Plant Biotechnology 25, 31–36 (2008)

Review

The utility of transcription factors for manipulation of floral traits

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Received November 15, 2007; accepted December 20, 2007 (Edited by Y. Ozeki)

Abstract Cross-pollination is an effective method of breeding flowering plants to produce novel variation. However, this strategy is often protracted or demands repeated crossing for several generations to obtain desired traits. Recently, gene-modification technologies have been utilized in plant breeding for manipulation of floral traits. Many homeotic genes that regulate flower development have been shown to encode transcription factors. In this review we describe the utility of transcription factors and a novel gene-silencing technology, the CRES-T system, to effectively manipulate floral traits. When *AGAMOUS* (*AG*) and *APETALA3* (*AP3*) were subjected to this system in *Arabidopsis*, *ag*-like and *ap3*-like phenotypes, respectively, were induced with high efficiency as a dominant trait. Plant transcription factors are conserved between different species to some extent and consequently the chimeric repressor derived from *Arabidopsis* can be applied to other species without any modification. By applying this system, new floral color and shape phenotypes were obtained in *Torenia fournieri* and *Ipomoea nil*. Since the CRES-T system is able to overcome the problem of gene redundancy, polyploid plants may be also manipulated with this system. In addition, with the CRES-T system modification of the flower morphology of plants for which limited genome sequence information is available can be expected.

Key words: ABC model, CRES-T system, floral trait, transcription factor.

Flowers are a characteristic organ in angiosperm and consist of sepals, petals, stamens and carpels. Petals are the most conspicuous organ in flowers because of their variation in colors and shapes. Some plants such as *Tulipa gesneriana* have tepals instead of sepals and petals and *Hydrangea* have degenerated petals and outstanding sepals in their flowers. The breeding and commercial production of cut flowers and ornamental plants is of major economic importance. Therefore, production of novel variation in flowers is an important focus of plant breeding. Through crossing different genotypes and selection for particular traits among the hybrid progeny, large numbers of phenotypically divergent varieties have been developed from the wild, less genetically diverse species. For example, wild *Eustoma grandiflorum* from North America has only purple-blue flowers, but a great number of color variations of this species are now available. Cross-pollination is a useful strategy for crop improvement and for production of genetically stable new varieties. However, this strategy may be protracted and require repeated crossing over several generations to acquire new and desired traits.

On the other hand, gene-modification technologies are a powerful tool to alter plant traits rapidly. Previously, mutagenesis by chemical or UV treatment was used to induce genetic variation. Recently, ion-beam irradiation has been shown to be a useful tool for mutagenesis (Shirley et al. 1992). However, due to the presence of redundant genes in the plant genome, this strategy is not always effective to obtain desired phenotypes especially in polyploid plants. Transgene expression has been shown to be an effective and more rapid means of introducing a desired phenotype into a target plant. This technology has been applied to plants to transfer agriculturally useful traits, such as insect or herbicide resistance (Comai and Shen 1983; Bates et al. 2005), as well as to manipulate floral traits. Alterations of petal color have been reported by ectopic expression or introducing antisense for genes related to pigmentation biosynthesis (Forkmann and Martens 2001). However, attempts to manipulate floral traits have not been fully achieved, probably due to limited availability of genomic information regarding specific floral traits in horticultural plants.

Plant gene expression is largely controlled at the transcription level and thus transcription factors play a pivotal role in gene regulation. Because transcription factors are capable of controlling the expression of multiple genes, some have been shown to act as a

Abbreviations: CaMV, cauliflower mosaic virus; CRES-T, chimeric repressor gene-silencing technology; RNAi, RNA interference
This article can be found at http://www.jspcmb.jp/

'master' regulator for various phenotypes. Many mutants with flower abnormalities have defective transcription factors (reviewed by Jack 2004). Therefore, it can be predicted that transcription factors are a potential tool for the easy and efficient manipulation of floral traits. Recently, we developed a novel gene-silencing system using the chimeric transcriptional repressor, which is designated chimeric repressor gene-silencing technology (CRES-T). This system can induce a phenotype that was not expressed by antisense or gene knockout lines (Hiratsu et al. 2003).

In this review, we highlight the various roles that transcription factors play in flower development and the utility of the CRES-T system for manipulation of floral traits.

