

## Creating ruffled flower petals in *Cyclamen persicum* by expression of the chimeric cyclamen TCP repressor

Yuri Tanaka<sup>1</sup>, Tomomichi Yamamura<sup>1</sup>, Yoshimi Oshima<sup>2</sup>, Nobutaka Mitsuda<sup>2</sup>, Tomotsugu Koyama<sup>3</sup>, Masaru Ohme-Takagi<sup>2</sup>, Teruhiko Terakawa<sup>1,\*</sup>

<sup>1</sup> Central Research Laboratories, Hokko Chemical Industry Co., Ltd., Atsugi, Kanagawa 243-0023, Japan;

<sup>2</sup> Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8562, Japan; <sup>3</sup> Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan

\* E-mail: terakawa-t@hokkochem.co.jp Tel: +81-46-228-5881 Fax: +81-46-228-0164

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**Abstract** *Cyclamen persicum* is among the most popular pot plants in the world. Creating new ruffled flower petals is an important breeding target in this plant. TCP transcription factors are involved in defining plant morphology in angiosperms. To modify flower shape in cyclamen, we isolated a *TCP* gene from cyclamen (*CpTCP1*), which is clustered in the same group as *TCP3* (*AtTCP3*) and *TCP4* (*AtTCP4*) in Arabidopsis and *CINCINNATA* (*CIN*) in *Antirrhinum majus*. A chimeric repressor gene construct for *CpTCP1* driven by the cauliflower mosaic virus (CaMV) 35S promoter (*35S:CpTCP1SRDX*) was transformed into Arabidopsis to confirm whether *CpTCP1* has a function similar to that of *AtTCP3* in Arabidopsis. The phenotypic changes resembled those of the chimeric repressor derived from *AtTCP3*. The *35S:CpTCP1SRDX* was introduced into cyclamen cv. Wink Pink. Finally, we produced and analyzed 106 independent transgenic plants. *CpTCP1SRDX* expression resulted in cyclamen with serrated wavy leaves and curled petals. Scanning electron microscopy showed that cells in petals and leaf margins of *35S:CpTCP1SRDX* plants were smaller than those of controls, and these cells also appeared immature. These results suggest that cyclamen *CpTCP1* possibly plays a role in regulating morphogenesis in floral and vegetative organs. We demonstrated that *CpTCP1SRDX* expression in cyclamen resulted in curly and ruffled flowers with a high ornamental value.

**Key words:** *Cyclamen persicum*, ruffled flower petals, TCP transcription factor.

Perennial plants in the family *Primulaceae*, genus *Cyclamen*, are among the most popular and important pot plants in the world. For many years, floral traits of *Cyclamen persicum* Mill. have been altered using cross-hybridization and mutation breeding techniques to produce new varieties. Flower shape is an important characteristic in ornamental plants. For example, creation of new ruffled flower petals in cyclamen is an important breeding target. However, these approaches are time consuming and require advanced technical skills since this plant shows a complicated pattern of inheritance because of its high chromosome number (2n=48 or more in some cultivars, Grey-Wilson 2002) and outbreeding characteristics. Recently, mutations caused by ion beam irradiation created a new phenotype in these cyclamen plants (Kondo et al. 2009; Sugiyama et al. 2008). However, no great change has been reported in flower shape.

Genetic transformation is a useful tool for breeding. This technique allows to obtain the intended phenotype more directly, and to create a new one, which cannot be achieved by mutation breeding or cross-hybridization. Several reports exist on genetic transformation in cyclamen. Transgenic *C. persicum* has been produced using *Agrobacterium tumefaciens*, and etiolated petioles of aseptic seedlings have been used as targets for transformation (Aida et al. 1999; Boase et al. 2002). We previously reported the development of an efficient method for plant regeneration using somatic embryos and *Agrobacterium*-mediated genetic transformation (Terakawa et al. 2008).

