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**Transgenic Note**

# Morphological changes of *Rosa×hybrida* by a chimeric repressor of *Arabidopsis* TCP3

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**Abstract** Chimeric repressor gene-silencing technology is a useful tool for changing morphology of ornamental plants. It has previously been demonstrated that the chimeric repressor TCP3SRDX, which consists of *Arabidopsis* TCP3 and an ERF-associated amphiphilic repression motif repression domain, perturbs the marginal morphology of *Arabidopsis* leaves and flowers. To obtain new rose cultivars that have ornamental values, we attempted to alter the morphology of *Rosa×hybrida* cv. Lavande with TCP3SRDX. The TCP3SRDX transgenic rose plants showed interesting phenotypes: the number of leaflets and the size of leaf teeth increased, the petals were wavy, and the sepals were compound-leafy. We succeeded in altering rose morphology using *Arabidopsis TCP3* without the sequence information of a *TCP3* homologue in the target plant species.

**Key words:** Compound leaf, leaflet, *Rosa×hybrida*, serration, TCP3.

Roses are one of the most important ornamental plants in the global flower market and have been a central attraction for consumers and breeders for hundreds of years. Modern roses (*Rosa×hybrida*) have resulted from extensive hybridization of wild rose species (Matsumoto et al. 1998). Flower traits such as floral architecture, petal color, and recurrent flowering are key characters that have been subjected to artificial selection pressure during domestication and crossbreeding. Recently, genetic engineering has enabled the development of novel traits which are not obtained by hybridization (Tanaka et al. 2005), for example, the blue rose (Katsumoto et al. 2007).

Chimeric repressor gene-silencing technology (CRES-T) has provided unique transgenic ornamental flowers (Nakatsuka et al. 2011; Shikata et al. 2011; Tanaka et al. 2011). CRES-T is a type of dominant-negative strategy for transcription factors in which a transcription factor fused to a modified ERF-associated amphiphilic repression (EAR)-motif repression domain functions as a dominant chimeric repressor in transgenic plants, which leads to a loss-of-function phenotype even in the presence of redundant transcription factors (Hiratsu et al. 2003). CRES-T is one of the most effective tools for manipulating plant traits.

The *TEOSINTE BRANCHED1*, *CYCLOIDEA*, and *PCF* (*TCP*) family encodes plant-specific transcription factors (Martín-Trillo and Cubas 2010). *TCPs* are classified in two types based on differences in TCP domains; Class I contains rice *PROLIFERATING CELL FACTORS 1* and *2* and class II includes *Antirrhinum CYCLOIDEA*, maize *teosinte branched1* (*TB1*), and *Antirrhinum CINCINNATA* (*CIN*). In *Arabidopsis thaliana*, 24 *TCP* genes were identified, and eight of them, including *TCP3*, were distinguished as *CIN* type. *CIN*-type *TCP* genes have redundant molecular functions to regulate margin development (Koyama et al. 2007). A chimeric repressor, TCP3SRDX, which consists of *Arabidopsis* TCP3 and the EAR-motif repression domain, induced serrated leaves and wavy petals (Koyama et al. 2007).

Modified margins of leaves and petals bring dynamic visual changes in the ornamental plants, leading to increases in ornamental value. To produce new rose cultivars, we attempted to change the rose marginal morphology using TCP3SRDX. The expression plasmid

Abbreviations: CIN, CINCINNATA; CRES-T, chimeric repressor gene-silencing technology; TCP, TEOSINTE BRANCHED1, CYCLOIDEA, and PCF

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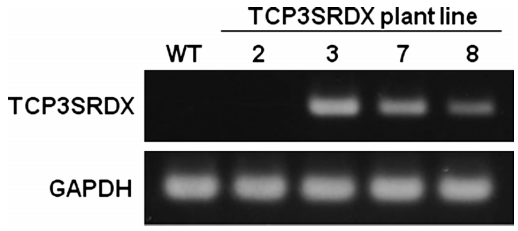