Many transcription factors regulate floral traits

Mutant analyses of *Arabidopsis thaliana* and *Antirrhinum majus* have revealed that a number of genes that regulate flower development encode transcription factors. Some of them are categorized into ABC genes of the well-known ABC model, which specify floral organ identities (Coen and Meyerowitz 1991). According to the ABC model, four floral organs (sepals, petals, stamens and carpels) are arranged in four concentric whorls from the outermost sepals to the innermost carpels. These organs are specified by a combination of three classes of genes, namely A, B and C (Figure 1A). Mutation in ABC genes causes homeotic conversion of floral organs. Most of ABC genes encode MADS-box transcription factors. One of the most striking phenotype is the mutation of the *AG* gene in *Arabidopsis*. Stamens of the *ag* mutant are converted into petals, and the fourth whorl bears another *ag* flower, resulting in an indeterminate flower with a repetitive pattern of sepals, petals and petals (Yanofsky et al. 1990). The flower of *ag* displays a similar appearance to that of a rose or double-flowered cherry blossom, which are believed to be a defect of a homologous gene of *AG* (Kitahara et al. 2004). One of the excellent aspects of the ABC model is that it can be adapted to most flowering plants with some modification. Many nongrass monocot such as *Tulipa gesneriana* have petaloid organs, called tepals, in first and second whorls and it is interpreted by modified ABC model which B class gene expression expanded to first whorl (Figure 1B; reviewed by Kanno et al. 2007). Thus, modification of ABC genes will make it possible to change floral traits. Besides *AG*, other ABC genes, namely *APETALA1* (*AP1*), *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), also encode MADS-box family transcription factors (Mandel et al. 1992; Goto and Meyerowitz 1994; Jack et al. 1992; Yanofsky et al. 1990), while *APETALA2* belongs to the AP2/ERF family (Jofuku et al. 1994).

A number of genes for transcription factors that do not belong to ABC genes also affect flower development.

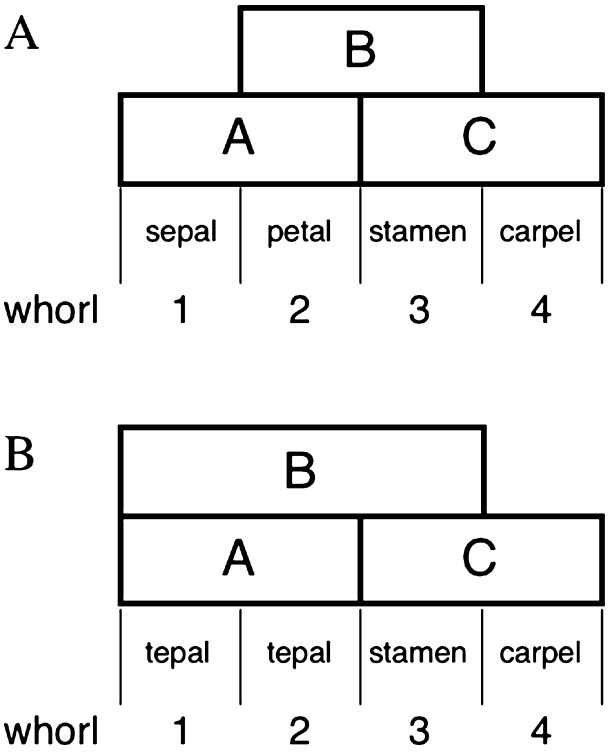

Figure 1. ABC model. (A) Classical ABC model. The combination of A, B and C class genes specifies respective floral organs. (B) Modified ABC model. Expression of class B gene expands to the first whorl, resulting similar type of floral organs to those in second whorl.

The *SUPERMAN* (*SUP*) gene that encodes the zinc-finger transcription factor negatively regulates expression of *AP3* and *PI*, and the *sup* mutation increases the number of stamens (Sakai et al. 1995). In contrast, mutation in the *HANABA TARANU* gene, which encodes the GATA-type zinc-finger protein, results in decreased number of floral organs (Zhao et al. 2004). *PERIANTHIA* (*PAN*) encodes a bZIP protein and the *pan* mutant flowers have five sepals, five petals and five stamens whereas wild type flowers have four sepals, four petals and six stamens (Running and Meyerowitz 1996; Cuang et al. 1999). Wild type *Antirrhinum* has zygomorphic flowers, whereas mutation in both *CYCLOIDEA* and *DICHOTOMA*, which encode TCP family transcription factors, results in actinomorphic flowers (Luo et al. 1996; Cubas et al. 1999). These studies illustrate that, because a number of transcription factors play a role in the control of flower development, transcription factors are a useful tool for the manipulation of floral traits and generation of novel variation.

