Review

Utilization of transcription factors for controlling floral morphogenesis in horticultural plants

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Introduction

Consumer preferences for commercially available ornamental flowers keep changing rapidly. Therefore, a large number of fascinating new cultivars are developed and commercialized every year. At present, these commercial cultivars are mainly generated by crossbreeding and mutation breeding aimed at altering characteristics, such as flower shape, petal color, petal shape, color pattern, and fragrance. Recently, progress in molecular breeding technology has enabled the generation of genetically modified (GM) plants, including ornamental flowers. For example, in Japan, bluish roses (Katsumoto et al. 2007) and bluish carnations (Tanaka et al. 2009) produced by molecular breeding are commercially available. Pigment synthesis-related genes were introduced into these GM flowers as the breeding aimed to generate new colors. However, consumers would also be interested in traits other than novel petal colors. Therefore, for developing new floral traits, there is an urgent need to establish next-generation molecular breeding technology and to identify new targets of floral traits. There is also a need to shorten the developmental period and reduce development costs for such ornamental GM flowers.

Transcription factors (TFs) play crucial roles in plants, such as in growth, hormone signaling, responses to biotic and abiotic stresses, and development and formation of organs including floral organs. TFs up- or downregulate the expression of downstream genes and also play important roles in determining various floral properties other than formation of floral organs, such as characterizing specific floral traits. Therefore, TFs are reasonable targets for modifying these floral traits and generating new flower cultivars. However, it has been difficult to control the functions of transcription factors because most plant genes, including those encoding transcription factors, exhibit redundancy. In particular, it has been difficult to understand the functions of these redundant genes by genetic analysis. Thus, a breakthrough silencing method called chimeric repressor gene silencing technology (CRES-T) was developed specifically for plant transcription factors. This method transforms transcriptional activators into dominant repressors, and the artificial chimeric repressors suppress the function of transcription factors regardless of their redundancy. Among these chimeric repressors, some were found to be inappropriate for expression throughout the plant body because they resulted in deformities. For these chimeric repressors, utilization of floral organ-specific promoters overcomes this problem by avoiding expression throughout the plant body. In contrast, attachment of viral activation domain VP16 to transcriptional repressors effectively alters into transcriptional activators. This review presents the importance of transcription factors for characterizing floral traits, describes techniques for controlling the functions of transcription factors.

Key Words: chimeric repressor, CRES-T, floral trait, promoter, transcription factor.

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TFs for floral organs and traits in plants

TFs regulate the expression of downstream genes by binding specific DNA sequences, called cis-elements, on the promoter region of target genes. Genetic and reverse genetic analyses in model plants have revealed that TFs play important roles not only in the development of floral organs (Coen and Meyerowitz 1991, Ma 1994, Soltis et al. 2007) but also in the formation of floral characteristics, such as flower shape, petal color, petal shape, color pattern, and floral fragrance, specifically observed in each plant species and cultivar (Colquhoun and Clark 2011, Petroni and Tonelli 2011, Preston and Hileman 2009, Sasaki et al. 2016, Soltis et al. 2007). Arabidopsis, which has been the most widely used model plant, contains 1726 TF loci genes in its genome (Sasaki et al. 2017; Table 1), and these TFs have been classified into 58 families by evaluation of the amino acid sequences of their DNA binding motifs. There are several plant TF databases, and the number of TF families is slightly different in each database (for review, see Jin et al. 2017, Mitsuda and Ohme-Takagi 2009). For protein-coding genes, Arabidopsis has a total of 27,416 loci genes (The Arabidopsis Information Resource, TAIR10; https://www.arabidopsis.org/portals/genAnnotation/gene_structural_annotation/annotation_data.jsp) in its genome, although the locus/gene number is also slightly different between resources. Considering that the number of protein coding loci genes is more than ten times larger than that of TF loci genes, one TF should regulate the expression of several target downstream genes.

One of the most well-known models in which TFs are involved is the ABC model, which was subsequently developed into the ABOCE model (Coen and Meyerowitz 1991, Ma 1994, and for review see ÖMaelieidigh et al. 2014, Soltis et al. 2007, Theißen 2001). This is a model of floral organ development that is commonly conserved in angiosperms, and the genes in the ABC model encode several TFs that are mainly classified into the MADS-box family (for review see Theißen et al. 2016). In the model, the development of sepals requires A-function genes, the petals require A- and B-function genes, the stamens require B- and C-function genes, and the carpels require C-function genes. In addition to these MADS-box genes, E-function genes SEPALLATA1–4 (SEP1–4) are essential for the development of these floral organs (Ditta et al. 2004, Honma and Goto 2001, Pelaz et al. 2000). These four types of MADS-box proteins form a different tetrameric complex, which is explained by the “quartet model,” to specify floral organs, sepals, petals, stamens, and carpels (for review see Theißen 2001, Theißen and Saedler 2001).

TFs contribute not only to morphogenesis commonly observed in angiosperms but also to additional characteristics exhibited in specific plant species, for example, symmetry.