TCP is a transcription factor derived from 3 founding members of this family, *TEOSINTE BRANCHED1* (*TB1*) of maize, *CYCLOIDEA* (*CYC*) of *Antirrhinum majus*, and the *PROLIFERATING CELL FACTOR* (*PCF*) of rice; all of which have been proven to play roles in

various aspects of development (Cubas et al. 1999; Doebley et al. 1997; Kosugi and Ohashi 1997; Luo et al. 1996). The TCP transcription factor family plays an important role in the evolution of key morphological characters in angiosperms. *Gerbera GhCYC2* regulates growth, and its overexpression in transgenic gerbera results in slower vegetative growth and reduced inflorescence size (Broholm et al. 2008). This family has been proven to act redundantly to control morphogenesis of shoot lateral organs and meristem growth by affecting cell proliferation in *Arabidopsis* (Koyama et al. 2007, 2010). In *A. majus*, *cinninata* (*CIN*) mutants, which are identified through inactivation of a *TCP* gene, have leaves with a crinkly lamina and flowers with a modified petal lobe shape and color (Crawford et al. 2004; Nath et al. 2003). A *CIN*-like subset of *TCP* harbors a microRNA319 (*miR319*)-target site. Leaf and floral organ morphogenesis is controlled by *miR319*-mediated degradation of *TCP* mRNAs (Nag et al. 2009; Palatnik et al. 2003). Determining whether the target sequence of microRNA is conserved is an important point in understanding the function of TCP family proteins (Martin-Trillo and Cubas 2010).

Expression of *TCP3SRDX* (*AtTCP3SRDX*), a chimeric repressor derived from *TCP3* (*AtTCP3*), in *Arabidopsis thaliana* resulted in formation of ectopic shoots on cotyledons and wavy and serrated organs (Koyama et al. 2007). *AtTCP3SRDX* construct consists of the coding region of *AtTCP3* and a modified EAR-like motif repression domain sequence called SRDX (Hiratsu et al. 2003). This repression domain-dependent gene silencing procedure is called Chimeric Repressor Gene-Silencing Technology (CRES-T). The resulting product dominantly suppresses target gene expression to confer loss-of-function phenotypes at high frequency, even in the presence of functionally redundant transcriptional activators (Hiratsu et al. 2003; Matsui et al. 2004, 2005; Mitsuda et al. 2005, 2006, 2007; Narumi et al. 2008).

In this study, we report the isolation of a *TCP* transcription factor gene from cyclamen and the modification of flower shape in cyclamen using the chimeric repressor from cyclamen *TCP*.

## Materials and methods

### Plant materials

*C. persicum* cvs. Fragrance Mini (FM) and Wink Pink (WP) were used in this study. FM has a wild-type flower shape, while WP is a homeotic double-flowered mutant (Grey-Wilson 2002). To generate transgenic plants, we employed cyclamen WP in our experiment. *In vitro* cyclamen plants were maintained under sterile conditions in a plant box.

### Isolation of the cyclamen *TCP* transcription factor

Total RNA was isolated from floral buds of cyclamen FM using the Plant RNA Reagent Kit (Invitrogen/Life Technologies,

Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was subjected to cDNA synthesis using the SMART PCR cDNA Synthesis Kit (Clontech/Takara Bio Inc., Shiga, Japan). An adapter primer (AP 5'-GGCCAGGCGTCGACTAGTAC-TTTTTTTTTTTTTTTT-3') was used instead of the 3'-end primer provided in the 3'-RACE System for Rapid Amplification of cDNA Ends Kit (Invitrogen/Life Technologies). To amplify the cyclamen *TCP* gene, RACE PCR was performed using degenerate primers. The reverse degenerate primer (first primer, 5'-TGTGTTAMTGGACTGAAGGGGWCC-3') and 5' PCR primer II A (5'-AAGCAGTGGTATCAACGCAGAGT-3', Clontech/Takara Bio Inc.) were used for 5'-RACE PCR. Forward degenerate (first primer, 5'-AGACMGMCAYAGYAA-RGTWTGYACWG-3' and nested primer, 5'-ATYCARTTYT-ACGAYGTKCAAGA-3') and AUAP (5'-GGCCAGGCGTCG-ACTAGTAC-3', Invitrogen/Life Technologies) primers were used for nested 3'-RACE PCR. These reactions were run for 35 cycles with denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 2 min using Takara LA Taq Hot Start Version (Takara Bio Inc., Shiga, Japan). PCR amplification with specific primers (forward primer, 5'-GGATAAATCCGACCCCAATCGACCCGA-3' and reverse primer, 5'-AGACTACAATTCATGCCCGGAGAGC-3') was performed to confirm the *CpTCP1* sequence. After an initial denaturation for 2 min at 94°C, PCR was run for 30 cycles with denaturation at 94°C for 30 s, annealing at 52°C for 1 min, and extension at 68°C for 2 min using KOD-Plus ver. 2.0 (Toyobo, Osaka, Japan). All PCR products were run on 0.8% agarose gel. Appropriate bands were excised from the gel and cloned into the pT7Blue vector (Novagen/Merck, Darmstadt, Germany). Sequencing was performed using DNA sequencing services from Fasmac Co. Ltd., and amino acid sequences were analyzed using the GENETYX ver. 9.0 software (Genetyx, Tokyo, Japan). Deduced amino acid sequences were aligned using ClustalW (Thompson et al. 1994).