Function of transcription factors is conserved between plant species

Recently, information from genomic and expressed sequence tags has accumulated in various plants, especially in model plants including *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Medicago truncatula*

and *Antirrhinum majus*. This information revealed that plants have similar sets of transcription factors. ABC genes or their homologues are conserved among plants and the ABC model can be applied to most flowering plants. For example, *Arabidopsis* AP1, AP3, PISTILLATA and AG correspond to *Antirrhinum* SQUAMOSA, DEFICIENS, GLOBOSA and PLENA, respectively (Huijser et al. 1992; Schwarz-Sommer et al. 1992; Tröbner et al. 1992; Bradley et al. 1993). These MADS-box transcription factors control floral organ identities by forming particular complexes and the patterns of combination of MADS-box transcription factors are also conserved between *Arabidopsis* and *Antirrinum* (Egea-Cortines et al. 1999; Honma and Goto 2001). In addition, formation of similar complexes was also reported in *Petunia* and *Chrysanthemum* (Ferrario et al. 2003; Shchennikova et al. 2004).

Conservation of the function of transcription factors between different species has been confirmed by complementation analysis between different species. If two homologous genes derived from different species have the same function, it is possible to complement the mutation of each other. For example, homologues of the *Arabidopsis LEAFY* (*LFY*) gene were found in various flowering plants and they could fully complement the *lfy* mutant (Maizel et al. 2005). These results indicate that transcription factors of *Arabidopsis* may be used for manipulation of floral traits in other flowering plants without any modification.

Application of a gene-silencing system for manipulation of floral organ development

Several transcription factors that identify floral organs have been shown to act as master regulators and are often conserved in many flowering plants. These suggest that modification of transcription factor that is expressed in flowers would be effective for the manipulation of flower traits. RNAi technology was applied to ABC genes to suppress *AG* in *Arabidopsis* and *SUPERWOMAN1* (*SPW1*) in rice (Chuang and Meyerowitz 2000; Xiao et al. 2003). However, it also revealed that a large number of plant genes are highly duplicated due to genome duplication or polyploidy (Moor and Purugganan 2005). In the presence of redundant genes, downregulation of a single gene, such as by gene knockout or RNAi, often fails to induce a visible phenotype (Figure 2A).

Recently, a novel gene-silencing system using a chimeric transcriptional repressor, designated CRES-T system, has been shown to be a useful tool to induce the defective phenotype. Moreover, the CRES-T system overcomes such difficulty of gene redundancy (Hiratsu et al. 2003; Matsui et al. 2005; Mitsuda et al. 2005; Mitsuda et al. 2007; Koyama et al. 2007). In this system, a chimeric repressor in which transcription factor fused to repression domain named SRDX, which derived from the ERF-associated amphiphilic repression (EAR) motif, suppresses expression of target genes (Figure 2B). As a result, the transgenic plant that expresses the chimeric repressor exhibits a phenotype similar to that of the loss-of-function allele of the transcription factor gene (Hiratsu et al. 2003). *Arabidopsis* transgenic plants that express the chimeric *AG* repressor (*35S:AGSRDX*) showed a similar phenotype to the *ag* mutant (Figure 2C; Mitsuda et al. 2006; Yanofsky et al. 1990). *Arabidopsis AP3*, a B class gene, specifies petal and stamen identities; development of these organs fails in the *ap3* mutant (Bowman et al. 1989; Jack et al. 1992). Transgenic plants that express the chimeric AP3 repressor (*35S:AP3SRDX*) mimicked the *ap3* flower in *Arabidopsis* (Figure 2D; Mitsuda et al. 2006).

The advantage of the CRES-T system to other gene-silencing technologies is that the chimeric repressor acts dominantly not only to endogenous transcription factors but also to functionally redundant factors, and that it can induce the defective phenotype as a dominant trait. We previously demonstrated that the CRES-T system could induce a defective phenotype even in the presence of redundant factors using the chimeric *CUC1* repressor as a model (Hiratsu et al. 2003). With regard to the phenotype in flowers, expression of the chimeric repressor for *Arabidopsis* TCP3, a member of the TCP family, induced wavy and serrated sepals and petals as well as leaf serration, although no visible phenotype was observed in their T-DNA inserted lines (Koyama et al. 2007). Functional redundancy is a major obstacle not only for functional analysis but also for manipulation of plant traits. Because the CRES-T system can induce a phenotype that was not expressed in antisense or knockout lines due to the presence of functionally redundant factors, this system is a powerful tool for manipulation of polyploid plants, such as hexaploid *Chrysanthemum morifolium*.

