|----------|--------------------------------|
| Target                    | 18–36 bp | 24–40 bp | 19–22 bp | 23bp | 24-30bp        |          |                              |
| Target site should be located 50–100 nt from ATG |          |          |         |      |                |          | (Khan et al. 2018) |
| Recognition mechanism     | Protein-DNA | Protein-DNA | RNA-DNA 2 RNA strands | RNA-DNA 1 RNA strands | ssRNA-DNA | RNA | RNA-Protein | gDNAs              | (Khan et al. 2018) |
| Targeting restrictions    | Non-G-rich sequences are difficult to target | T in the start and A at the end | Protospacer adjacent motif (PAM) must be present (NGG) | 5′-TTTV-3′ | 3′ non G PFS | Only targets mRNA | Only targets mRNA | No specific sequence require only 5′ phosphorylation | (Khan et al. 2018) |
| Nuclease                  | FokI | FokI | Cas9 (Blunt ends) | Cpf1 endonucleases (Sticky ends) | 2X HEPN | Dicer and Argonaute proteins | RNase H | NgAgo endonuclease | (Khan et al. 2018) |
| Gene knockout             | Yes | Yes | Yes | Yes | No | No | (Khan et al. 2018) |
| Gene knockdown            | 75-85% | >90% | Yes | Yes | (Khan et al. 2018) |
| Off targeting             | Small | Very few | High | Markedly more specific | High | High | Lower | (Khan et al. 2018) |
| Limitation                | Both expensive and time consuming to construct | Takes long to construct | Off targets | Off targets | Off targets, Instability and polarity of oligonucleotides, CpG motifs may reduce specificity Toxicity cause by nonantisense effects | Randomly remove 1-20 nt from the cleavage site require superb experimental skills Not have ability to edit gene | (Khan et al. 2018) |
| Cytotoxicity              | Low | Variable to high | Low PAM sequence often destroy during genome editing | PAM site may be preserved after genome editing | Variable to high | Variable to high | (Khan et al. 2017) |
Prime editing technology is relatively new, and it promises a lot of potential for genome editing in not only ornamentals but also other plants.

Genome editing tools have been successfully used to modify various fruits and ornamental species (Karkute, Singh, Gupta, Singh, & Singh, 2017). But, so far in the case of ornamentals, very little progress has been made for various reasons, like lack of whole-genome sequencing in most of the ornamentals and not having proper information about the exact pathways of many volatiles associated with these traits, especially flower fragrance. The lack of research is mainly due to the fact that there are thousands of ornamental flowers and the market value of one variety as a single is very low while modification and then regulation of modified plants require a lot of money and time.

In genome editing tools, only CRISPR has been used to harvest color modification in ornamentals, maybe due to the simplicity in its designing (Cong et al., 2013). Whereas, no attempt to modify floral fragrance in ornamentals using genome editing tools has been made.

*Torenia* spp., Japanese morning glory, and *P. hybridia* have been reported to have modified color using CRISPR. In Japanese morning glory knockout of carotenoid cleavage dioxygenase (*CCD4*) using the CRISPR/Cas9 system resulted in the pale-yellow colored flowers (Watanabe, Oda-Yamamizo, Sage-Ono, Ohmiya, & Ono, 2018). While knockdown of dihydroflavonol-4-reductase-B (*DFR-B*) gene in Japanese morning glory using CRISPR/Cas9 system yielded white-colored flowers instead of purple (Watanabe et al., 2017). While in *Torenia* spp., knock out of flavanone 3-hydroxylase (*F3H*) gene resulted in the creation of pale blue colored flowers (Nishihara, Higuchi, Watanabe, & Tasaki, 2018) and similarly in *P. hybridia* flavanone 3-hydroxylase (*F3H*) was knockdown using CRISPR/Cas9 system (Subburaj et al., 2018).

4. Concluding remarks

The modifications done in ornamentals using antisense, sense or RNAi technology were most of the times proved to be unstable and reversible. So, in the future usage of genome editing tools for genetic modifications can be proved worthy. Several ways could be adopted to bring stable modifications in ornamentals using genome editing tools. Like, 3,5-dimethoxytoluene (DMT) contributes a significant part in Tea rose scent (Scalliet et al., 2008) and *OOMT1* and *OOMT2* were found playing role in DMT formation (Scalliet et al., 2008). So, by precisely knocking out or knocking down these enzymes using genome editing tools, we can have a modified form of fragrance in these flowers. Similarly, upregulation of *ODO1* (Verdonk et al., 2005), having a role in the shikimate pathway, using CRISPRa will be promising for enhancing fragrance in it.

