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Title

Generation of serrated and wavy petals by inhibition of the activity of TCP transcription factors in *Arabidopsis*

Running title

Petal development regulated by TCP transcription factors

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TCP transcription factors regulate the activities of ASYMMETRIC LEAVES1 and miR164, as well as the auxin response, during the differentiation of leaves in *Arabidopsis*. Plant Cell 2010; 22:3574-3588.
Abstract

The final shape of shoot lateral organs, namely, leaves and flowers, is determined by coordinated growth after the initiation of primordia from shoot meristems in seed plants. This coordination is achieved by the complex action of many transcription factors, which include the TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP) family. We have recently reported that CINCINNATA-like (CIN-like) TCP genes act dose-dependently to regulate the flat and smooth morphology of leaves in Arabidopsis thaliana. In contrast, the roles of CIN-like TCP genes in flower development are poorly understood. In this report, using multiple tcp mutants and transgenic plants in which the activity of CIN-like TCP transcription factors is dominantly inhibited, we found that these TCPs regulate the smooth and flat morphology of petals. Based on these findings, we discuss a possible strategy to generate a fringed morphology in floricultural plants.

Key words

TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP); petal; SRDX; Arabidopsis; fringe; floriculture

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TCP genes encode proteins that contain a plant-specific DNA binding domain and regulate diverse aspects of plant development.\(^1\) Eight of the 24 TCP genes in the Arabidopsis genome are classified as CIN-like TCP genes, since these genes have similar nucleotide sequences and functions in planta to CIN of Antirrhinum majus.\(^2\) We have demonstrated that the differential activity of CIN-like TCP genes generates a graded change in leaf forms.\(^3\) Our molecular analyses have clarified that CIN-like TCP transcription factors directly regulate genes involved in the promotion of leaf differentiation, production of miRNA, and the response to the plant hormone auxin. The products of these target genes negatively and cooperatively regulate the expression of CUP-SHAPED COTYLEDON (CUC) genes, which regulate the formation of shoot meristem and the morphological separation of an organ from adjacent organs. Considering these observations, we have proposed that the negative regulation of CUC genes by CIN-like TCP transcription factors is the core process in the development of leaves. In contrast, TCP1, a CYCLOIDEA-like TCP gene, regulates the longitudinal elongation of leaves,\(^5\)\(^-\)\(^7\) suggesting that the individual TCP genes of the different subgroups have specific functions in leaf development. In addition to the clarification of the roles of TCP genes during leaf development,\(^3\)\(^4\) we present here some insights regarding roles of CIN-like TCP genes in flower development.

We found that CIN-like TCP genes regulate petal development, using various lines of Arabidopsis tcp mutants\(^3\) (Figures 1A to 1H). Wild-type petals had a smooth margin and flat surface (Figures 1A and 1E), whereas the quadruple and quintuple mutants tcp3 tcp4 tcp5 tcp10 (tcp3/4/5/10) and tcp3 tcp4 tcp5 tcp10 tcp13 (tcp3/4/5/10/13), respectively, exhibited wavy and serrated petals (Figures 1B, 1C, 1F and 1G). Moreover, ectopic expression of miR319A under the control of the Cauliflower Mosaic Virus (CaMV) 35S
promoter in tcp3/4/5/10/13 (Pro35S:miR319A tcp3/4/5/10/13), which resulted in suppression of TCP2 and TCP24, as well as the inactivation of TCP3/4/5/10/13 genes, induced severely wavy and serrated petals (Figures 1D and 1H). These results suggest that CIN-like TCP genes regulate the smoothness of the surface and margin of petals. In contrast, each single mutant, and the double mutants tcp3/tcp4, tcp3/tcp10, and tcp3/tcp5, exhibited normal flowers, while the triple mutant tcp3/tcp4/tcp10 showed a small sign of wavy petals (data not shown). Therefore, CIN-like TCP genes may redundantly regulate flower development, as was observed in leaf development.