Application of CRES-T system to various plants

The CRES-T system functions also in plants other than *Arabidopsis*. Rice *SPW1* is an ortholog of *AP3* and in the *spw1* mutant, lodicules and stamens were transformed to palea-like organs and carpels, respectively (Nagasawa et al. 2003). When the chimeric SPW1 repressor was expressed in rice, transgenic lines have *spw1*-like flowers (Mitsuda et al. 2006). In these transgenic plants, the rice *SPW1* gene was used for construction of the chimeric repressor, while the sequence of the repression domain was identical to the sequence used in *Arabidopsis*. As described earlier, the function of transcription factors is usually conserved between plant species. This suggests that the chimeric repressor constructed with an *Arabidopsis* transcription factor can be applied to other plants without any modification or with minor modification such as alteration of the promoter or

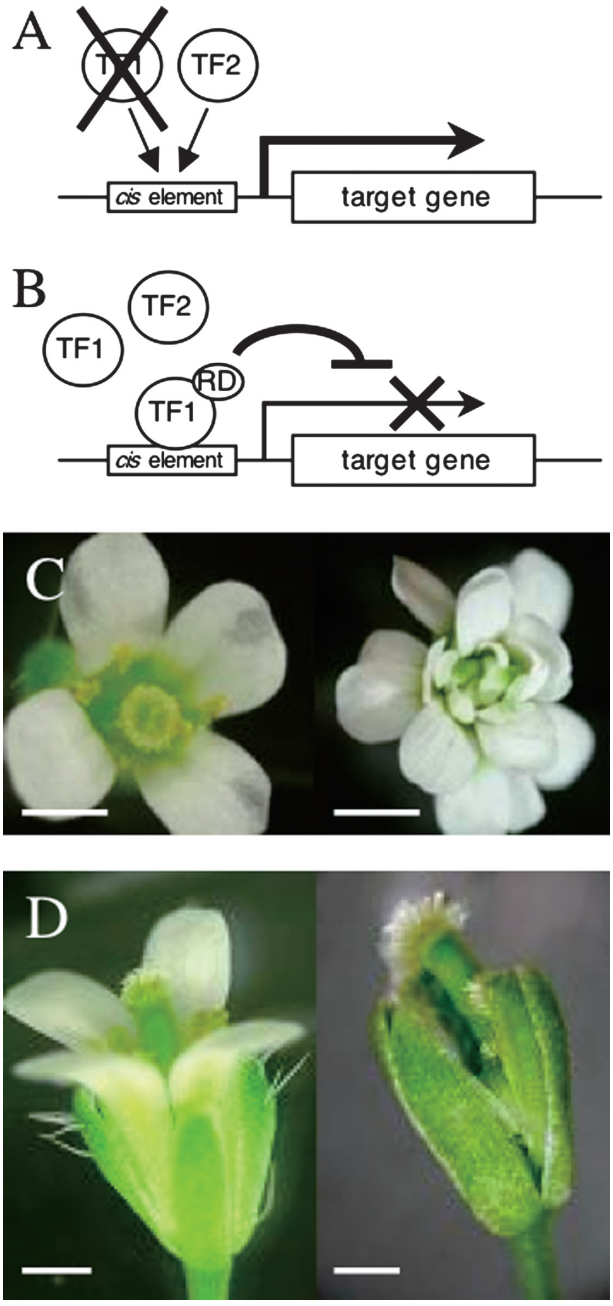

Figure 2. CRES-T system. (A) Existence of the functional redundancy of genes for plant transcription factors (TFs) often results in failure to show defective phenotype in single mutant (represented as X). (B) A chimeric repressor in which transcription factor fused to repression domain (RD) act dominantly not only endogenous gene (TF1) but also functionally redundant gene (TF2), resulting in defective phenotypes. (C) Wild type flower (left) and *35S:AGSRDX* flower (right). (D) Wild type flower (left) and *35S:AP3SRDX* flower (right). Bars = 0.5 mm.

selection marker. Although it would be better to use the gene of the host plant for maximal efficiency of the chimeric repressor, the genomic information necessary for construction of transgenes is not always available, especially in horticultural plants. For this reason RNAi technology cannot be applied to a plant for which limited sequence information is available because relatively

