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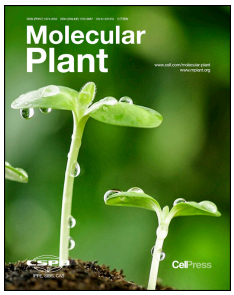

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Letter to the Editor

Class I and class II TCP transcription factors modulate SOC1-dependent flowering at multiple levels

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Dear Editor,

Teosinte branched 1, cycloidea, PCF (TCP) transcription factors regulate several aspects of plant development (reviewed in Uberti-Manassero et al., 2013). In Arabidopsis, the 24 members of this family are classified into classes I and II according to the structure of the TCP domain, a conserved region involved in DNA binding and dimerization. Class II is further divided into two clades: CINCINNATA (CIN) TCPs that are involved in several developmental processes, including regulation of leaf morphology, and CYC/TB1 TCPs that regulate branching and floral symmetry. Class I TCPs have diverse functions, some of them also related to leaf development. The fact that class I and class II (especially CIN) TCPs participate in similar processes led to the idea that they may share regulatory pathways, possibly acting as antagonistic factors on the same group of target genes. For example, it was proposed that class I TCPs stimulate cell division and growth during early stages of leaf development while CIN TCPs negatively regulate these processes outside the proliferation zone. There is also evidence that TCPs may regulate flowering, but little is known about the mechanisms involved. Here, we analyzed the role of the class I protein TCP15 during flowering.

Flowering, measured both as days-to-flowering and number of leaves at the vegetative-reproductive transition, showed a significant delay in the *tcp15-3* loss-of-function mutant when compared to wild-type plants (Figure 1A and 1B). In addition, plants expressing *TCP15* under the *35SCaMV* promoter (*35S:TCP15*) flowered earlier than wild-type plants by both criteria (Figure 1A and 1B), hinting at a positive role of TCP15 during flowering. We also analyzed plants impaired in the related class I TCPs TCP14, TCP8 and TCP22, which are known to interact with TCP15. A *tcp14-4 tcp15-3* double mutant behaved like *tcp15-3*, in agreement with the fact that the single *tcp14-4* mutant did not show differences with wild-type (Figure 1A and 1B). This may indicate a less relevant role of TCP14 during flowering. However, the fact that the *tcp14-4* mutant may not be a complete knock-out (Davière et al., 2014) may also explain these results. Plants expressing an artificial miRNA directed against the class I TCPs *TCP8* and *TCP22* in a *tcp14-4 tcp15-3* background (*amiR2.4* plants; Davière et al. 2014) showed a further delay (Figure 1A and 1B), suggesting that TCP8 and/or TCP22 also participate in flowering time regulation.

Since *TCP15* is expressed in meristems and young leaf primordia (Figure 1C), the same expression niches as the flowering integrator SUPPRESSOR OF OVEREXPRESSION

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OF CONSTANS 1 (*SOC1*), we measured *SOC1* expression in mutant and transgenic plants. *SOC1* transcript levels were significantly reduced in *tcp15*, *tcp14 tcp15* and *amiR2.4* plants, while a ca. 8-fold induction was observed in *35S:TCP15* plants (Figure 1D), suggesting that TCP15 acts through the regulation of *SOC1* expression. Overexpression of *TCP15* did not revert the late-flowering phenotype of the *soc1-2* loss-of-function mutant (Figure 1E), thus confirming that TCP15 function in flowering is upstream of SOC1.

An *in silico* analysis of the *SOC1* promoter revealed the presence of a TCP consensus target site (GGGACCA) at position –687 from the transcription start site. We assessed whether TCP15 is able to bind the *SOC1* promoter *in vivo* by chromatin immunoprecipitation (ChIP) followed by qPCR. To this end, we used plants expressing TCP15 fused to the red fluorescent protein (TCP15-RFP). Two different fragments of the *SOC1* promoter located near the TCP binding site exhibited a significant enrichment compared to a fragment located in the *SOC1* coding region (Figure 1F). No enrichment was observed for *ACTIN* genes, used as controls. Thus, our results point to *SOC1* as a potential direct target of TCP15.