Figure 1. Transcript levels of TCP3SRDX in transgenic rose plants. Total RNA was isolated from the wild-type (WT) and transgenic rose leaves, and subjected to first-strand cDNA synthesis. PCR was performed with the primers specific to the *Arabidopsis* TCP3 (5′-ATGGCACCAGATAACGACCA-3′) and SRDX (5′-GAGTTCTAG-ATCCAGATC-3′), or rose GAPDH (5′-TGTCATCTCTGCCCCA-AGTAAGG-3′ and 5′-CAACATCCTCATCGGTGTAACCC-3′). PCR conditions were as follows: 95°C for 5 min, and then 30 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, and finally 72°C for 7 min.

of *35S:TCP3SRDX* (Koyama et al. 2007) was introduced into *Agrobacterium tumefaciens* Agl0 (Lazo et al. 1991), and *Rosa×hybrida* cv. Lavande was transformed as described previously (Firoozabady et al. 1994). Four transgenic plant lines were obtained, and *TCP3SRDX* mRNA was detected in three of these lines, TCP3SRDX-3, -7, and -8 (Figure 1).

A wild-type compound leaf is composed of a midvein, a terminal leaflet, and two to three pairs of lateral, petiolated, and serrated leaflets (Figure 2A). The transgenic rose TCP3SRDX-3, which showed the severest phenotype, increased the number of leaflets and the number and size of leaf teeth (Figure 2B, C, D). The transgenic rose TCP3SRDX-7, whose phenotypic change was milder than the TCP3SRDX-3 plant, changed the size and pattern of the serrations, although the number of leaflets was five to seven, the same as the wild type (Figure 2E). The stipules of the TCP3SRDX-3 plant were deeply serrated (Figure 2F, G), but those of the TCP3SRDX-7 plant were not (data not shown). The serrations in TCP3SRDX-7 leaves were deeper than those in the wild type (Figure 2H, I). The serrations on the medial axis of wild-type leaflets consisted of a big tooth with small teeth on both lateral sides. The serrations on the lateral sides of the leaflets consisted of a big tooth with small teeth only on the lateral side (Figure 2H). On the other hand, both the medial and lateral serrations in the TCP3SRDX-7 leaves resembled the medial serration pattern of the wild type (Figure 2I), as if further leaflets developed in the leaflets. The secondary veins in the wild-type leaves formed loops, while those in the TCP3SRDX-7 leaves extended linearly to the margins (Figure 2J, K, L, M). The wild-type cells in the leaf adaxial epidermis were organized in a specific pavement-like pattern, while the epidermal cells of the TCP3SRDX-3 leaf were rounded with undifferentiated cell features (Figure 2N, O). The patterns of xylem and phloem in the leaves and petals of the TCP3SRDX rose plants did not change (data not shown), implying that TCP was not involved in the adaxial-abaxial polarity. TCP3SRDX transgenic rose plants maintained apical dominance (data not shown), implying that TCP3SRDX did not affect the gene expression downstream of a TB1-type TCP which are involved in the maintenance of apical dominance (Aguilar-Martínez et al. 2007; Doebley et al. 1997).

In the wild-type rose Lavande, the petals were pink and rolled back with pointed edges, and the sepals were drop-shaped and leafy at the tops (Figure 3A, B, C). A wavy petal and a petal-fused sepal sometimes appear in very young rose plants. In the TCP3SRDX-7 rose plant, the petals were light pink, serrated, and wavy (Figure 3D). The sepals were crooked and partly compound-leafy (Figure 3F). In the TCP3SRDX-3 rose plant, the petals were small, colorless, serrated, and wavy (Figure 3G). The sepals of the TCP3SRDX-3 plant were severely compound-leafy (Figure 3I), which is a new morphology. Notably, compound leaves were induced anywhere in leafy tissues in the TCP3SRDX-3 rose plants.