Table 1. Classification of TF families on PlantTFDB v4.0*

| TF Family | Arabidopsis | Arabidopsis |
|-----------|-------------|-------------|
| AP2       | 18          | 30          |
| ARF       | 22          | 31          |
| ARR-B     | 14          | 32          |
| B3        | 66          | 33          |
| BBR-BPC   | 7           | 34          |
| BEs1      | 8           | 35          |
| bHLH      | 153         | 36          |
| bZIP      | 74          | 37          |
| C2H2      | 100         | 38          |
| C3H       | 50          | 39          |
| CAMTA     | 6           | 40          |
| CO-like   | 17          | 41          |
| CPP       | 8           | 42          |
| DBB       | 11          | 43          |
| Dof       | 36          | 44          |
| E2F/DP    | 8           | 45          |
| EIL       | 6           | 46          |
| ERF       | 123         | 47          |
| FAR1      | 17          | 48          |
| G2-like   | 42          | 49          |
| GATA      | 30          | 50          |
| GeBP      | 22          | 51          |
| GRAS      | 34          | 52          |
| GRF       | 9           | 53          |
| HB-other  | 7           | 54          |
| HB-PHD    | 2           | 55          |
| HD-ZIP    | 48          | 56          |
| HRT-like  | 2           | 57          |
| HSF       | 24          | 58          |
| total     | 1726        |             |

*The data were referred to Jin et al. (2017).
This Table is licensed under CC BY, modified from original paper (Sasaki et al. 2017).
In *Antirrhinum majus*, which has flowers with bilateral symmetry, TF genes that regulate this trait have been studied by genetic analyses. Upper (dorsal) petals of the bilaterally symmetric flowers are specified by the CYCLOIDEA (CYC) and DICHOTOMA (DICH) TFs. CYC and DICH activate RADIALIS (RAD; for review see Hileman 2014, Preston and Hileman 2009). Lower (ventral) petals are specified by DIVARICATA (DIV) function. Moreover, the expression of *DIV* is eliminated from the dorsal region through negative regulation by RAD function.

As described above, TFs play important roles not only in floral organ development but also in characterizing specific floral traits. Thus, the functional modification of these TFs should be effective for changing floral traits and/or generating new traits. To date, the functions of TFs in floral organ identity have been mainly analyzed by genetics in *Arabidopsis*. However, genetic analyses of all genes of interest are not always possible in higher plants because many plant genes including those encoding TFs exhibit redundancy in the plant genome (Moore and Purugganan 2005; for review see Soltis et al. 2007). In these redundant genes, the specific mutant phenotype would not be caused by a single mutation. Therefore, functional analysis of the gene of interest by genetic analysis alone has been very difficult because one or more functionally redundant genes compensate for the function of the single target gene that has been mutated. For example, single-gene mutations in *SEP* genes, in which the *Arabidopsis* genome contains four redundant genes, caused subtle phenotypic changes; however, the triple mutation *sep1 sep2 sep3* caused a significant phenotypic change that altered all floral organs into sepal-like organs (Pelaz et al. 2000). The whole genome sequences of many plant species, including *Arabidopsis* (*Arabidopsis Genome Initiative 2000*) and rice (*International Rice Genome Sequencing Project 2005*), have now been released, and genetic analysis by multiple mutations is making it increasingly clear that functionally redundant TFs are present in these plants. In such model plants, the generation of multiple variants would be possible, but not easy. However, genetic analysis is very difficult or almost impossible in horticultural plants and their cultivars because there has been little information on the whole genomes in these plants; furthermore, many horticultural plants exhibit characteristics making them unsuitable for genetic analysis, such as higher polyploidy, self-incompatibility, and vegetative reproduction.

**Modification of floral traits using TFs, chimeric repressors, and activators**

Ectopic overexpression of TFs using the 3S promoter from cauliflower mosaic virus (hereafter called the “3S promoter”) has also revealed many TF functions and has led to modification of floral traits. In the case of TFs related to morphogenesis in floral organs, flowers of transgenic plants overexpressing two *Arabidopsis* B-function genes, *PISTILLATA* (*PI*) and *APEALLA3* (*AP3*), leaded two outer whorls to petals and two inner whorls to stamens (Krizek and Meyerowitz 1996). This result indicated that *PI* and *AP3* play important roles in petal development, and co-overexpression of *PI* and *AP3* is sufficient to convert sepals into petals. In the case of TFs related to pigment biosynthesis, overexpression of *PRODUCTION OF ANTHOCYANIN PIGMENT 1* (*PAP1*) and *PAP2*, which encode *Arabidopsis* MYB transcription factors, enhanced anthocyanin purple pigmentation in leaves and petals not only in *Arabidopsis* but also in tobacco (Borevitz et al. 2000). Overexpression of *ANTHOCYANIN2*, a petunia *PAP1* homolog, also caused strong accumulation of pigmentation in petunia flowers (Hoballah et al. 2007).

As a tool for the loss-of-function analysis of redundant transcriptional activators, the plant-TF-specific silencing technology CRES-T has been developed (Hiratsu et al. 2003, Mitsuda et al. 2011b). In this method, the plant-specific ERF-associated amphiphilic repression (EAR) motif, which is commonly observed in tobacco ERF3 (Ohta et al. 2001), *Arabidopsis* SUPERMAN (SUP; Hiratsu et al. 2002), *Arabidopsis* AUXIN/INDOLE-3-ACETIC ACID (Szemenyi et al. 2008, Tiwari et al. 2004), and *Arabidopsis* NJNA (Pauwels et al. 2010), was used. The repression domain containing the EAR motif from SUP was further optimized for suppressing transcriptional activators, and the designed 12-amino acid sequence called SRDX (Hiratsu et al. 2003) was attached to target TFs in order to transform the transcriptional activators into strong transcriptional repressors. In *Arabidopsis*, transcriptional activators account for approximately 80% of all TFs (Mitsuda and Ohme-Takagi 2009). This artificial chimeric repressor exerts pronounced effects and suppresses the activities of transcriptional activators despite the presence of functionally redundant TFs with homologous amino acid sequences (Fig. 1A). For example, the *Arabidopsis* genome contains eight CINCINNATA-like (CIN-like) TCP genes that exhibit functional redundancy and amino acid sequence similarity. Transgenic plants with RNAi of *Arabidopsis* TCP3 (*AtTCP3*), which is one of these CIN-like TCP genes, and a single tcp3 mutant (T-DNA-tagged lines) basically showed a normal phenotype (Koyama et al. 2007). In contrast, overexpression of *AtTCP3-SRDX* caused a severely defective leaf phenotype (Koyama et al. 2007) and repressed the expression of downstream genes, such as miR164, *ASYMETRIC LEAVES*, and INDOLE-3ACETIC ACID/SHORT HYPOCOTYL2 (Koyama et al. 2010). In addition, CRES-T is also useful in higher polyploidy plants, such as hexaploid chrysanthemum (Narumi et al. 2011). CRES-T has been used in basic research in model monocot and dicot plants, and many reports on its use in rice and *Arabidopsis* have been published (Hiratsu et al. 2003, Koyama et al. 2007, 2010, Mitsuda et al. 2006).

