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Original Paper

Morphological changes in *Ipomoea nil* using chimeric repressors of *Arabidopsis* TCP3 and TCP5

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Abstract Chimeric REpressor gene-Silencing Technology (CRES-T) is a reverse genetic method that converts transcriptional factors (TFs) to chimeric repressors by fusion with an ethylene-responsive element-binding factor (ERF)-associated amphiphilic repression domain. The plant expressing chimeric repressor is expected to show loss-of-function phenotype of the original TF even in the presence of other functionally redundant TFs. We used the CRES-T system for modification of flower shape in transgenic *Ipomoea nil* (formally *Pharbitis nil*). *I. nil* is emerging as a model plant for ornamental flowers because it has produced a wide variety of historical mutants, and it has been chosen to be a part of the National BioResource Project in Japan. We used cDNAs of TFs of *Arabidopsis thaliana* with the CRES-T system because *A. thaliana* TFs are well characterized compared with *I. nil* TFs. For this study, we selected two TCP (*TEOSINTE BRANCHED1*, *CYCLOIDEA*, and *PCF*) TFs, TCP3 and TCP5, because overexpression of these chimeric repressors TCP3SRDX and TCP5SRDX causes severe morphological alterations in *A. thaliana*. We found that these chimeric repressors cause morphological alterations, an undeveloped corolla and wavy petals in *I. nil*. In addition, the sympetalous corolla was easily disrupted to form choripetalous corolla. Although several *TCP3SRDX* transgenic ornamental flowers were reported, typical sympetalous flowers like *I. nil* have not yet been presented.

Key words: Chimeric repressor, *Ipomoea nil*, *Pharbitis nil*, sympetalous corolla, TCP

The Japanese morning glory (*Ipomoea nil*) is emerging as a model ornamental plant: many spontaneous mutants have been isolated and collected since the Edo period (approximately 200 years ago), libraries of expression sequence tags (ESTs) and bacterial artificial chromosomes have been created, genetic and molecular maps have subsequently been made available, transformation systems have been established, and whole-genome sequencing is in progress. These experimental results are available through the National BioResource Project (NBRP) in Japan (Yamazaki et al. 2010). The flowers of *I. nil* have a typical sympetalous corolla and possess several advantages for use as an ornamental flower, such as variation in color, color patterning, and corolla shape. Extensive research has revealed that most of the spontaneous mutations are due to transposon insertions. Most of the genes that encode the pigment biosynthetic enzymes and some genes known to cause morphological mutations have been isolated and characterized (Nitasaka 2003; Suzuki et al. 2003; Iida et al. 2004; Kitazawa et al. 2005; Iwasaki et al. 2006; Hoshino et al. 2009).

Chimeric REpressor gene-Silencing Technology (CRES-T) system is a powerful reverse genetic method that uses a transcriptional factor (TF) fused with an ethylene-responsive element-binding factor (ERF)-associated amphiphilic repression (EAR)-like motif repression domain (Ohta et al. 2001; Hiratsu et al. 2002, 2003). Several of the transgenic plants created using the CRES-T system exhibited recognizable phenotypes and among these, the alteration of flower shape is particularly well-suited to experimental purposes (Mitsuda et al. 2008; Narumi et al. 2008; Ohtsubo 2011). We used *A. thaliana* TFs for the CRES-T system because *A.*

Abbreviations: CRES-T, Chimeric REpressor gene-Silencing Technology; GM, genetically modified; NBRP, the National BioResource Project; NT, non-transformant; TCP, TEOSINTE BRANCHED1, CYCLOIDEA, and PCF; TF, transcriptional factor.
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Table 1. PCR primers used in this study

Gene	Sense primer	Antisense primer	Annealing temperature (°C)	Product length (bp)
PnAP3	5′-AGAGCAGTGAATTGAAGATAGACTCG-3′	5′-TTTACATACCCAAACCAGTATTATTTG-3′	55	951
CD	5′-GGGAAAAATGGGGAGAGGAAGGATTGAG-3′	5′-TAACTGATAATGATAATGGCACCATGCA-3′	55	721
DP	5′-AATTGATAGGTACAAGAAGGC-3′	5′-TAGTCGATTTCTGCAAACAGGAG-3′	57	238
PN	5′-CGATTGACAGGTACAAGAAACATC-3′	5′-GATACATGTTGGCATTTTGCAGTT-3′	57	196
PnUBQ	5′-CTCCATCTCGTGCTCCGTTTGA-3′	5′-GCCCTCCTTGTCCTGAATCTTG-3′	60	363
TCP3	5′-ATGGCACCAGATAACGACCATTTC-3′	5′-ATGGCAAGAATCGGATGAAGC-3′	60	1017
TCP5	5′-ATGAGATCAGGAGAATGTGATG-3′	5′-AGAATCCTGATTCATTATCGCTAC-3′	60	1095
*SRDX**		5′-GAGTTCTAGATCCAGATCGAGCCC-3′	—	—