### Expression analysis of endogenous *CpTCP1*

Various tissues from sepals, petals, stamens, carpels, receptacles, flower stalks, leaves, and leaf stems of cyclamen FM were sampled. Total RNAs were isolated as described above. Each total RNA sample was treated with DNase using the Turbo DNA-free kit (Ambion/Life Technologies, Carlsbad, CA, USA) and reverse transcribed into cDNA using the oligo(dT)<sub>20</sub> primer and ReverTra Ace- $\alpha$  (Toyobo). These cDNAs were subjected to reverse transcription (RT)-PCR using KOD-Plus ver. 2.0. The *CpTCP1* gene was amplified using forward (5'-GAATGGAGAAGCTCTGACGCC-3') and reverse (5'-TGTGTTACTGGACTGAAGGGGTCC-3') primers. A fragment of the actin gene was amplified from the same cDNA as a standard control to normalize the cDNA amount used in RT-PCR. The following actin gene primers were used: forward (5'-CCTAGGCACACTGGTGTCATGGTT-3') and reverse (5'-GCAGTGATCTCCTTGCTCATTCCGGT-3'). After an initial denaturation at 94°C for 2 min, RT-PCR was run for 30 cycles (for *CpTCP1*) or 24 cycles (for actin) of amplification, with denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 68°C for 1 min.

### Transformation of cyclamen and *Arabidopsis*

The full-length coding region of cyclamen *CpTCP1* cDNA

## A

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CpTCP1 1 MGEALTPHR-QPAPSRLLGMR-----APAAGAGEIVEVHGHHIVRSTGRKDRHSKVCTAK
AtTCP3 1 MAPDNDFLDSESPPLLEMRHHQSATENGGGCGEIVEVQGGHIVRSTGRKDRHSKVCTAK

CpTCP1 53 GPRDRRVRLSAHTAIQFYDVQDRLCYDRPSKAVDWLTKKAQAIDELELPPWNEAAVQ
AtTCP3 61 GPRDRRVRLSAPTAIQFYDVQDRLCFDRPSKAVDWLTKAKSAIDDLAQLPPWNEADTLR

CpTCP1 113 EERESGSIQENLGHEIGASS-----NNYSNSQQN-PSFLPPSMDSDSIADTIKSF
AtTCP3 121 QHAAAAAAKPRKTKTLISPPPPQPEETEHRIGEEEDNESFLPASMDSDSIADTIKSF

CpTCP1 163 FFMGAAVEPNAAVQFPELISRANSHSQDLW-LSLHHHHAPE----SSASGHS--DST
AtTCP3 181 FQVASTQQS-----YHQPPSRGNNTQNQDLLRLSLQSFQNGPFFPNQTEPALFSGQSNN

CpTCP1 216 LIGFEHGSSGWPDPFP-----RMGGWNPGGDSATGLYNLQMAAF-----LLFGNQ
AtTCP3 235 QLAFDSSSTASWEQSHQSPEFGKIQRLVSWNNVGAESA GSTGGVFVAFSESSLHPVYSQSQ

CpTCP1 263 FASQRGPLQSSNTQSIRAWIDP-----VAAPMAYAGADHHSQFPVH---HPTMFQGM DL
AtTCP3 295 LLSQRGPLQSSINTPMIRAWFDPHHHHHHHQSSMTTDDLHHHHPYHIPPGI HQSAIPGIAF
.....