In recent years, the molecular mechanism of CRES-T on the repressing function for transcriptional activators in plants has been gradually elucidated. In *Arabidopsis*, repression domains, including the EAR motif, directly interacted with TOPELESS (TPL) and TPL-related (TPR) proteins...
of VP16 to a transcriptional repressor transformed it into a transcriptional activator. This indicates that VP16 could be used for the analysis of transcriptional repressors in a similar way to use of CRES-T for analyzing transcriptional activators (Fig. 1B). For example, overexpression of VP16-attached transcriptional repressor FLOWERING LOCUS C (FLC) caused early flowering, and simple overexpression of FLC had the opposite result of late flowering in Arabidopsis (Fujisawa et al. 2014). Furthermore, the phenotype by the VP16-attached FLC was stronger than that by a multiple-knockout mutation in Arabidopsis (Fujisawa et al. 2014). In another example, although Arabidopsis MYB106 (AtMYB106) is not a transcriptional repressor, for the analysis of the AtMYB106 in Arabidopsis, Oshima et al. (2013) demonstrated that transgenic plants overexpressing the VP16-attached AtMYB106 showed less-branched trichomes and slightly shiny leaves, and these phenotypes were opposite to those overexpressing AtMYB106-SRDX. However, VP16 did not seem to be universally applicable because the attachment of VP16 to ERF3, a transcriptional repressor, did not overcome ERF3’s repressive activity (Ohta et al. 2001). Thus, caution should be applied when planning to use VP16 for such research. If modification or optimization enabling the use of VP16 for every transcriptional repressor is achieved, VP16 would also become a widely used research tool like CRES-T.

CRES-T can also be used in ornamental plants for basic research. As an example of this, in torenia (Torenia fournieri), the function of DEFISIENCE (TfDEF) and GLOBOSA (TfGLO), class B MADS-box genes (Sasaki et al. 2010), was analyzed using the repression domain SRDX. Co-overexpression of chimeric repressors of TfDEF-SRDX/TfGLO-SRDX in torenia altered petals into sepal-like petals, and the sepaloid petals strongly resembled sepal morphologically (Sasaki et al. 2014). Conversely, co-overexpression of TfDEF/TfGLO altered petals into petal-like sepal, and the petaloid sepal quite closely resembled petals not only morphologically but also qualitatively, such as in their color, color pattern, and shape, except that the petaloid sepal had no stamens, unlike petals of wild-type torenia (Sasaki et al. 2014). A similar phenomenon was seen in the study of class B genes in Japanese gentian (Gentiana scabra). Overexpression of gentian P12 (GsP12) partially altered sepal organs into petaloid organs in gentian and Arabidopsis (Nakatsuka et al. 2016). The petaloid phenotype was further accelerated by co-overexpression of gentian AP3a (GsAP3a) with GsP12 in Arabidopsis.

CRES-T has been used not only for such basic research but also for applied studies in ornamental plants that are unsuitable for genetic analyses, such as torenia, rose, gentian, lisanthus, carnation, cyclamen, chrysanthemum, and morning glory (Mitsuda et al. 2008, 2011a; Fiore DB; http://www.cres-t.org/foire/public_db/index.shtml). At present (October 2017), whole genomic sequences of these plant species have not been released except for carnation (Yagi et al. 2014) and morning glory (Hoshino et al. 2016). Therefore, CRES-T is
effective for these ornamental crops whose whole genome information is poorly understood. Among these ornamental plants, many Arabidopsis chimeric repressors have been applied in torenia without any change of the chimeric repressor plasmids that were originally used for Arabidopsis, and these Arabidopsis chimeric repressors enabled alteration of the morphology of the floral organs of torenia (Narumi et al. 2008, Shikata et al. 2011). Furthermore, a more efficient screening method has been developed for plant use. The CT method has been used to generate many varied floral phenotypes in one transformation via 40–50 plant use. The CT method has been used to generate many more efficient screening method has been developed for the introduction of the morphology of the floral organs of torenia and these chimeric repressor plasmids that were originally used for Arabidopsis chimeric repressors have been applied in torenia without any change of the chimeric repressor plasmids. Against these backgrounds, versatile systems (or sets of versatile plasmids) of chimeric repressors, which could commonly function in a variety of ornamental plants, would be expected to achieve the efficient modification of floral traits in ornamental flowers. For such versatility, searching for versatile TFs and/or promoters that are able to work in many plant species would be required. With regard to versatile TF genes, the introduction of a chimeric repressor of AtTCP3 caused similar morphological modification in several ornamental plants, such as rose, torenia, cyclamen, and chrysanthemum (Mitsuda et al. 2011a). In these ornamental plants, AtTCP3-SRDX causes phenotypes similar to those shown in Arabidopsis, such as a serrated margin in leaves and petals (Koyama et al. 2007, 2010). As a general versatile promoter, the 35S promoter was commonly and widely used for overexpression of a transgene in many plant species. This promoter is well known to be expressed constitutively and throughout the plant body with high activity in many plant species, and was also mainly used for the expression of chimeric repressors in previous studies. Another general versatile promoter, InMYBI promoter, which is a petal-specific promoter of Japanese morning glory (Ipomoea nil), is also utilisable in various plant species, such as Arabidopsis, eustoma, carnation, chrysanthemum, and Japanese gentian (Azuma et al. 2016). The accumulation of information on these versatile TFs and promoters would be important for efficient progress in the molecular breeding of ornamental plants. In addition, research using the CT method in ornamental plants may enable the screening of TFs that cause novel floral phenotypes that would be hard to find in Arabidopsis, whose flowers are small and white. Flower architecture is also known to differ among plant species, and TFs also function in specifying a variety of flower architectures, such as radial symmetric flowers in Arabidopsis (Matsumoto and Okada 2001), bilateral symmetric flowers in snapdragon (for review see Hileman 2014, Preston and Theißen 2008), compound flowers in gerbera (Broholm et al. 2008), and inner and outer tepal structures in orchid (for review, see Mondragón-Palomino and Theißen 2008). The functions of TF in this research field may still remain unknown. These analyses would lead to the identification of novel TF functions that are specific to a certain plant species.