* An antisense primer used with corresponding sense primer.

thaliana TFs are well studied compared with those of *I. nil*. In this study, we used two *TCP* (*TEOSINTE BRANCHED1*, *CYCLOIDEA*, and *PCF*) TFs, TCP3SRDX and TCP5SRDX, that had been fused with the repression domain. TCPs are an essential group of plant TFs that are responsible for the maintenance of undifferentiated fates in the shoot apical meristem and the promotion of cell differentiation in leaves (Luo et al. 1996; Cubas et al. 1999; Koyama et al. 2007, 2010). As a transgene, *TCP3SRDX* causes various kinds of morphological alterations, from the formation of ectopic shoots on cotyledons to various defects in organ development in *A. thaliana*. In particular, moderate effects of *TCP3SRDX* produced wavy and serrated rosette leaves and severely abnormal flowers (Koyama et al. 2007). Recently, the effects of *TCP3SRDX* on morphology were demonstrated in *Torenia fournieri*, *Chrysanthemum morifolium*, and *Rosa×hybrida* (Narumi et al. 2011; Gion et al. 2011). The resultant phenotypes were similar to those of *A. thaliana,* but were characteristic of each species. Koyama et al. (2011) reported that *TCP5SRDX* caused severely serrated margins of sepals and petals in transgenic *A. thaliana* plants. In *Cyclamen persicum*, *A. thaliana TCP3SRDX* and *TCP5SRDX* did not induce effective phenotypic changes. Tanaka et al. (2011) isolated a *TCP* gene, *CpTCP1*, clustered in the same group as *TCP3* from *Cyclamen persicum* and observed the phenotypic changes caused by *CpTCP1SRDX* in *Cyclamen persicum* and in *A. thaliana*. *CpTCP1SRDX* in *Cyclamen persicum* created curly and ruffled flowers with a high ornamental value (Tanaka et al. 2011). To date however, typical sympetalous flowers have not been used to observe the effects of *TCP3SRDX* and *TCP5SRDX*.

In this study, we generated transgenic *I. nil* plants harboring *TCP3SRDX* or *TCP5SRDX* as a transgene. As *I. nil* is a typical sympetalous flower, the effects of *TCP3SRDX* and *TCP5SRDX* on connate petals were demonstrated.

Materials and methods

Construction of plasmids for overexpression of chimeric repressor

Constitutive and overexpression constructs (pBCKK-35S-cDNA-RD) with *TCP* cDNAs of *A. thaliana* were used in all experiments (Mitsuda et al. 2006). The *Arabidopsis* Genome Initiative numbers of the two cDNAs used are as follows: AT1G53230 (*TCP3*) and AT5G60970 (*TCP5*). We used chimeric repressors, *TCP3SRDX* and *TCP5SRDX* previously reported (Koyama et al. 2007). All the constructs, detailed gene information, and the appearance of the transgenic flower obtained in this study are available on the web-based interface of the "FioreDB" database (http://www.cres-t.org/fiore/public_db/; Mitsuda et al. 2008, 2011).

Transformation of I. nil

All the constructs in binary vectors were used to transform the *A. tumefaciens* strain LBA4404 that was equipped with the constitutive *virG*N54D allele (van der Fits et al. 2000). The transgenic *Agrobacterium* preparations were then used to transform the secondary embryo culture of *I. nil* strain Violet as previously described (Kikuchi et al. 2005). As all the transformants were sterile, all the observations were made using the first generation transformants. In each case, at least three independent transformants were observed.

Reverse transcription polymerase chain reaction (RT-PCR)

To examine the expression levels of the transgenes and internal MADS-box genes, total RNAs were isolated from transformed and non-transformed (NT) plants using a Get pure RNA Kit (Dojindo, Kumamoto, Japan). First-strand cDNA was synthesized from 1 μg of each RNA sample in a 20-μl reaction solution using a SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. PCR was carried out under standard conditions using a thermal cycler (TP600, Takara Bio Inc., Ohtsu, Japan), with an initial denaturation step at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. The primers used in this study were designed using existing information from two homologs of *AGAMOUS* (*AG*), *DUPLICATED* (*DP*), and *PEONY* (*PN*) (Nitasaka et al. 2003), and homologs of *APETALLA3* (*AP3*) and *PISTILLATA* (*PI*), *P. nil APETALA3* (*PnAP3*) and *CONTORTED* (*CD*) (Nitasaka, unpublished), from an EST library of *I. nil* cv. Tokyo Kokei Standard established by the NBRP for Japanese morning glory (http://www.nbrp.jp/) and from the web-based interface of the "FioreDB" database. A *P. nil UBIQUITIN* gene (*PnUBQ*) was used as an internal standard. The primer sequences, annealing temperatures, product length, and number of amplification cycles are listed in Table 1. The PCR products were separated on 1.5% agarose gels.