CpTCP1 314 SS---FSGFRIPAWIQGEFEHHDGISDKPSSASSDSL SRH
AtTCP3 355 ASSGGEFSGFRIPARFQGEQEEHGSD-NKPSASSD--SRH

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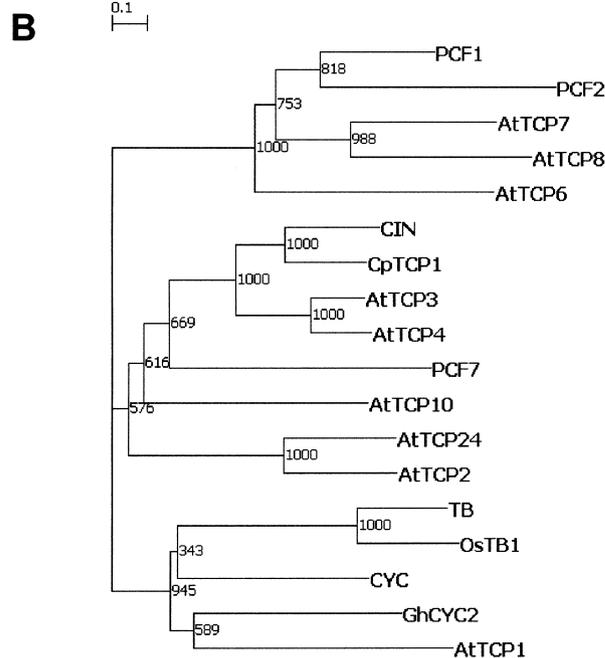


Figure 1. Comparison of the amino acid sequences. (A) Alignment of primary structures of CpTCP1 and AtTCP3. White letters on the black background indicate residues identical to the sequences. The TCP domain is underlined. The region encoded by the microRNA target sequence is dotted-underlined. (B) Phylogenetic analysis with full-length amino acid sequences of the TCP family transcription factor. The neighbor-joining tree was constructed using the GENETYX ver. 9.0 software. Bootstrap values are denoted at nodes. Bar=0.1 amino acid substitution/site. Accession numbers: *Arabidopsis thaliana* (AtTCP1, NP\_001154454; AtTCP2, NP\_567553; AtTCP3, NP\_564624; AtTCP4, NP\_188121; AtTCP6, NP\_198919; AtTCP7, NP\_197719; AtTCP8, NP\_176107; AtTCP10, NP\_565712; and AtTCP24, NP\_849730), *Antirrhinum majus* (CYC, CAA76176 and CIN, AAO43102), *Gerbera hybrida* (GhCYC2, ACC54347), *Zea mays* (TB, AAL66761), and *Oryza sativa* (PCF7, Q8LT05; OsTB1, NP\_001051028; PCF1, NP\_001052212; and PCF2, NP\_001062409).

was cloned and used for transformation. Chimeric 35S: *CpTCP1SRDX* was constructed as described previously (Mitsuda *et al.* 2006). Etiolated petioles were used as transformation materials. Leaf segments were placed onto 1/3 Murashige and Skoog medium (Murashige and Skoog 1962) supplemented with  $0.1 \text{ mg l}^{-1}$   $\alpha$ -naphthaleneacetic acid,  $5 \text{ mg l}^{-1}$  benzylaminopurine, 3% (w/v) sucrose (pH 5.8), and 0.2% gellan gum (Wako Pure Chemical Industries, Osaka,

Japan) and were cultured in the dark at  $25^\circ\text{C}$ . Etiolated petioles were induced after 3 months. Transgenic plants were obtained by modified *Agrobacterium*-mediated transformation as described previously (Aida *et al.* 1999). For flowering, transgenic plants were grown in a closed, special-netted greenhouse. WP plants, used as controls, were grown in a greenhouse.

*A. thaliana* Col-0 were maintained and transformed as described previously (Mitsuda *et al.* 2006).

### Scanning electron microscopy (SEM) analysis

Fresh samples of leaves and petals at the flowering stage were collected from the transgenic *35S:CpTCP1SRDX* plant and the non-transgenic WP plant, and were observed using a scanning electron microscope (Keyence model VE-8800 real 3D system) at an accelerating voltage of 2 kV.