(1) Construction of chimeric-repressor plasmids

(2) Mixture of various chimeric-repressor plasmids

(3) Co-transformation of the mixture into ornamental plants (Agrobacterium method)

(4) Screening of floral traits

Fig. 2. Method flow of CT system with TFs. Flow of the CT system for selecting target floral traits through massive screening of plant TFs (Shikata et al. 2011). A variety of floral traits are obtained by the CT system.
Recent studies with chimeric repressors mainly used the constitutive 35S promoter for their expression in plants. However, some research revealed that the 35S promoter sometimes causes problems for the expression of chimeric repressors. The ectopic overexpression of some chimeric repressors throughout the plant body causes not only alteration of floral morphology but also certain abnormalities, such as morphological defects in leaves and dwarfing (Narumi et al. 2011). In CRES-T, the chimeric repressor strongly and dominantly suppresses the function of redundant genes. Thus, it would be reasonable to assume that expression of the chimeric repressor throughout the plant body using the 35S promoter had an effect on several parts other than floral organs.

For example, the overexpression of the chimeric repressor of Arabidopsis MYB24 (AtMYB24-SRDX) with the 35S promoter in torenia resulted in the alteration of leaf phenotype, with glossing off the surface and curling of the leaf margin (Shikata et al. 2011). However, at the same time, the transgenic torenia plants did not come into bloom, although they formed flower buds (Fig. 3A; Sasaki et al. 2011). Then, the floral organ-specific Arabidopsis APETALAI (AtAP1) promoter was used for the expression of AtMYB24-SRDX in torenia. In the transgenic torenia plants with the AtAP1 promoter driving AtMYB24-SRDX, the opening of flowers and sterically waved petals were identified (Fig. 3B), the configuration of which is not usually observed in wild-type torenia. In addition, the transgenic torenia avoided morphological alterations in the leaves, and showed a normal leaf phenotype (Sasaki et al. 2011). In Arabidopsis, the floral organs specific InMYB1 promoter was used for modification of the epidermal cell shapes of petals through regulation of the function of AtMYB106 (Azuma et al. 2016). Utilization of the 35S promoter for overexpression of AtMYB106, which regulates epidermal cell morphology in Arabidopsis (Oshima et al. 2013), resulted in undesirable phenotypes in organs other than petals. Overexpression of AtMYB106-SRDX not only changed petal cell morphology but also caused fusion of leaves and buds, and overexpression of AtMYB106-VP16 resulted in slightly shiny leaves with cutin nanoridges, which are usually developed in petals (Oshima et al. 2013). Then, the InMYB1 promoter was used for expression of AtMYB106-SRDX and AtMYB106-VP16 to avoid these unfavorable phenotypes in organs other than petals (Azuma et al. 2016). When the InMYB1 promoter was used instead of the 35S promoter, transgenic plants with AtMYB106-SRDX exhibited wrinkled petals, and those with AtMYB106-VP16 showed inward-curved petals; these phenotypes were not observed in wild-type torenia. Utilization of the InMYB1 promoter for expression of AtMYB106-SRDX and AtMYB106-VP16 resulted in no other phenotypical alteration in these transgenic plants. As another example, the AtTCP3-SRDX was expressed in torenia. The expression throughout the plant body with the 35S promoter caused specific floral phenotypes, such as serrated petal margins and cracked petals, which were not observed in wild-type torenia (Narumi et al. 2011, Sasaki et al. 2016). However, the overexpression of AtTCP3-SRDX simultaneously resulted in serrated leaves and dwarfing, which were unfavorable phenotypes. To use the morphological alteration in petals and avoid these unfavorable phenotypes in other parts of plants besides the floral organs, five floral organ-specific promoters, which possess different properties, were used for the expression of the AtTCP3 chimeric repressor (Sasaki et al. 2016). One of these was the AtAP1 promoter and the other four were derived from torenia, two of which were derived from anthocyanin-biosynthesis-related genes and the other two from class B MADS-box genes. As expected, specific expression of the chimeric repressor of AtTCP3 in floral organs avoided the leaf morphological defects (Fig. 4A) and dwarfing in torenia.

At the same time, an interesting phenomenon was also
observed in these transgenic torenia plants expressing the *AtTCP3-SRDX* gene. Six promoters, consisting of the 35S promoter and five floral organ-specific ones, seemed to cause different floral phenotypes (Fig. 4B; Sasaki et al. 2016). Although these transgenic plants exhibited various color variations, the six different combinations led to morphologically similar phenotypes within the same constructs. Because these six promoters exhibited different promoter activity and properties, such variation would cause the different varieties of floral traits. Another interesting phenotype was also observed in these *AtTCP3-SRDX* transgenic torenia plants. These transgenic plants, which were derived from the same plasmid construct, exhibited various color variations (Fig. 4B). The *Antirrhinum CIN*, into which TCP3 is also classified, contributes to the differentiation of epidermal cells and growth of petals (Crawford et al. 2004). Then, the strength of suppression of TCPs and/or the amount of expression of the *AtTCP3-SRDX* gene at the growth stage of petal color synthesis may affect epidermal cell shape, and pigment metabolism and accumulation in petals. This may in turn lead to different varieties of cell shape and petal color, even though the same plasmid was introduced. Indeed, three patterns of epidermal cell shape were observed by SEM in petals of these six types of transgenic plants (Sasaki et al. 2016). Although the difference of the cell shapes observed by Sasaki et al. (2016) was due to the different properties of these six promoters, petal color variations with the same plasmid construct may have been caused by such factors. At the present time, we do not know whether the color variation derived from six types of promoters is caused directly or indirectly; therefore, more detailed research on this issue is required.