Scanning electron microscopy

The surfaces of fresh leaves were directly observed using scanning electron microscopy (SEM: VE-7000, Keyence Co., Osaka, Japan) without fixing.

Results and discussion

Generation of transgenic I. nil expressing TCP3SRDX and TCP5SRDX

We introduced *TCP3SRDX* or *TCP5SRDX* overexpressed by the CaMV35S promoter (Figure 1A) into *I. nil* using transformation with *Agrobacterium*. In all transgenic plants, transcripts of *TCP3SRDX* or *TCP5SRDX* were detectable by RT-PCR. Results of three independent transformants are presented in Figures 1B and 1C. We observed phenotypic variation among transgenic lines, but severe phenotypes were not always correlated with higher expression levels of the transgenes (data not shown). It has been reported that when using the CRES-T system, differences in expression level of SRDX fused cDNA do not reflect the severity of the phenotype (Narumi et al. 2011; Sage-Ono et al. 2011), and we noticed a similar effect in *TCP3SRDX* and *TCP5SRDX* transformants.

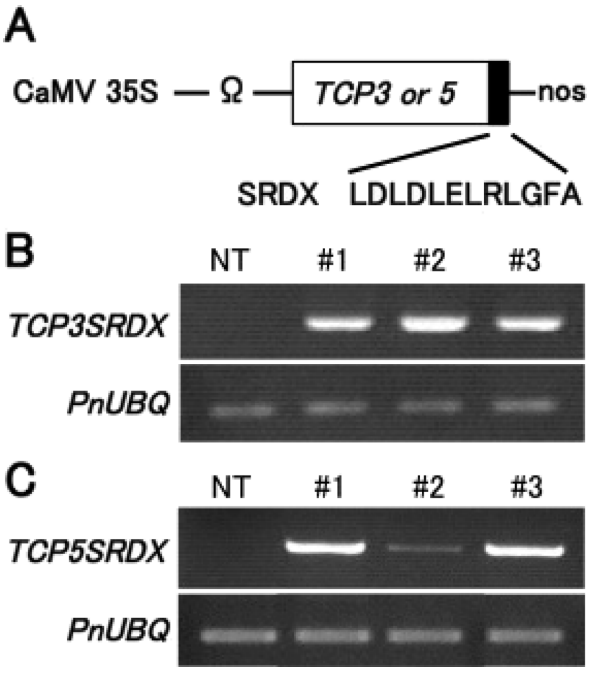

Figure 1. Detection of *TCP3SRDX* and *TCP5SRDX* mRNA in transgenic *I. nil* plants. (A) Schematic representation of the transgene. CaMV 35S, Ω, *TCP3* or *5*, SRDX, and nos represent the CaMV 35S promoter, the translational enhancer from the *Tobacco mosaic virus*, the cDNA of *TCP3* or *TCP5*, the repression domain (12 amino acids long), and the terminator sequence of the *NOS* gene, respectively. (B) and (C) Transcript levels of *TCP3SRDX* and *TCP5SRDX* in transgenic plants. RT-PCR analysis was performed to examine the expression levels of *TCP3SRDX* and *TCP5SRDX*. PCR products were electrophoresed in agarose gels and ethidium bromide-stained bands were photographed under ultraviolet light. *PnUBQ* was used as an internal control. NT: non-transformed plant. The numerals after the # symbols indicate the line number of the independent transformants.

Morphological changes in vegetative organs of TCP3SRDX and TCP5SRDX transformants

TCP3SRDX leaves showed deeper indentations with narrow lobes compared with NT leaves (Figure 2A, 2F). Overall, leaf morphology was somewhat similar to that observed in the dominant mutation co^H (*cordate-Hederacea-leaf*) originally introgressed from *Ipomoea hederacea* to *I. nil* (Nitasaka, unpublished results); however, development of leaf lobes was more strongly suppressed in *TCP3SRDX* transformants. We performed SEM on epidermal cells. Although the differences in the shapes of cells were subtle, the epidermal cells on the adaxial surface were relatively smaller in *TCP3SRDX* plants than those in NT plants (Figure 2G,