## Results and discussion

### Isolation of the cyclamen TCP homolog and phylogenetic analysis

Our preliminary studies using chimeric repressors of Arabidopsis *TCP3* and *TCP5* could not induce effective phenotypic changes in young cyclamen plants, therefore we attempted to isolate the native *TCP* gene from cyclamen (data not shown). The *TCP* homolog gene in cyclamen was isolated by RACE PCR using degenerate primers. 5'-RACE and nested 3'-RACE PCR products were cloned and sequenced. After assembly of overlapping fragments to obtain the full-length cDNA, PCR amplification was performed with gene-specific primers to confirm the sequence. Cloned cDNA was 1,632 bp in length and contained an open reading frame of 1,053 bp. This gene, termed as *CpTCP1* (accession number, AB597521), was deduced to encode 350 amino acid residues sharing 42% identity with *AtTCP3* from Arabidopsis. Figure 1A shows the amino acid alignment of *CpTCP1* with *AtTCP3* of Arabidopsis. Both the encoding sequence of the TCP domain and the micro RNA target sequence were conserved in *CpTCP1*. The phylogenetic tree of the TCP family shows that *CpTCP1* is clustered in the same group as *CIN* (*A. majus*) and *AtTCP3* and *TCP4* (*AtTCP4*) of Arabidopsis (Figure 1B). Analysis of expression in various tissues of the wild-type plant revealed that *CpTCP1* was expressed in floral and leaf tissues, but the signal was weak in the stamen, receptacle, flower stalk, and leaf stem (Figure 2). However, a large difference was not observed in the expression profiles of *AtTCP3* of Arabidopsis (Koyama et al. 2007). These gene features suggest that the protein encoded by *CpTCP1* plays a role in cell proliferation and is involved in defining plant morphology, similar to the role played by the TCP family in Arabidopsis and *A. majus*.

### Expression of the chimeric *CpTCP1* repressor in Arabidopsis

To confirm the function of *CpTCP1*, a binary vector to express *CpTCP1SRDX* under a constitutive CaMV35S promoter was constructed and introduced in Arabidopsis. The resulting transgenic Arabidopsis plants had similar defects as those observed in *35S:AtTCP3SRDX* plants (Koyama et al. 2007; Figure 3). Forty-six out of 48 *35S:CpTCP1SRDX* seedlings had defects in terms of cotyledons with serrated margins and formation of ectopic shoots (Figure 3A, B, C). Three lines formed irregular flowers, whereas the others died during culture (Figure 3D, E). Petals of *35S:CpTCP1SRDX* plants were clearly serrated (Figure 3F, G). These phenotypic changes resemble those of *35S:AtTCP3SRDX*.

### Expression of the chimeric *CpTCP1* repressor in cyclamen

The same construct p35S:CpTCP1SRDX was introduced in cyclamen WP. Two hundred etiolated petiole segments were infected by *A. tumefaciens* LBA4404 harboring p35S:CpTCP1SRDX. After 2 months of culture on selection medium containing 5 mg l<sup>-1</sup> hygromycin, 146 resistant calli were obtained in 2 experiments (hygromycin-resistant rate: 73%, Table 1). Regeneration frequency was 80%, which was the same as that in non-transgenic cyclamen plants, suggesting that *CpTCP1SRDX* did not affect regeneration. Finally, a total of 106 *CpTCP1SRDX* lines were identified using PCR analysis (Table 1). Based on the number of leaf segments in this study, the efficiency of genetic transformation was 53%. Regenerated shoots were transferred to a rooting medium. After acclimatization, they were grown in a closed incubator to observe their morphology.

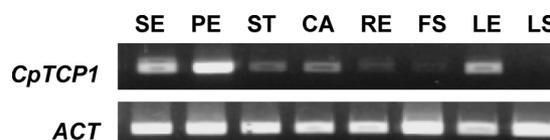


Figure 2. Analysis of *CpTCP1* expression in various organs using RT-PCR. SE, sepal; PE, petal; ST, stamen; CA, carpel; RE, receptacle; FS, flower stalk; LE, leaf; and LS, leaf stem. Gene names are indicated on the left. *ACT*, *ACTIN*.

Table 1. Efficiency and phenotype in transgenic *35S:CpTCP1SRDX* cyclamen plants

Transgene	Exp.	No. of Hyg resistant calli	No. of regenerating calli	No. of PCR (+) calli *	No. of transgenic plants		
					normal	mild	severe
<i>35S:CpTCP1SRDX</i>	1	73	53	49	1	21	27
	2	73	63	57	2	46	9
total		146	116	106	3 (3%)	67 (63%)	36 (34%)

\* PCR(+) calli: Genomic DNA was isolated from Hyg-resistant calli by the CTAB method. Integration of *hpt* was confirmed by the polymerase chain reaction (PCR) analysis with the *hpt*-specific primers. PCR(+) calli were PCR positive.