**Contribution on basic and applied researches by utilization of floral organ-specific promoters and TFs**

The utilization of six different types of promoter led to various floral phenotypes in torenia transgenic plants, even though only one chimeric TF was used (Sasaki et al. 2016). This was an interesting finding in terms of both basic and applied research aspects.

With regard to basic research, the diversity of the alteration of floral traits is interesting because it suggests that torenia TCP3 orthologs and/or homologs have temporospatially specific and multilateral functions in floral organ formation. In other words, various promoters with different functions caused multilateral alterations of floral traits. This diversity would be derived from temporospatially wide-ranging activities, which were observed in different tissues, organs, timings, and intensities at various growth stages of...
floral organ development and formation (Fig. 5). The alteration of floral organs in these transgenic torenia plants could not be observed in mutants because the suppression in transgenic plants is temporospatially limited. However, activities of the organ-specific promoters were not always completely consistent with the expression of redundant genes of intrinsic torenia TCP3 orthologs and/or homologs in floral organs. Therefore, it would be difficult to obtain a comprehensive overview of the spatiotemporally specific function of a TF of interest simply by using these floral organ-specific promoters without modifying promoters. However, if the regulation and/or detection of spatiotemporally specific expression of target TFs became possible, it would expand our understanding of the detailed intact function of not only TCP3 but also other TFs of interest.

On another front, some plant TFs are also regulated by microRNAs (miRNAs), small non-coding RNAs. Recent research has revealed that many miRNAs contribute to the regulation of plant growth and development as well as to responses to biotic and abiotic stresses (for review see Samad et al. 2017). Therefore, for research on intact temporospatial functions of TFs of interest in vivo, the regulation of TFs by miRNAs should also be taken into consideration.

With regard to applied research, it is anticipated that floral organ-specific promoters will be used as horticultural molecular breeding technology for the modification of floral traits in only floral organs, without affecting non-target organs. For example, the utilization of constitutive promoters that are active throughout the plant body may cause morphological abnormalities, such as deformation of leaves and dwarfing. Such phenotypic alterations may prevent the use of cultivation techniques that involve cultivating and developing a select parental line. On the other hand, adding and/or modifying the traits in only floral organs using a floral organ-specific promoter would enable the utilization of cultivation methods of parental lines without any change. To date, genes including those encoding TFs have mainly been used for molecular breeding to modify floral traits. However, if the alteration of promoters could induce changes of a variety of floral traits, such as petal color, petal shape, and color pattern, this approach would also be viable. In addition to the floral organ-specific promoters, other “organ-specific”, “inducible”, and “stress-responsive” promoters would also be among the options for modifying and/or adding favorable plant traits (Fig. 6). For example, if the goal is to achieve a glossy leaf phenotype, the combination of a leaf-specific promoter and AtMYB24-SRDX (Sasaki et al. 2011) would be a good candidate. Coloring in specific organs, such as, leaves, stems, and sepals, may also provide new targets for the modification of plant traits. In fact, coloring in sepals is possible in torenia (Fig. 7). For example, this was achieved by the overexpression of TfGLO alone, resulting in the accumulation of purple pigment anthocyanins in sepals (Sasaki et al. 2010). In this case, the 35S promoter could be used because this overexpression of TfGLO did not seem to affect other traits. However, it would be desirable to use sepal-specific or native promoters to avoid an effect on non-targeted organs and/or plant traits and other TFs, the overexpression of which could lead to unfavorable plant traits in undesirable organs. Combinations of some type of “specific” promoter and TFs (including chimeric repressors/activators) would have unlimited possibilities for the creation of novel flowers that have never previously been seen.

In recent years, there has been significant progress in

![Fig. 5. Differences of properties of floral organ-specific promoters. Floral organ-specific promoters have different properties. Utilization of these different promoters would generate a variety of floral traits, as shown in Fig. 4, even though only one TF was used as a chimeric repressor.](image)

![Fig. 6. Generation of “customized flowers”. Combination of “specific” promoters and TF (with or without CRES-T, VP16) or non-TF genes would enable the generation of “customized flowers”.](image)
Consumer preferences for commercially available ornamental flowers keep changing rapidly. Therefore, it is important to achieve prompt cultivar improvement and modification of floral traits designed for consumers, farm producers, buyers at distribution centers, and people at flower markets and retail stores. Although molecular breeding had already provided new opportunities in the flower industry in Japan, the combination of “specific” promoters and TFs (chimeric repressors/activators) would provide new possibilities for “customized flowers” that meet new and more specific consumer demands. In the future, it is expected that these combinations will be applied in various flower species, with the expectation that this will generate more choice (genes and promoters) for the modification of floral traits. Molecular breeding is a biotechnology that can provide new floral traits that could not be provided by traditional crossbreeding, mutation breeding, and genome editing breeding, affecting only a single targeted plant organ. Each breeding method introduced in this review, including molecular breeding technology, has its own advantages and disadvantages in basic and applied research for a particular targeted flower species. Moreover, each ornamental plant species would be associated with different problems when utilizing respective breeding techniques, for example, difficulty of using the technology, a time-intensive process of developing the technique, and a high cost of such development. Therefore, these breeding techniques should be used in careful consideration of the task to be performed, and molecular breeding is also anticipated to contribute to attractive flower cultivars in the future.