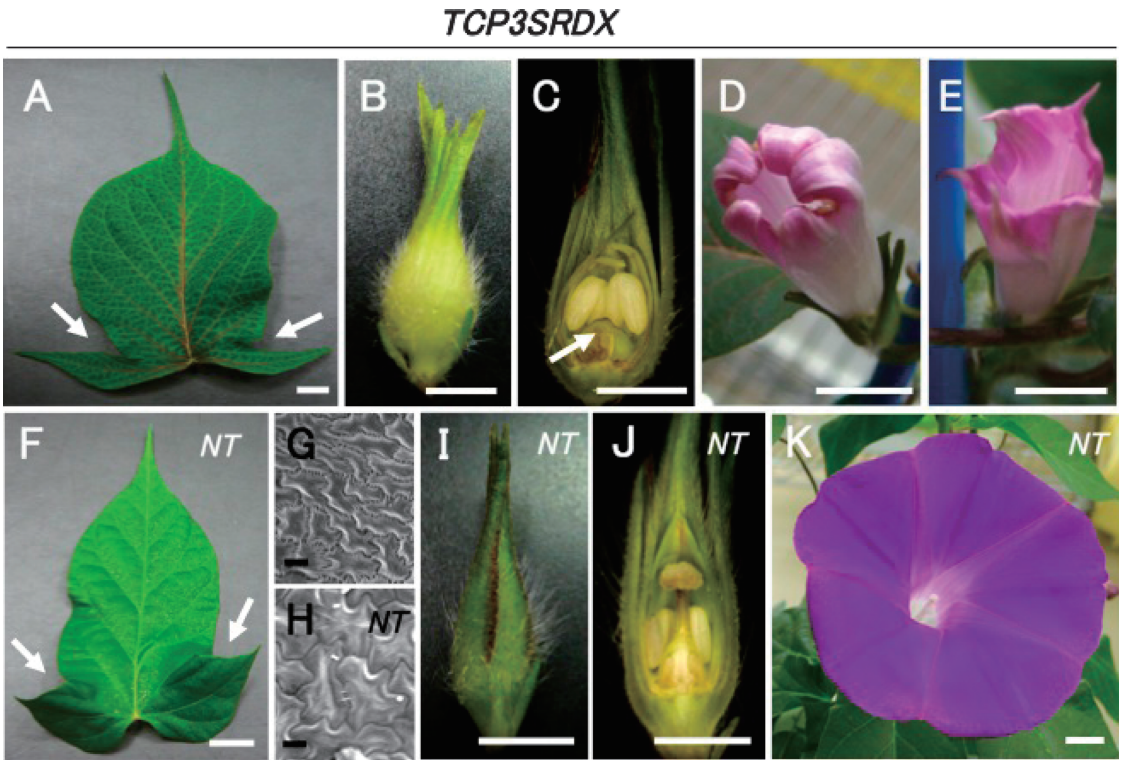

Figure 2. Morphological changes induced by *TCP3SRDX*. Various organs of *TCP3SRDX* transformants (A, B, C, D, E, G) were compared with those of NT plants (F, H, I, J, K). A fully expanded *TCP3SRDX* leaf (A) and an NT leaf of similar stage (F). The arrows indicate the differences in the depth of indentations. Leaf epidermal cells of *TCP3SRDX* leaf (G) were compared with those of NT leaf (H). An undeveloped flower bud of *TCP3SRDX* transformant (B) was compared with an NT bud at a similar developmental stage (I). A vertical section of a *TCP3SRDX* flower bud (C) was compared with that of an NT plant (J). The arrow in (C) indicates the lack of pistil in the *TCP3SRDX* flower bud. Unexpanded funnel (D) and no funnel (E) of corolla in *TCP3SRDX* flowers compared with an NT flower (K). White scale bars represent 1 cm, whereas the black scale bars in (G) and (H) represent 50 μm.