The excessive compound-leaf formation in TCP3SRDX rose plants resembles the phenotype of the transgenic tomato plant, in which ectopic expression of *miR319* represses *LANCEOLATE* (*LA*) of the *TCP* family (Ori et al. 2007). Class I *KNOTTED*-like homeobox (*KNOX1*) genes are major regulators of compound-leaf formation. Overexpresion of *KNOX1* in tomato results in excessive compound-leaf formation, and this phenotype is suppressed by overexpression of *LA* (Ori et al. 2007). An increase in *CUP-SHAPED COTYLEDON 2* (*CUC2*) expression results in deeply serrated leaves in *Arabidopsis* (Nikovics et al. 2006). Accumulation of miR164, which negatively regulates *CUC2* expression (Nikovics et al. 2006), was reduced in TCP3SRDX-transgenic *Arabidopsis* (Koyama et al. 2007; Koyama et al. 2010). Comparisons between the mild and severe phenotypes of the TCP3SRDX rose plants suggested that CIN-type TCPs may control the molecular mechanisms for the both leaf-tooth and leaf/leaflet formation. The formation process of leaf teeth is similar to that of simple leaves and leaflets (Kawamura et al. 2010); the *CUC* genes are involved in the development of both leaf teeth (Nikovics et al. 2006) and leaves and leaflets (Aida et al. 1997; Blein et al. 2008). These results imply that rose *KNOX1* and *CUC2* homologues may be involved in excessive compound-leaf formations and the deep serrations observed in TCP3SRDX rose plants.

We succeeded in producing the new transgenic rose cultivar with the serrated leaves and petals, suggesting that CRES-T using *Arabidopsis* transcription factors is a useful tool for the improvement of ornamental plants with limited genomic and sequence information. It is notable that CRES-T showed dominant effect in *Rosa×hybrida* which is a tetraploid. The constitutive expression