Future directions

Consumer preferences for commercially available ornamental flowers keep changing rapidly. Therefore, it is important to achieve prompt cultivar improvement and modification of floral traits designed for consumers, farm producers, buyers at distribution centers, and people at flower markets and retail stores. Although molecular breeding had already provided new opportunities in the flower industry in Japan, the combination of “specific” promoters and TFs (chimeric repressors/activators) would provide new possibilities for “customized flowers” that meet new and more specific consumer demands. In the future, it is expected that these combinations will be applied in various flower species, with the expectation that this will generate more choice (genes and promoters) for the modification of floral traits. Molecular breeding is a biotechnology that can provide new floral traits that could not be provided by traditional crossbreeding, mutation breeding, and genome editing breeding, affecting only a single targeted plant organ. Each breeding method introduced in this review, including molecular breeding technology, has its own advantages and disadvantages in basic and applied research for a particular targeted flower species. Moreover, each ornamental plant species would be associated with different problems when utilizing respective breeding techniques, for example, difficulty of using the technology, a time-intensive process of developing the technique, and a high cost of such development. Therefore, these breeding techniques should be used in careful consideration of the task to be performed, and molecular breeding is also anticipated to contribute to attractive flower cultivars in the future.

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Literature Cited

Agarwal, M., P. Kumar and S.J. Mathew (2015) The Groucho/Transducin-like enhancer of split protein family in animal development. IUBMB Life 67: 472–481.

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408: 796–815.

Azuma, M., R. Morimoto, M. Hirose, Y. Morita, A. Hoshino, S. Iida, Y. Oshima, N. Mitsuda, M. Ohme-Takagi and K. Shiratake (2016) A petal-specific InMYB1 promoter from Japanese morning glory: a useful tool for molecular breeding of floricultural crops. Plant Biotechnol. J. 14: 354–363.

Borevitz, J.O., Y. Xia, J. Blount, R.A. Dixon and C. Lamb (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12: 2383–2394.

Broholm, S.K., S. Tähtiharju, R.A. Laitinen, V.A. Albert, T.H. Teeri and P. Elomaa (2008) A TCP domain transcription factor controls flower type specification along the radial axis of the Gerbera (Asteraceae) inflorescence. Proc. Natl. Acad. Sci. USA 105: 9117–9122.

Causier, B., M. Ashworth, W. Guo and B. Davies (2012) The TOPLESS interactome: a framework for gene repression in Arabidopsis. Plant
Utilization of transcription factors in horticultural plants

Physiol. 158: 423–438.

Čermák, T., S.J. Curtin, J. Gil-Humanes, R. Čegan, T.J.Y. Kono, E. Konečná, J.J. Belanto, C.G. Starker, J.W. Mathre, R.L. Greenstein et al. (2017) A multipurpose toolkit to enable advanced genome engineering in plants. Plant Cell 29: 1196–1217.

Coen, E.S. and E.M. Meyerowitz (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353: 31–37.

Colquhoun, T.A. and D.G. Clark (2011) Unraveling the regulation of floral fragrance biosynthesis. Plant Signal. Behav. 6: 378–381.

Crawford, B.C., U. Nath, R. Carpenter and E.S. Coen (2004) CINCINNATA controls both cell differentiation and growth in petal lobes and leaves of Antirrhinum. Plant Physiol. 135: 244–253.

Ditta, G., A. Pinyopich, P. Robles, S. Pelaz and M.F. Yanofsky (2004) The SEPAL gene of Arabidopsis thaliana functions in floral organ and meristem identity. Curr. Biol. 14: 1935–1940.

Fujiwara, S., S. Sakamoto, K. Kigoshi, K. Suzuki and M. Ohme-Takagi (2014) VP16 fusion induces the multiple-knockout phenotype of redundant transcriptional repressors partly by Med25-independent mechanisms in Arabidopsis. FEBS Lett. 588: 3665–3672.

Hileman, L.C. (2014) Bilateral flower symmetry—how, when and why? Curr. Opin. Plant Biol. 17: 146–152.

Hiratsu, K., M. Ohta, K. Matsu and M. Ohme-Takagi (2002) The SUPERMAN protein is an active repressor whose carboxy-terminal repression domain is required for the development of normal flowers. FEBS Lett. 514: 351–354.

Hiratsu, K., K. Matsu, T. Koyama and M. Ohme-Takagi (2003) Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in Arabidopsis. Plant J. 34: 733–739.

Hoballah, M.E., T. Gütib, J. Stuurman, L. Broger, M. Barone, T. Mandel, A. Dell’Olivo, M. Arnold and C. Kuhlemeyer (2007) Single gene-mediated shift in pollinator attraction in Petunia. Plant Cell 19: 799–790.

Homma, T. and K. Goto (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 409: 525–529.

Hoshino, A., V. Jayakumar, E. Nitasaka, A. Toyoda, H. Noguchi, T. Itoh, T. Shin-I, Y. Minakuchi, Y. Koda, A.J. Nagano et al. (2016) Genomic sequence and analysis of the Japanese morning glory Ipomoea nil. Nat. Commun. 7: 13295.

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature 436: 793–800.

Jin, J.P., F. Tian, D.C. Yang, Y.Q. Meng, L. Kong, J.C. Luo and G. Gao (2017) PlantTFDB 4.0: toward a central hub for transcription factors in Arabidopsis. FEBS Lett. 588: 3665–3672.

Katsumoto, Y., M. Fukuchi-Mizutani, Y. Fukui, F. Bruglier, T.A. Holton, M. Karan, N. Nakamura, K. Yonekura-Sakakibara, J. Togami, A. Pigai et al. (2007) Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. Plant Cell Physiol. 48: 1589–1600.