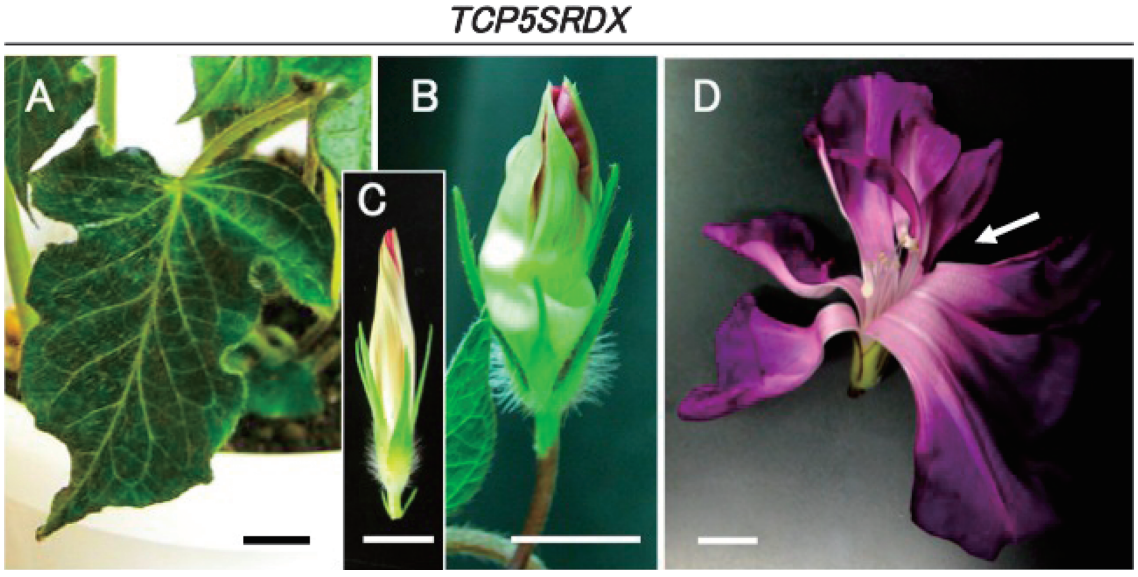

Figure 3. Morphological changes induced by *TCP5SRDX*. A leaf (A), a flower bud (B), and a fully opened flower (D) are presented. A flower bud of an NT plant (C) at an equivalent stage of (B) is presented for comparison. The arrow in (D) indicates a petaloid stamen. All scale bars represent 1 cm.

2H), suggesting that these cells remained undeveloped. Undeveloped cells caused by *TCP3SRDX* were reported in *A. thaliana*, *Chrysanthemum morifolium*, and *Cyclamen persicum* (Koyama et al. 2007; Narumi et al. 2011; Tanaka et al. 2011). On the contrary, *TCP5SRDX* leaves showed a wavy margin phenotype (Figure 3A).

Wavy margin phenotypes were relatively common to *TCP3SRDX* in *A. thaliana*, *Torenia fournieri*, *Chrysanthemum morifolium*, *Cyclamen persicum*, and *Rosa×hybrida* (Koyama et al. 2007, 2011; Narumi et al. 2011; Tanaka et al. 2011; Gion et al. 2011). However, we also observed epidermal cells of *TCP5SRDX* leaves using SEM and found no difference compared with NT epidermal cells (data not shown).

Morphological changes in reproductive organs of TCP3SRDX and TCP5SRDX transformants

In plants transformed with *TCP3SRDX*, flower buds showed an immature round shape compared with NT (Figure 2B, 2I). Vertical sections of flower buds showed a lack of pistil and morphologically normal stamens in *TCP3SRDX* transformants, in contrast with those in NT plants (Figure 2C, 2J). The flower buds of *TCP5SRDX* transformants showed a wavy margin phenotype similar to their leaves, in contrast to the smooth outline of NT flower buds (Figure 3B, 3C). These modifications in flower buds resulted in changes in flower morphology. *TCP3SRDX* flowers exhibited divided and unexpanded petals (Figure 2D) or loss of the corolla funnel (Figure 2E), compared with flowers of NT plants (Figure 2K). In contrast, *TCP5SRDX* flowers showed a divided corolla with wavy petals (Figure 3D).

The funnel-shaped corolla of *I. nil* is composed of a tube and five connate petals. *I. nil* shows "late sympetaly" and the corolla is initiated as five separate primordia and the fused portion appears later. The corolla tube is initiated by cooperation of interprimordial growth and marginal growth of the petal primordia. The corolla funnel is formed during later development of the interprimordial region of the petals (Nishino, 1976). In *TCP3SRDX* flowers, the development of five connate petals was suppressed, but morphologically normal corolla tubes were formed. *TCP3SRDX* specifically suppressed the later development of the interprimordial region of the petal primordia and of the petal primordia themselves, whereas in *TCP5SRDX* flowers, the well-developed wavy corolla was choripetalous and the tube of the corolla was frequently separated to the base. *TCP5SRDX* did not suppress cell division and growth during flower development, but may have disrupted synchronous growth. In *TCP3SRDX* and *TCP5SRDX*, the sympetalous corolla was easily disrupted to form choripetalous corolla.