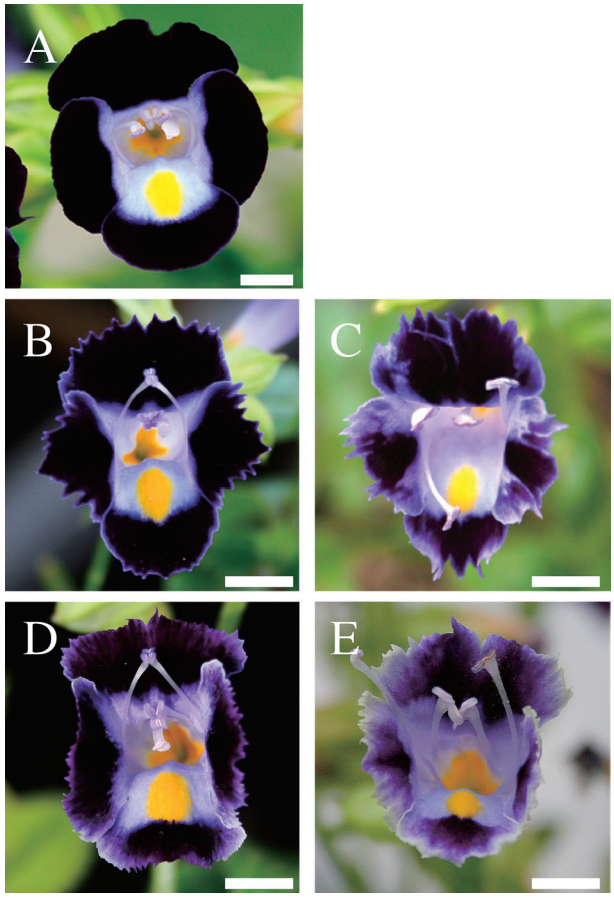

Figure 3. Phenotypic variation in Transgenic *Torenia*. Wild type *Torenia* (A) and different lines of transgenic *Torenia* expressing *SEP3SRDX* (B–E). Same construct induce variations in colors and degrees of serration in corolla. Bars = 5 mm.

accurate sequence data are required to make effective RNAi constructs (Watson et al. 2005). Expression of the *Arabidopsis* chimeric repressor was shown to induce interesting flower phenotypes in *Torenia fournieri* and *Ipomoea nil* (Figure 3; T. Narumi and N. Ohtsubo, unpublished results; S. Hiyama and M. Ono, personal communication). Therefore, because of the high conservation of transcription factors between different species, it is possible to apply the CRES-T system to plants for which genomic information is unavailable.

Advantage of transgenic plants for manipulation of floral traits

Generally, gene mutation, in most cases null mutants, usually leads to stable and invariable phenotypes between generations or lines. On the other hand, phenotypes induced by a transgene are often varied mainly because of the different level of expression between lines. These variations are sometimes troublesome due to low phenotypic stability but at the same time such variation is useful to isolate a diversity of phenotypes. Such variation derived from a single construct can be used to obtain flowers with a new color or shape. For example, a

chimeric repressor of SEP3, floral organ identity gene, has been shown to induce a range of floral colors and degrees of serration in corolla in transgenic *Torenia* (Figure 3; T. Narumi and N. Ohtsubo, unpublished results).

It is possible to manipulate and confer a new trait in a specific tissue by contriving the spatial and temporal expression of the transgene, such as that driven by a tissue-specific promoter. Moreover, alteration of the promoter sequence may induce another phenotype that will not be induced by the constitutive promoter, such as the CaMV35S promoter. In *Arabidopsis*, several chimeric repressor constructs are reported to induce a phenotype only when it was driven by its own promoter (Mitsuda et al. 2005; Ito et al. 2007).

Some plants with interesting floral phenotypes are sterile. It is difficult to maintain such sterile lines even if the floral phenotype is very rare and worth preserving. In contrast, the sterile phenotype induced by a transgene is reproducible.

Conclusion

Arabidopsis contains about 2,000 genes for transcription factors (Guo et al. 2005). More than 250 of these are highly expressed in the floral meristem and floral organs (Schmid et al. 2005). Chimeric repressors derived from the transcription factors that are expressed in floral organs may induce novel phenotypes that have not been expressed in screening with previous mutant lines, such as gene knockout lines, because the CRES-T system overcomes the problem of functional redundancy of transcription factors. It is predicted that it will be possible to create plant varieties with new floral traits by applying the CRES-T system to a diversity of flowering plants. In addition, comparison of the phenotype in diverse species in which the same chimeric repressor was transformed may reveal the function of the transcription factor and enhance our understanding of the evolution of flowers.

Acknowledgements

This work was supported by a Grant-in-Aid “Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries” from the Research Council, Ministry of Agriculture, Forestry and Fisheries of Japan, grant No.1782.

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