Monoterpenes constitute a major part of rose fragrance. GPP is found to be the precursor of monoterpenes (Magnard et al., 2015). So, by enhancing the production of GPPPs and CaGGPPs (enzymes catalyzing productions of GPP and GGPP respectively) using CRISPRa, an enhance in the rose fragrance is expected which will be commercially beneficial.

Geraniol is also a significant part of rose fragrance. An enzyme RhNUDX1 was found to be a precursor of geraniol formation in roses (Magnard et al., 2015). The upregulation of this enzyme, using CRISPRa, is expected to enhance fragrance in roses.

Chrysanthemum has carotenoids but they have white-colored flowers because they have a carotenoid degrading enzyme called carotenoid cleavage dioxygenase (*CCD4*) (Ohmiya, Kishimoto, Aida, Yoshioka, & Sumitomo, 2006). By precise targeting of *CCD4* through genome editing tools, we can yield carotenoid related colors in this flower.

The blue color of the gentian is found to be due to anthocyanins (Nakatsuka et al., 2008c). So, by precise targeting of anthocyanidin glucosyltransferase through genome editing tools, we can expect a noble color formation in gentian flowers.

Likewise, Tiger Lilly has b-carotene based carotenoids (Deli, 1999). So, by targeting enzymes involved in the b-carotene formation like lycopene B-cylose, a significant change in the flower color is expected.

Ornamental plants are under continuous threat from pests; be it aphids (Peronti and Sousa-Silva, 2002), bugs (Wheeler and Henry, 1976), whiteflies (Simala et al., 2009), sawflies (Miles, 1962) or moths (Evenden, 2009). The future work on ornamentals could be directed towards using genome editing tools for decreasing the infestation of pests on ornamental plants. It can be started from analyzing the parameters which are considered by these pests for attacking an ornamental. Recently, we have seen that knocking down *PDS* gene of carotenoid pathway resulted in less growth of caterpillars on the
modified plants (Zheng et al., 2010). Similarly, other important genes involved in the color and fragrance formation in ornamental plants can be targeted via genome editing tools and we can have less infestation of these harmful insects.

Another effective method for color modification using genome editing tools could be knock out/knockdown of transcriptional factors associated with regulation and synthesis of various floral pigments. Many such have been identified in various ornamentals (Davies et al., 2012; Morita et al., 2006; Naing & Kim, 2018; Nakatsuka et al., 2008a; Schwinn et al., 2014; Shi et al., 2015; Yamagishi et al., 2010).

Shikimate pathway is the precursor to the phenyl propanoid pathway (Herrmann & Weaver, 1999). So, any precise disturbance induced in the enzymes of this pathway like TYRB or PDT using genome editing tools could result in the disturbance of phenylpropanoid leading to a significant change in both color and fragrance. Apart from these possible editions, many other ways are waiting to be explored.

We are optimistic that in the future scientists would be successful in exploring more and more paradigms behind the formation of flower color and fragrance. As color and fragrance formation is a complex phenomenon and we had already seen that manipulation of the genes, involved in pigments biosynthesis, negatively affected the other traits of the plant besides color (Van Der Meer, Stam et al. 1992; Li, Zhang et al. 2013). So only a healthy cooperation between fields of structural and functional genomics along with transcriptomics and bioinformatics can open new dimensions in the identification and then efficient genome editing of the factors producing color and fragrance in ornamental plants.

The prime editing and base editing will be the frontline tools to achieve subtle fragrance and color modifications. Also, the future optimization of prime editing and base editing complexes will play a decisive role in determining their part in the advancement of ornamental industry.

As in the future we will be equipped with more efficient genome editing tools and genome modification would be easier than ever, a close eye should be kept on the possible repercussions of these modifications. The modification in the color and fragrance of ornamentals will lead to the change in the selection pattern of their pollinators and their pests. It would be necessary then to be clear in the objectives of genome editing. The economic gain at the cost of ecological disruption will be proven nothing but an illusion.

In the end, the issue of regulation of gene-edited crops would be worth discussing. As apart from the aforementioned reasons, scientists were moving towards genome editing tools because these tools were out of the jurisdiction of regulatory authorities of genetically modified crops. However, the Court of Justice of the European Union (ECJ) recent ruling that all gene-edited crops should be regulated under the same laws as genetically modified organisms are being regulated, can be a huge set back to the prospering industry of gene-edited ornamentals. The demand for minimizing regulatory procedures at-least for the non-food plants, especially the ornamentals, can now be heard louder than ever.

**Data availability**

No data are associated with this article.

**Author contributions**

Z.K. planned the manuscript outline. A.R. and T.S. wrote the draft and created the figures and tables. S.H.K., A.A., Z.A. and A.A.K. revised and proofread the manuscript. All authors read and approved the final manuscript.

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