Using a *I. nil maple-willow* (m^w) mutant showing a choripetalous flower, Kajita and Nishino (2009) studied the morphological changes in the funnel-shaped sympetalous corolla necessary to produce almost choripetalous corolla. They showed that m^w caused pleiotropic morphological phenotypes of leaf and floral organs by reducing their lateral growth and fusing regions in the calyx, corolla, and gynoecium. However, m^w does not affect formation of the lower corolla tube. Despite differences in overall morphological changes, *TCP3SRDX* transformants partially resembled the m^w mutant phenotype, especially in the undeveloped petals and the normal corolla tube. The gene responsible for the m^w mutant *MAPLE* may work with or communicate with *TCP* genes.

Comparison of TCP3SRDX and TCP5SRDX transformants in other plants

Compared with the *TCP3SRDX*-transformed phenotypes observed in other plant species (Koyama et al. 2007, 2011; Narumi et al. 2011; Tanaka et al. 2011; Gion et al. 2011), our results were rather mild. Our transformants grew normally, but the regeneration efficiencies of *TCP3SRDX* and *TCP5SRDX* were relatively lower than those reported for other genes. Because our transformation system for *I. nil* utilized secondary embryo formation from immature embryos (Kikuchi et al. 2005), the transformed cells with more severe phenotypes could not regenerate. Although moderate phenotypes were observed in comparison with those observed in other plant species, all the *TCP3SRDX* and *TCP5SRDX* transformants were sterile. In *A. thaliana*, more severely transformed *TCP3SRDX* and *TCP5SRDX* flowers can produce vital seeds (Koyama et al. 2007, 2011), suggesting that the fertility of *I. nil* is affected by *TCP3SRDX* and *TCP5SRDX*.

In *I. nil*, a strong wavy margin phenotype was observed not in *TCP3SRDX* transformants but in *TCP5SRDX* transformants. However, strong wavy margin phenotypes were observed in *TCP3SRDX* transformants in *A. thaliana*, *Torenia fournieri*, *Chrysanthemum morifolium*, *Cyclamen persicum*, and *Rosa×hybrida* (Koyama et al. 2007, 2011; Narumi et al. 2011; Tanaka et al. 2011; Gion et al. 2011). Moreover, in *TCP3SRDX* transformants in *I. nil*, petal growth was strongly suppressed and even inhibited. Similar growth suppressions were more evident in *TCP5SRDX* transformants than in *TCP3SRDX* transformants in *A. thaliana* (Koyama et al. 2011). *TCP3* and *TCP5* belong to a same clade of the *TCP* gene family and the phenotypes of their mutations are similar but there are a few different points in details in *A. thaliana* (Koyama et al. 2007, 2010, 2011). The reason for these differences between *I. nil* and other plants is not clear, but they could be caused by unique interactions between genes in the different species used. Further studies should pursue the isolation of internal *TCP* genes and analysis of the specific mutants of each *TCP* gene in *I. nil*.

In *A. thaliana*, 24 TCPs were identified and eight of them including TCP3 and TCP5 were distinguished as CIN-type. CIN-type TCPs have redundant molecular functions to regulate margin development (Koyama et al. 2007). We searched the EST library of *I. nil* (http://

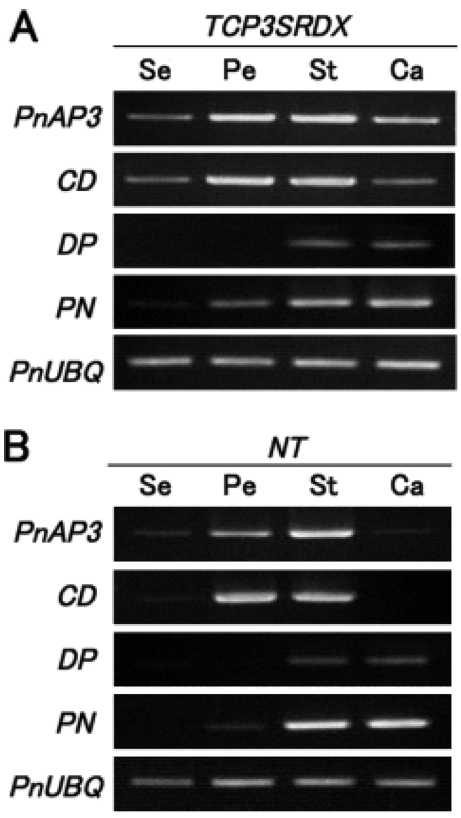

Figure 4. Changes in mRNA level of internal MADS-box genes in *TCP3SRDX* flower buds. Expression of *P. nil APETALA3* (*PnAP3*), *CONTORTED* (*CD*), *DUPLICATED* (*DP*), and *PEONY* (*PN*) in flower buds of *TCP3SRDX* (A) compared with that of NT plants (B). RT-PCR was performed as described in Figure 1. Total RNA was extracted from floral organs (Se: sepal, Pe: petal, St: stamen, Ca: carpel). *PnUBQ* was used as an internal control.