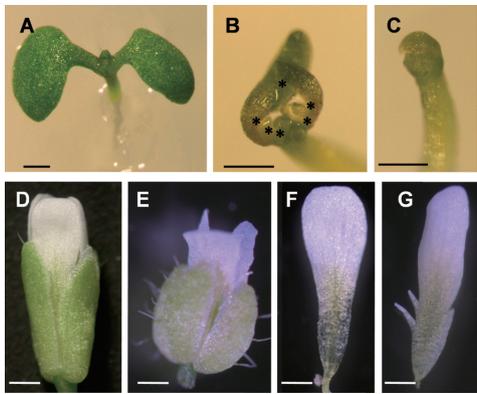


Figure 3. Comparison of phenotypes in wild-type and *35S:CpTCP1SRDX* Arabidopsis transgenic plants. (A) The seedling of wild-type Arabidopsis. (B) and (C) Seedlings of *35S:CpTCP1SRDX* Arabidopsis with moderate (B) and severe (C) defects. Asterisks in (B) indicate ectopic shoots. (C) The flower of wild-type Arabidopsis. (D) The flower of *35S:CpTCP1SRDX* Arabidopsis. (E) The petal of wild-type Arabidopsis. (F) The petal of *35S:CpTCP1SRDX* Arabidopsis. Scale bars indicate 0.5 mm.

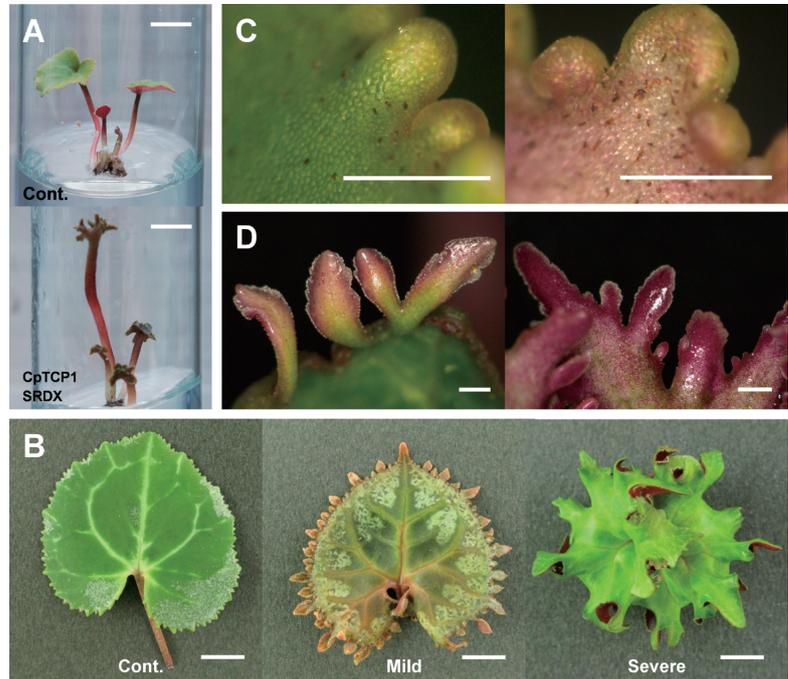


Figure 4. Comparison of phenotypes in controls and *35S:CpTCP1SRDX* transgenic plants. (A) Seedlings in a test tube. The top panel shows non-transgenic plants for controls. The bottom panel shows the *35S:CpTCP1SRDX* plant. Scale bars indicate 1 cm. (B) Comparison of the leaf phenotype. Leaves of non-transgenic plants for controls (left) and *35S:CpTCP1SRDX* plants with mild (center) and severe (right) phenotypes are shown. Scale bars indicate 1 cm. (C) and (D) The morphology of the leaf margin of non-transgenic (C) and *35S:CpTCP1SRDX* plants (D). Adaxial (left) and abaxial (right) surfaces are shown. Scale bars indicate 1 mm. Cont., controls.