We further examined the roles of CIN-like TCP genes in petal development, using the TCP5SRDX gene, in which TCP5 was fused with SRDX, a strong transcriptional repression domain (Figures 1I to 1P). When we expressed TCP5SRDX under the control of the CaMV 35S promoter (Pro35S:TCP5SRDX) in Arabidopsis plants, we found that Pro35S:TCP5SRDX plants had severely serrated margins of sepalas and petals (Figures 1I to 1L), in addition to wavy and serrated leaves. This abnormality was possibly caused by the dominant repression of expression of the putative target genes of CIN-like TCP transcription factors. Since the phenotype of Pro35S:TCP5SRDX petals resembled—-but was more severe than—Pro35S:TCP3SRDX petals, we compared the abaxial epidermis of Pro35S:TCP5SRDX and wild type petals by scanning electron microscopy. The wild-type petals had rounded cells in the distal region and flat cells elongate according to the proximodistal axis in the proximal region (Figures 1M and 1O). In contrast, Pro35S:TCP5SRDX petals had flat and elongated cells in all regions examined, and did not have rounded cells in the distal region (Figures 1N and 1P). The flat cells in Pro35S:TCP5SRDX petals resemble those in the cin mutant. The altered regulation of cell differentiation in the distal region of Pro35S:TCP5SRDX petals might
interfere with the patterning of the smooth margin.

Extending our finding that inhibition of the activity of CIN-like TCP transcription factors induces a wavy and serrated margin of Arabidopsis petals, we introduced the TCPSRDX genes into several floricultural plants, and have recently generated transgenic plants with modified petals. In Cyclamen persicum, expression of CpTCP1SRDX, which is derived from an endogenous CIN-like TCP gene, induces ruffled petals.\(^\text{11}\) Furthermore, expression of TCP3SRDX, even if it is derived from the Arabidopsis genome, induces fringed and wavy petals in Torenia fournieri and Rosa x hybrida, respectively.\(^\text{12,13}\) In addition, these modified petals exhibit a variation of coloring pattern. Interestingly, TCP3SRDX induces a specific morphology of petals with suppressed development in Chrysanthemum morifolium.\(^\text{12}\) Chrysanthemum plants have a pseudanthium composed of a disk and ray florets, and the effect of TCP3SRDX could result in a different morphology of the chrysanthemum pseudanthium. With respect to leaf morphology, expression of the TCPSRDX genes induces undulation, serration, or dissection with different severity in the plants that we examined.\(^\text{11-13}\) Therefore, using the TCPSRDX genes, we provide a simple methodology for generating leaves and petals with different margins and surfaces.

In this report, we demonstrate that CIN-like TCP genes regulate the smooth morphology of petals, and these genes act redundantly to regulate petal development in Arabidopsis. Considering the similarity in the roles of CIN-like TCP genes during the development of both leaves and petals,\(^\text{3,4}\) we postulate that the molecular basis downstream of CIN-like TCP transcription factors is conserved, in part, among several different plants. One possible conserved gene downstream of CIN-like TCP transcription factors is that involved in the separation of organs.\(^\text{3,4}\) Although CUC genes are involved in the separation of leaves as well as sepals and stamens,\(^\text{14}\) it is important to identify genes
responsible for the separation of petals.
References


Figure.legend

Figure 1. Flowers of wild type, tcp mutants, and Pro35S:TCP5SRDX plants.

Top views of wild-type (A), tcp3/4/5/10 (B), tcp3/4/5/10/13 (C), and Pro35S:miR319A tcp3/4/5/10/13 (D) flowers. Petals of wild type (E), tcp3/4/5/10 (F), tcp3/4/5/10/13 (G), and Pro35S:miR319A tcp3/4/5/10/13 (H) plants. The photos in (E) to (H) are presented in back and white for detailed visualization of the margin. Side views of wild-type (I) and Pro35S:TCP5SRDX flowers (J). Petals of wild type (K) and Pro35S:TCP5SRDX plants (L). The moderate and severe phenotypes of Pro35S:TCP5SRDX petals are presented at left and right, respectively, in (L). The epidermal cells at the distal region of wild-type (M) and Pro35S:TCP5SRDX (N) petals. The epidermal cells at the proximal region of wild-type (O) and Pro35S:TCP5SRDX (P) petals. Bars = 0.5 mm in (A) to (L) and 10 µm in (M) to (P).