ipomoeanil.nibb.ac.jp, Hoshino unpublished) by BLAST analysis (Altschul et al. 1997). There were at least 4 incomplete contigs containing 12 ESTs, and they showed limited homology to TCP3 and TCP5. These data suggested that *I. nil* genome has some homologous genes to *TCP3* and *TCP5*. In a near future, the whole-genome sequencing in *I. nil* will show all the *CIN*-type *TCP* family members. Then, we will be able to make multiple mutants of *CIN*-type *TCPs* and obtain similar morphological phenotypes to *TCP-SRDX*.

Changes in MADS-box expression in TCP3SRDX transformants

To elucidate the cause of the morphological changes in the *TCP3SRDX* and *TCP5SRDX* flowers, we examined the expression of the MADS-box type floral homoeotic genes, *PnAP3*, *CD*, *DP*, and *PN* in mature flower buds using RT-PCR. The results for *TCP3SRDX* are presented in Figure 4A. *PnAP3* and *CD* are two of the four class B homoeotic genes in *I. nil* (Nitasaka unpublished), whereas *DP* and *PN* are the two class C homoeotic genes in this species (Nitasaka, 2003). In *A. thaliana*, defects in these genes have been extensively demonstrated to result in phenotypes with differences in determination of floral organ identity (Bowman et al. 1989; Yanofsky et al. 1990; Coen and Meyerowitz 1991; Immink et al. 2010). In the *TCP3SRDX* flowers, expression of class C genes was unchanged, but class B genes were likely to be expressed ubiquitously among the four floral organs compared with NT flowers (Figure 4A, 4B). In *TCP3SRDX* flowers, these changes in expression pattern may cause undeveloped petals, lack of stigma, and pollen sterility. Unfortunately, reports on *TCP3SRDX* flowers in other plants have not analyzed expression of MADS-box genes, and these changes need to be examined in other plant systems. On the contrary, *TCP5SRDX* transformants sometimes bore petaloid stamens, but we could not identify any difference in expression of the MADS-box genes in the four floral organs compared with those of NT (data not shown). Studies on the target genes of *TCP* genes, including their candidate MADS-box genes, will be important for understanding the molecular function of TCP TFs and also for artificial modification of flower architecture.

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402

Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in *Arabidopsis*. *Plant Cell* 1: 37–52

Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353: 31–37

Cubas P, Lauter N, Doebley J, Coen E (1999) The TCP domain: a motif found in proteins regulating plant growth and development. *Plant J* 18: 215–222

Gion K, Suzuri R, Shikata M, Mitsuda N, Oshia Y, Koyama T, Ohme-Takagi M, Ohtsubo N, Tanaka Y (2011) Morphological change of *Rosa×hybrida* by a chimeric repressor of *Arabidopsis* TCP3. *Plant Biotechnol* 28: 149–152

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

Hoshino A, Park KI, Iida S (2009) Identification of r mutations conferring white flowers in the Japanese morning glory (*Ipomoea nil*). *J Plant Res* 122: 215–222

Iida S, Morita Y, Choi JD, Park KI, Hoshino A (2004) Genetics and epigenetics in flower pigmentation associated with transposable elements in morning glories. *Adv Biophys* 38: 141–159

Immink RG, Kaufmann K, Angenent GC (2010) The 'ABC' of MADS domain protein behaviour and interactions. *Semin Cell Dev Biol* 21: 87–93

Iwasaki M, Nitasaka E (2006) The *FEATHERED* gene is required for polarity establishment in lateral organs especially flowers of the Japanese morning glory (*Ipomoea nil*). *Plant Mol Biol* 62: 913–925

Kajita Y, Nishino E (2009) Development of leaves and flowers in the wild type and pleiotopic *maple-willow* mutant of Japanese Morning Glory (*Ipomoea nil*). *J Japan Soc Hort Sci* 78: 4690477

Kitazawa D, Hatakeda Y, Kamada M, Fujii N, Miyazawa Y, Hoshino A, Iida S, Fukaki H, Morita MT, Tasaka M, Suge H, Takahashi H (2005) Shoot circumnutation and winding movements require gravisensing cells. *Proc Natl Acad Sci USA* 102: 18742–18747

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

Koyama T, Ohme-Takagi M, Sato F (2011) Generation of serrated and wavy petals by inhibition of the activity of TCP transcription factors in *Arabidopsis thaliana*. *Plant Signal Behav* 6: 697–699