Ke, J., H. Ma, X. Gu, A. Thelen, J.S. Brunzelle, J.Li, H.E. Xu and K. Melcher (2015) Structural basis for recognition of diverse transcriptional repressors by the TOPLESS family of corepressors. Sci. Adv. 1: e1500107.

Kishi-Kaboshi, M., R. Aida and K. Sasaki (2017) Generation of gene-edited Chrysanthemum morifolium using multicopy transgenes as targets and markers. Plant Cell Physiol. 58: 216–226.

Koyama, T., M. Furutani, M. Tasaka and M. Ohme-Takagi (2007) TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis. Plant Cell 19: 473–484.

Koyama, T., N. Mitsuda, M. Seki, K. Shinozaki and M. Ohme-Takagi (2010) TCP transcription factors regulate the activities of ASYMMETRICAL LEAVES1 and miR164, as well as the auxin response, during differentiation of leaves in Arabidopsis. Plant Cell 22: 3574–3588.

Krzek, B.A. and E.M. Meyerowitz (1996) The Arabidopsis homeotic genes APETALA3 and PISTILLATA are sufficient to provide the B class organ identity function. Development 122: 11–22.

Kui, L., H. Chen, W. Zhang, S. He, Z. Xiong, Y. Zhang, L. Yan, C. Zhong, F. He, J. Chen et al. (2017) Building a genetic manipulation tool box for orchid biology: identification of constitutive promoters and application of CRISPR/Cas9 in the orchid, Dendrobium officinale. Front. Plant Sci. 7: 2036.

Lee, J.E. and J.F. Golz (2012) Diverse roles of Groucho/Tup1 co-repressors in plant growth and development. Plant Signal. Behav. 7: 86–92.

Ma, H. (1994) The unfolding drama of flower development: recent results from genetic and molecular analyses. Genes Dev. 8: 745–756.

Ma, H., J. Duan, J. Ke, Y. He, X. Gu, T.H. Xu, H. Yu, Y.Wang, J.S. Brunzelle, Y. Jiang et al. (2017) A D53 repression motif induces oligomerization of TOPLESS corepressors and promotes assembly of a corepressor-nucleosome complex. Sci. Adv. 3: e1601217.

Ma, X., Z. Zhu, Y. Chen and Y.G. Liu (2016) CRISPR/Cas9 platforms for genome editing in plants: developments and applications. Mol. Plant 9: 961–974.

Matsumoto, N. and K. Okada (2001) A homeobox gene, PRESSES FLOWER, regulates lateral axis-dependent development of Arabidopsis flowers. Genes Dev. 15: 3355–3364.

Mitsuda, N., K. Hiratsugu, D. Todaka, K. Nakashima, K. Yamagushi-Shinozaki and M. Ohme-Takagi (2006) Efficient production of male and female sterile plants by expression of a chimeric repressor in Arabidopsis and rice. Plant Biotechnol. J. 4: 325–332.

Mitsuda, N., Y. Umemura, M. Ikeda, M. Shikata, T. Koyama, K. Matsu, T. Narumi, R. Aida, K. Sasaki, T. Hiyama et al. (2008) FioreDB: a database of phenotypic information induced by the chimeric repressor silencing technology (CRES-T) in Arabidopsis and floricultural plants. Plant Biotechnol. 25: 37–43.

Mitsuda, N. and M. Ohme-Takagi (2009) Functional analysis of transcription factors in Arabidopsis. Plant Cell Physiol. 50: 1232–1248.

Mitsuda, N., Y. Takiguchi, M. Shikata, K. Sage-Ono, M. Oono, K. Sasaki, H. Yamaguchi, T. Narumi, Y. Tanaka, M. Sugiyama et al. (2011a) The new FioreDB database provides comprehensive information on plant transcription factors and phenotypes induced by CRES-T in ornamental and model plants. Plant Biotechnol. 28: 123–130.

Mitsuda, N., K. Matsu, M. Ikeda, M. Nakata, Y. Oshima, Y. Nagatomi and M. Ohme-Takagi (2011b) CRES-T, an effective gene silencing system utilizing chimeric repressors. Methods Mol. Biol. 754: 87–105.

Mondragon-Palomino, M. and G. Theissen (2008) MADS about the evolution of orchid flowers. Trends Plant Sci. 13: 51–59.

Moore, R.C. and M.D. Purugganan (2005) The evolutionary dynamics of plant duplicate genes. Curr. Opin. Plant Biol. 8: 122–128.

Nakatsuoka, T., M. Saito and M. Nishihara (2016) Functional characterization of duplicated B-class MADS-box genes in Japanese gentian. Plant Cell Rep. 35: 895–904.

Narumi, T., R. Aida, T. Niki, T. Nishihija, N. Mitsuda, K. Hiratsugu, M. Ohme-Takagi and N. Ohtsubo (2008) Chimeric AGAMOUS repressor induces serrated petal phenotype in Torenia fournieri similar to that induced by cytokinin application. Plant Biotechnol. 25: 45–53.
Narumi, T., R. Aida, T. Koyama, H. Yamaguchi, K. Sasaki, M. Shikata, M. Nakayama, M. Ohme-Takagi and N. Ohtsubo (2011) *Arabidopsis* chimeric TCP3 repressor produces novel floral traits in *Torenia fournieri* and *Chrysanthemum morifolium*. Plant Biotechnol. 28: 131–140.

Ohta, M., K. Matsui, K. Hiratsu, H. Shinshi and M. Ohme-Takagi (2001) Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. Plant Cell 13: 1959–1968.

O’Maoiléidigh, D.S., E. Graciet and F. Wellmer (2014) Gene networks controlling *Arabidopsis thaliana* flower development. New Phytol. 201: 16–30.