MiR319-mediated silencing of class II TCPs was reported to produce a delay in flowering (Palatnik et al., 2003). In agreement, the *suppressor of jaw-D 8* (*soj8*) mutant, which expresses a miR319-resistant version of *TCP4*, showed accelerated flowering (Figure 1G). Unlike the results obtained when overexpressing *TCP15*, the *soj8* mutation was able to revert, almost completely, the late-flowering phenotype of *soc1-2* plants (Figure 1G), suggesting that TCP4 is able to act either downstream or independently of SOC1. Partial reversion of the *soc1-2* late-flowering phenotype by *soj8* was also observed under short-day conditions (Supplemental Figure 1). The *soc1-2* mutant showed reduced transcript levels of *TCP4* and the related gene *TCP3*, and the same was observed in *tcp15*, *tcp14 tcp15* and *amiR2.4* plants (Figure 1H), as expected from reduced *SOC1* expression in these plants. *TCP15* overexpression in a wild-type background, but not in a *soc1-2* background, caused an increase in *TCP3* and *TCP4* transcript levels (Figure 1H), pointing to SOC1 as a necessary node in the TCP15-mediated regulation of *TCP3* and *TCP4*. Conversely, neither *TCP15* nor SOC1 transcript levels were modified in *soj8* and miR319-overexpressing *jagged and wavy-D* (*jaw-D*) plants (Supplemental Figure 2).

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Since TCP3 and TCP4 are regulated by miR319, we wondered whether the TCP15-SOC1 regulatory node could be acting on this miRNA. Small RNA blots quantifying miRNA levels revealed an over-accumulation of miR319 in *soc1-2* and *tcp15* mutant lines (Figure 1I). Furthermore, a reduction in miR319 levels was detected in *TCP15* overexpressing plants (Figure 1I). The levels of the miR319c precursor followed a similar pattern (Supplemental Figure 3). Since miR319 is similar to miR159 and there may be cross-hybridization in blots, we also measured by RT-qPCR transcript levels of pri-miR159a and *MYB33*, a miR159 target, and found no significant differences among the different lines under study (Supplemental Figures 3 and 4). Altogether, our observations suggest that it is miR319 the actual target of SOC1 regulation.

It was described that TCP15 and related class I TCPs are targets of DELLA proteins and participate in gibberellin (GA)-dependent regulation of germination and stem elongation (Davière et al., 2014; Resentini et al., 2015). GAs also induce flowering by regulating, among others, the expression of *SOC1*. We then wondered whether TCP15 participates in GA-mediated *SOC1* induction. Overexpression of *TCP15-RFP* alleviated the characteristic late-flowering phenotype of the GA-deficient mutant *ga1* (Figure 1J). In addition, GA treatment did not significantly affect flowering in *35S:TCP15* plants as it did in wild-type plants and failed to completely revert the late-flowering phenotype of *tcp15* mutant plants (Figure 1K). These results suggest that TCP15 has a role in GA-mediated flowering, probably through the regulation of *SOC1* expression. Indeed, GA-dependent induction of *SOC1* transcription was severely compromised in *tcp15*, *tcp14 tcp15* and *amiR2.4* plants (Figure 1L), pointing to TCP15 as key mediator of the response of *SOC1* to GAs. A nuclear factor Y (NF-Y) protein was also reported to mediate the activation of the *SOC1* promoter in response to GA through interaction with DELLAs (Hou et al., 2014). Whether both proteins act in the same signaling pathway deserves further investigation. GAs also act downstream of SOC1 (http://www.phytosystems.ulg.ac.be/florid/networks/hormones) and this may explain the fact that *tcp15* mutants are not completely insensitive to the hormone.

We showed here that TCP15 promotes flowering upstream of the flowering integrator SOC1 (Figure 1M). This differs from class I proteins TCP20 and TCP22, which were reported to delay flowering acting on the expression of the clock gene *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) (Wu et al., 2016). According to our results, TCP15 directly induces the expression of the flowering integrator SOC1. *SOC1* expression is

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regulated in meristems by another flowering integrator, FLOWERING LOCUS T (FT), which is induced in leaves and translocated to meristems to induce flowering (Corbesier et al., 2007). TCP15 was shown to interact with FT (Ho et al., 2014), but the functional significance of this interaction remains unknown. We observed that *FT* expression is not significantly affected in *tcp15* mutants or *35S:TCP15* plants and that overexpression of *TCP15* was not able to revert the late flowering phenotype of the *ft-10* mutant (Supplemental Figure 5), suggesting that TCP15 may act in combination with FT to activate *SOC1* expression. Further studies are required to confirm such hypothesis. Notably, class II proteins also interact with FT (Ho et al., 2014). In Arabidopsis, the CYC/TB1 TCP BRANCHED1/TCP18 (BRC1) interacts with FT to inhibit flowering in axillary meristems (Niwa et al., 2013). The relevance of the interaction of TCP4 and other CIN TCPs with FT is still unclear. Adding complexity to the system, two recent studies showed that CIN TCPs induce photoperiodic flowering by directly activating *CONSTANS* (*CO*) transcription in leaves and also induce *FT* transcription through CO-independent pathways (Kubota et al., 2017; Liu et al., 2017). While *FT* transcript levels were reduced in *jaw-D* plants, as reported by Liu et al. (2017), we did not detect *FT* expression changes in the *soj8* mutant (Supplemental Figure 6). Relative to this, Kubota et al. (2017) were unable to observe differences in *FT* expression in *35S:TCP4* plants, while Liu et al. (2017) did. In our case, the use of a miR319-resistant *TCP4* under its natural promoter allowed the expression of TCP4 in places where miR319 is abundant, while the contrary may have occurred when overexpressing the native *TCP4*, as the other authors did. Our results showed that, in addition to their role in the regulation of *FT* in leaves, TCP4 and other CIN TCPs are subjected to regulation by SOC1 through miR319 (Figure 1M), most likely in meristematic regions where SOC1 is expressed. TCP4 and related CIN TCPs are considered as “maturation” factors that determine developmental timing at the organ and organismal level. During the vegetative phase, precocious expression of CIN TCPs leads to early meristem termination and the development of miniature leaves. In wild-type plants, this is mainly avoided through negative regulation by miR319 in meristems and leaf primordia. Once flowering is induced, downregulation of miR319 by SOC1 may be required to accelerate plant development at the organismal level. Indeed, SOC1 may directly regulate the expression of *MIR319* genes, as suggested by the fact that *MIR319b* and *MIR319c* were identified as possible SOC1 targets in a global ChIP analysis (Immink et al., 2012).