Kikuchi R, Sage-Ono K, Kamada H, Ono M (2005) Efficient transformation mediated by *Agrobacterium tumefaciens* with a ternary plasmid in *Pharbitis nil*. *Plant Biotechnol* 22: 295–302

Luo D, Carpenter R, Vincent C, Copsey L, Coen E (1996) Origin of floral asymmetry in *Antirrhinum*. *Nature* 383: 794–799

Mitsuda N, Hiratsu K, Todaka D, Nakashima K, Yamaguchi-Shinozaki K, Ohme-Takagi M (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, Umemura Y, Ikeda M, Shikata M, Koyama T, Matsui K, Narumi T, Aida R, Sasaki K, Hiyama S, Higuchi Y, Ono M, Isuzugawa K, Saitoh K, Endo R, Ikeda K, Nakatsuka T, Nishihara M, Yamamura S, Yamamura T, Terakawa T, Ohtsubo N, Ohme-Takagi M (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, Takiguchi Y, Shikata M, Sage-Ono K, Ono M, Sasaki K, Yamaguchi H, Narumi T, Tanaka Y, Sugiyama M, Yamamura T, Terakawa T, Gion K, Suzuri R, Tanaka Y, Nakatsuka T, Kimura S, Nishihara M, Sakai T, Endo-Onodera R, Saitoh K, Isuzugawa K, Oshima Y, Koyama T, Ikeda M, Narukawa M, Matsui K, Nakata M, Ohtsubo N, Ohme-Takagi M (2011) 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

Narumi T, Aida R, Niki T, Nishijima T, Mitsuda N, Hiratsu K, Ohme-Takagi M, Ohtsubo N (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, Aida R, Koyama T, Yamaguchi H, Sasaki K, Shikata M, Nakayama M, Ohme-Takagi M, Ohtsubo N (2011) *Arabidopsis* chimeric TCP3 repressor produces novel floral traits in *Torenia fournieri* and *Chrysanthemum morifolium*. *Plant Biotechnol* 28: 131–140

Nitasaka E (2003) Insertion of an En/Spm-related transposable element into a floral homeotic gene *DUPLICATED* causes a double flower phenotype in the Japanese morning glory. *Plant J* 36: 522–531

Nishino E (1976) Developmental anatomy of foliage leaves, bracts, calyx and corolla in *Pharbitis nil*. *Bot Mag Tokyo* 89: 191–209

Ohtsubo N (2011) Beyond the blue rose: Modification of floral architecture with plant-specific chimeric repressors. *Plant Biotechnol* 28: 113–121

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

Sage-Ono K, Ozeki Y, Hiyama S, Higuchi Y, Kamada H, Mitsuda N, Ohme-Takagi M, Ono M (2011) Induction of double flowers in *Pharbitis nil* using a class-C MADS-box transcription factor with Chimeric REpressor gene-Silencing Technology. *Plant Biotechnol* 26: 153–165

Suzuki Y, Saso K, Fujioka S, Yoshida S, Nitasaka E, Nagata S, Nagasawa H, Takatsuto S, Yamaguchi I (2003) A dwarf mutant strain of *Pharbitis nil*, Uzukobito (*kobito*), has defective brassinosteroid biosynthesis. *Plant J* 36: 401–410

Tanaka Y, Yamamura T, Oshima Y, Mitsuda N, Koyama T, Ohme-Takagi M, Terakawa T (2011) Creating ruffled flower petals in *Cyclamen persicum* by expression of the chimeric cyclamen TCP repressor. *Plant Biotechnol* 28: 141–147

Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldmann KA, Meyerowitz EM (1990) The protein encoded by the *Arabidopsis* homeotic gene agamous resembles transcription factors. *Nature* 346: 35–39

Yamazaki Y, Akashi R, Banno Y, Endo T, Ezura H, Fukami-Kobayashi K, Inaba K, Isa T, Kamei K, Kasai F, Kobayashi M, Kurata N, Kusaba M, Matuzawa T, Mitani S, Nakamura T, Nakamura Y, Nakatsuji N, Naruse K, Niki H, Nitasaka E, Obata Y, Okamoto H, Okuma M, Sato K, Serikawa T, Shiroishi T, Sugawara H, Urushibara H, Yamamoto M, Yaoita Y, Yoshiki A, Kohara Y (2010) NBRP databases: databases of biological resources in Japan. *Nucleic Acids Res* 38(Database issue): D26–D32

van der Fits L, Deakin EA, Hoge JH, Memelink J (2000) The ternary transformation system: constitutive *virG* on a compatible plasmid dramatically increases *Agrobacterium*-mediated plant transformation. *Plant Mol Biol* 43: 495–502