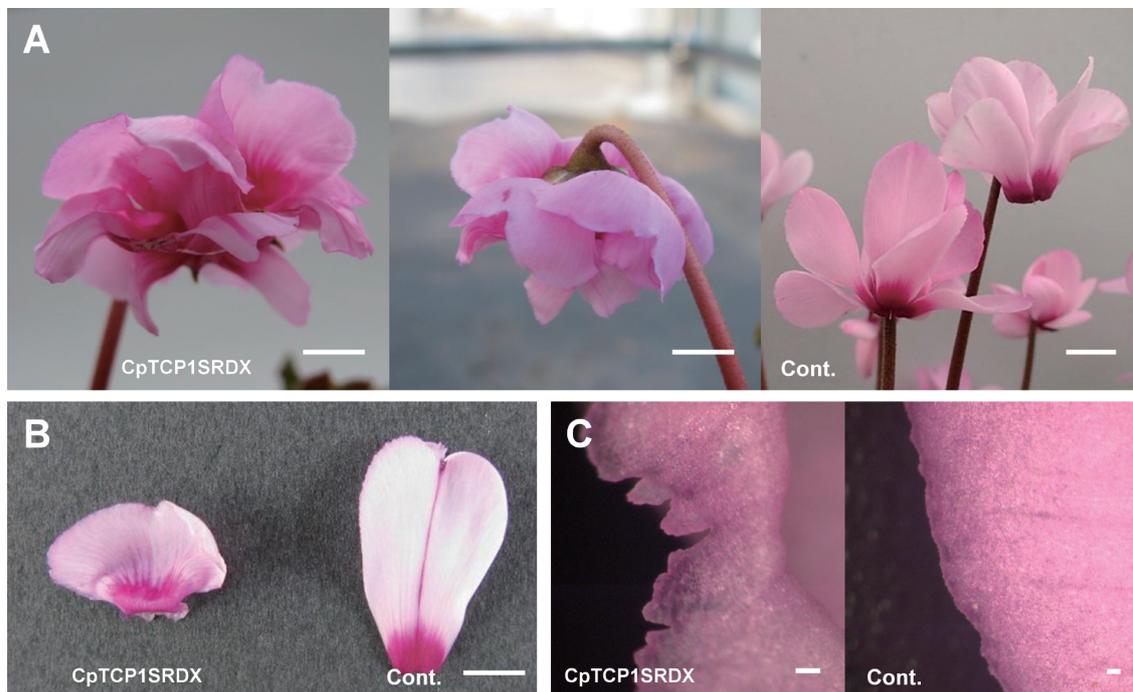


Figure 5. Comparison of flower shapes in controls and *35S:CpTCP1SRDX* transgenic plants. (A) The flower morphology of *35S:CpTCP1SRDX* transgenic (line no. 26) (left, center) and non-transgenic (right) plants. Scale bars indicate 1 cm. (B) Comparison of the petal phenotype. Petals of the *35S:CpTCP1SRDX* plant (left) and the non-transgenic plant for controls (right) are shown. Scale bar indicates 1 cm. (C) The marginal region of the adaxial surface of petals of *35S:CpTCP1SRDX* (left) and non-transgenic plants (right). Scale bars indicate 100  $\mu$ m. CpTCP1SRDX, *35S:CpTCP1SRDX*; Cont., controls.

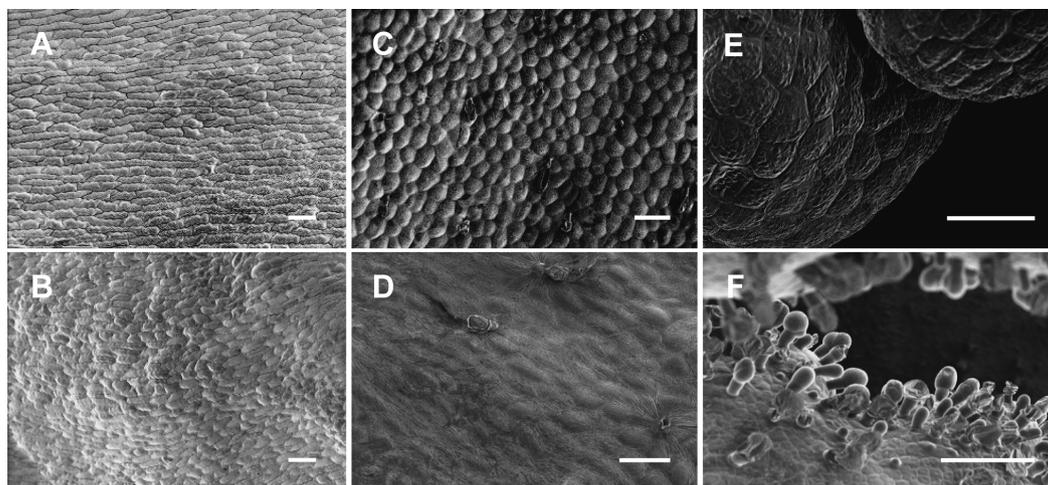


Figure 6. Morphological changes on the surface of *35S:CpTCP1SRDX* transgenic plants. The epidermal cell morphology of the petal and leaf in both non-transgenic (A–C) and *35S:CpTCP1SRDX* plants (D–F) by SEM. (A) and (B) adaxial petal surface. (C) and (D) adaxial leaf surface. (E) and (F) marginal region of the leaf. Scale bars indicate 100  $\mu\text{m}$ .