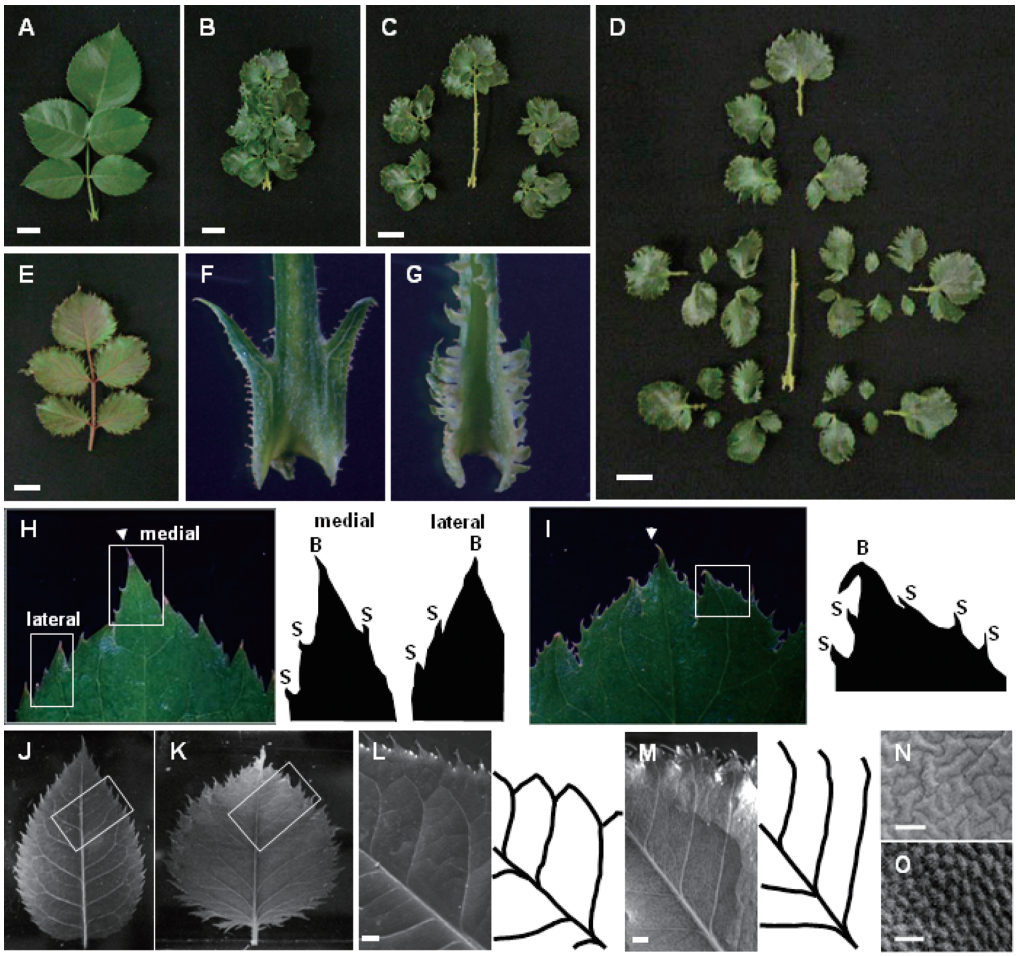


Figure 2. Phenotypes of leaves of TCP3SRDX rose plants. Compound leaves of wild type (A) and TCP3SRDX-3 with the severe phenotype (B). Leaves of TCP3SRDX-3 excised from a midvein (C) and further from lateral veins (D). Compound leaves of TCP3SRDX-7 with the mild phenotype (E). Stipules of wild type (F) and TCP3SRDX-3 (G). Leaf tooth patterns of terminal leaves of wild type (H) and TCP3SRDX-7 (I). Arrowheads indicate the position of the medial axis in the terminal leaflet. B and S indicate big and small teeth, respectively. Vascular patterns of cleared terminal leaflets of wild type (J) and TCP3SRDX-7 (K). Magnified views of the veins of wild type (L) and TCP3SRDX-7 (M) corresponding white squares in J and K. Scanning electron microscopy images of leaf adaxial surface of wild type (N) and TCP3SRDX-3 (O). Scale bars indicate 1 cm in A to E, 1 mm in L and M, and 50 $\mu$m in N and O.

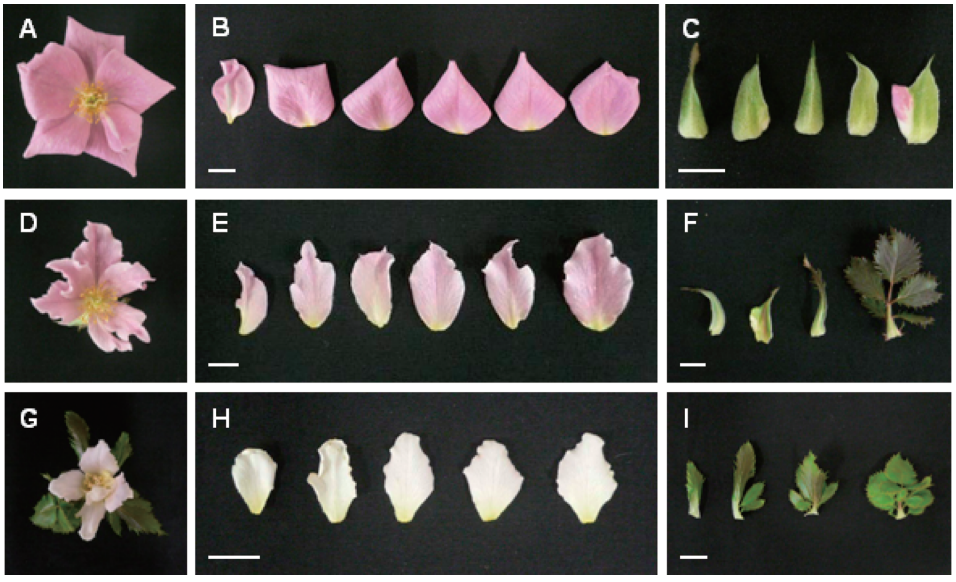


Figure 3. Phenotypes of flowers of TCP3SRDX rose plants. Flower (A), petals (B), and sepals (C) of wild type. Flower (D), petals (E), and sepals (F) of TCP3SRDX-7. Flower (G), petals (H), and sepals (I) of TCP3SRDX-3. Scale bars indicate 1 cm.

of TCP3SRDX in rose resulted in a severe delay in growth and flower bud initiation. It took more than six months to produce the second flower from the first flower in the case of the TCP3SRDX-3 plant, while approximately three months in the case of non-transgenic plants (data not show). This slow growth and flowering should be resolved in order to make TCP3SRDX commercially applicable. For example, it is one of effective strategies to change the 35S promoter to an inducible promoter. TCP3SRDX would make the production of crinkly and wavy ornamental plants feasible.

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