Oshima, Y., M. Shikata, T. Koyama, N. Ohtsubo, N. Mitsuda and M. Ohme-Takagi (2013) MIXTA-like transcription factors and WAX INDUCER1/SHINE1 coordinately regulate cuticle development in *Arabidopsis* and *Torenia fournieri*. Plant Cell 25: 1609–1624.

Otani, M., A. Sharifi, S. Kubota, K. Ozumi, F. Uetake, M. Hirai, Y. Hoshino, A. Kanno and M. Nakano (2016) Suppression of B function strongly supports the modified ABCE model in *Tricyrtis* sp. (Liliaceae). Sci. Rep. 6: 24549.

Pauwels, L., G.F. Barbero, J. Geerinck, S. Tilleman, W. Grunewald, A.C. Pérez, J.M. Chico, R.V. Bossche, J. Sewell, E. Gil et al. (2010) NINJA connects the co-repressor TOPLESS to jasmonate signal transduction. Nature 464: 788–791.

Pelaz, S., G.S. Ditta, E. Baumann, E. Wisman and M.F. Yanofsky (2000) B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature 405: 200–203.

Petroni, K. and C. Tonelli (2011) Recent advances on the regulation of anthocyanin synthesis in reproductive organs. Plant Sci. 181: 219–229.

Preston, J.C. and L.C. Hileman (2009) Developmental genetics of floral symmetry evolution. Trends Plant Sci. 14: 147–154.

Riechmann, J.L., I. Heard, G. Martin, L. Reuber, C. Jiang, J. Keddie, L. Adam, O. Pineda, O.J. Ratcliffe, R.R. Samaha et al. (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 290: 2105–2110.

Samad, A.F.A., M. Sajad, N. Nazaruddin, I.A. Fauzi, A.M.A. Murad, Z. Zainal and I. Ismail (2017) MicroRNA and transcription factor: key players in plant regulatory network. Front. Plant Sci. 8: 565.

Sasaki, K., R. Aida, H. Yamaguchi, M. Shikata, T. Niki, T. Nishijima and N. Ohtsubo (2010) Functional divergence within class B MADS-box genes *TfGLO* and *TfDEF* in *Torenia fournieri* Lind. Mol. Genet. Genomics 284: 399–414.

Sasaki, K., H. Yamaguchi, M. Nakayama, R. Aida and N. Ohtsubo (2011) Generation of novel floral traits using a combination of floral organ-specific promoters and a chimeric repressor in *Torenia fournieri* Lind. Plant Cell Physiol. 57: 1319–1331.

Sasaki, K., N. Mitsuda, K. Nashima, K. Kishimoto, Y. Katayose, H. Kanamori and A. Ohmiya (2017) Generation of expressed sequence tags for discovery of genes responsible for floral traits of *Chrysanthemum morifolium* by next-generation sequencing technology. BMC Genomics 18: 683.

Shikata, M., T. Narumi, H. Yamaguchi, K. Sasaki, R. Aida, Y. Oshima, Y. Takiguchi, M. Ohme-Takagi, N. Mitsuda and N. Ohtsubo (2011) Efficient production of novel floral traits in torenia by collective transformation with chimeric repressors of *Arabidopsis* transcription factors. Plant Biotechnol. 28: 189–199.

Soltis, D.E., H. Ma, M.W. Frohlich, P.S. Soltis, V.A. Albert, D.G. Oppenheimer, N.S. Altman, C. dePamphilis and J. Leebens-Mack (2007) The floral genome: an evolutionary history of gene duplication and shifting patterns of gene expression. Trends Plant Sci. 12: 358–367.

Szemenyesi, H., M. Hannon and J.A. Long (2008) TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. Science 319: 1384–1386.

Tanaka, Y., F. Bruglieria and S. Chandler (2009) Recent progress of flower colour modification by biotechnology. Int. J. Mol. Sci. 10: 5350–5369.

Tanaka, Y., O. Yamamura, M. Sugiyama, N. Mitsuda, N. Ohtsubo, M. Ohme-Takagi and T. Terakawa (2013) Multi-petal cyclamen flowers produced by AGAMOUS chimeric repressor expression. Sci. Rep. 3: 2641.

Theißen, G. (2001) Development of floral organ identity: stories from the MADS house. Curr. Opin. Plant Biol. 4: 75–85.

Theißen, G. and H. Saedler (2001) Plant biology. Floral quartets. Nature 409: 469–471.

Theißen, G., R. Melzer and F. Rümpker (2016) MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. Development 143: 3259–3271.

Tiwari, S.B., G. Hagen and T.J. Guilfoyle (2004) Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell 16: 533–543.

Triezenberg, S.J., R.C. Kingsbury and S.L. McKnight (1988) Functional dissection of VP16, the trans-activator of herpes simplex virus immediate early gene expression. Genes Dev. 2: 718–729.

Watanabe, K., A. Kobayashi, M. Endo, K. Sage-Ono, S. Toki and M. Ono (2017) CRISPR/Cas9-mediated mutagenesis of the dihydroflavonol-4-reductase-B (*DFR-B*) locus in the Japanese morning glory *Ipomoea (Pharbitis) nil*. Sci. Rep. 7: 10028.

Yagi, M., S. Kosugi, H. Hiraakawa, A. Ohmiya, K. Tanase, T. Harada, K. Kishimoto, M. Nakayama, K. Ichimura, T. Onozaki et al. (2014) Sequence analysis of the genome of carnation (*Dianthus caryophyllus* L.). DNA Res. 21: 231–241.

Yin, K., C. Gao and J.L. Qiu (2017) Progress and prospects in plant genome editing. Nat. Plants 3: 17107.

Zhang, B., X. Yang, C. Yang, M. Li and Y. Guo (2016) Exploiting the CRISPR/Cas9 system for targeted genome mutagenesis in petunia. Sci. Rep. 6: 20315.