### Morphological changes observed in *35S:CpTCP1SRDX* cyclamen plants

The obtained 106 transgenic lines were grouped into 3 classes according to the severity of phenotypic abnormalities observed in adult leaves (Table 1). The no change phenotype (“normal”) was observed in only 3 lines (3%). Thus, 103 of 106 transgenic plant lines had altered morphology. The appearance rate of altered morphology was extremely high. In lines with altered morphology, leaves of young seedlings of *35S:CpTCP1SRDX* plants were serrated and wavy (Figure 4A). The transgenic plants classified in the “mild” group had leaves with mildly abnormal phenotypes. These leaves exhibited serrated margins (Figure 4B). The plants classified in the “severe” group had crumpled leaves with highly serrated margins and wavy blades, showing a drastic change compared with leaves in controls. To clarify the detailed features of serration in these leaves, the marginal region of transgenic leaves was observed under higher magnification. The margin of non-transgenic WP leaves had an array of dome-shaped structures (Figure 4C). In contrast, leaves of the *35S:CpTCP1SRDX* plant had irregular protrusions of acicular and branched shapes (Figure 4D). To observe the flower shape, 2 selected transgenic lines from the mild group were cultivated in the closed, special-netted greenhouse (Nos. 26 and 59). *CpTCP1SRDX* expression was confirmed by RT-PCR in these transgenic lines (Supplemental Figure 1). The plants with the severe phenotype tended to grow badly, such that all plants failed to attain the flowering stage. Flowers in the 2 transgenic lines showed similar phenotypes; *35S:CpTCP1SRDX* flowers had curled and ruffled petals (Figure 5A). The petals of WP flowers showed straight and elongated fringe. However, the petals of *35S:CpTCP1SRDX* plants were curved and reduced in

length compared with those of controls (Figure 5B). In addition, serrate margins were observed in the petals of transgenic plants (Figure 5C).

To investigate the influence of *CpTCP1SRDX* on cell morphology, epidermal cells of petals and leaves were analyzed using SEM. On the adaxial surface of petals, puzzle-shaped slender cells parallel to the proximal–distal axis were observed in controls (Figure 6A). In contrast, the cells of *35S:CpTCP1SRDX* plants were rounded and irregularly positioned (Figure 6B). The cells on the adaxial surface of leaves in controls were clearly protuberant, but those on the adaxial surface of leaves in *35S:CpTCP1SRDX* plants were flattened (Figure 6C, D). Leaf margin in controls was surrounded by ball-shaped protrusions and had penta- or hexagonal-shaped cells (Figure 6E). In contrast, leaf margin in transgenic plants had irregular protrusions and similar shaped cells; these cells were smaller than those in controls (cell length; 30  $\mu\text{m}$  or more in controls and 10–15  $\mu\text{m}$  in transgenic plants), with numerous globular head trichomes (Figure 6F). Trichomes of these shapes were observed in young leaves, suggesting that they were young trichomes (Supplemental Figure 2). These results suggest that undifferentiated cells developed in petals and leaves, especially in the marginal region of leaves in *35S:CpTCP1SRDX* plants. Differentiation could be repressed in these cells, which might contribute to the formation of immature organs on the surface of petals and leaves.

In Arabidopsis, expression of a chimeric repressor derived from *AtTCP3* induced wavy and serrated rosette leaves (Koyama et al. 2007). *cin* mutants exhibited larger leaves with an undulating edge because of excessive growth in the marginal region, and flowers with small, slightly curled and pale petals (Crawford et al. 2004; Nath et al. 2003). In this study, the phenotype of the

35S:CpTCP1SRDX plant resembled that of AtTCP3SRDX in Arabidopsis and *cin* mutant in *A. majus*. These results suggest that cyclamen CpTCP1 possibly plays a role in regulating morphogenesis in floral and vegetative organs by suppressing boundary-specific gene expression. However, the phenotype was slightly different, exhibiting curled and ruffled petals in cyclamen. This flower shape observed in the 35S:CpTCP1SRDX plant is a novel phenotype in cyclamen.

In this study, we obtained transgenic cyclamen plants expressing a cyclamen chimeric TCP repressor, CpTCP1SRDX. These plants have modified flower-shape, which exhibits curved and ruffled petals. We succeeded to demonstrate that the introduction of CpTCP1SRDX into cyclamen created flowers with a novel phenotype and a high ornamental value. This suggests that ruffled flower petals could be obtained in other commercial varieties using CpTCP1SRDX.

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