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Our results not only introduced TCP15 as a new component of the flowering regulatory network but also established a new mode of hierarchical interaction of TCPs from different classes in a single signaling pathway (Figure 1M). Even acting at different levels, the class I protein TCP15 and the class II protein TCP4 positively regulate flowering. Our findings challenge the current concept of antagonistic functions exerted by class I and class II TCPs. This idea arose mainly from studies of leaf development, where class I and class II TCPs stimulate and repress proliferation, respectively. Considering the diversity of TCP proteins and the many processes in which they participate, it becomes evident that different functional interactions between proteins from both classes were established, reaching a wide range of developmental outputs.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

L.E.L. and D.H.G. conceived the project. L.E.L., P.A.M., D.E.G., and F.D.A. performed experiments. All authors designed experiments, analyzed data and contributed to writing.

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REFERENCES

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C., and Coupland, G. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science **316:**1030-1033.

Davière, J.M., Wild, M., Regnault, T., Baumberger, N., Eisler, H., Genschik, P., and Achard, P. (2014). Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height. Curr. Biol. **24:**1923-1928.

Ho, W.W., and Weigel, D. (2014). Structural features determining flower-promoting activity of Arabidopsis FLOWERING LOCUS T. Plant Cell **26:**552-564.

Hou, X., Zhou, J., Liu, C., Liu, L., Shen, L., and Yu, H. (2014). Nuclear factor Y-mediated H3K27me3 demethylation of the SOC1 locus orchestrates flowering responses of Arabidopsis. Nat. Commun. **5:**4601. doi: 10.1038/ncomms5601.

Immink, R.G., Posé, D., Ferrario, S., Ott, F., Kaufmann, K., Valentim, F.L., de Folter, S., van der Wal, F., van Dijk, A.D., Schmid, M., and Angenent, G.C. (2012). Characterization of SOC1's central role in flowering by the identification of its upstream and downstream regulators. Plant Physiol. **160:**433-449.

Kubota, A., Ito, S., Shim, J.S., Johnson, R.S., Song, Y.H., Breton, G., Goralogia, G.S., Kwon, M.S., Laboy Cintrón, D., Koyama, T., Ohme-Takagi, M., Pruneda-Paz, J.L., Kay, S.A., MacCoss, M.J., and Imaizumi, T. (2017). TCP4-dependent induction of CONSTANS transcription requires GIGANTEA in photoperiodic flowering in Arabidopsis. PLoS Genet. **13:**e1006856.

Liu, J., Cheng, X., Liu, P., Li, D., Chen, T., Gu, X., and Sun, J. (2017) MicroRNA319-regulated TCPs interact with FBHs and PFT1 to activate CO transcription and control flowering time in Arabidopsis. PLoS Genet. **13:**e1006833.

Niwa, M., Daimon, Y., Kurotani, K., Higo, A., Pruneda-Paz, J.L., Breton, G., Mitsuda, N., Kay, S.A., Ohme-Takagi, M., Endo, M., and Araki, T. (2013). BRANCHED1 interacts with FLOWERING LOCUS T to repress the floral transition of the axillary meristems in Arabidopsis. Plant Cell **25:**1228-1242.

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Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C., and Weigel, D. (2003). Control of leaf morphogenesis by microRNAs. Nature **425:**257-263.

Resentini, F., Felipo-Benavent, A., Colombo, L., Blázquez, M.A., Alabadí, D., and Masiero, S. (2015). TCP14 and TCP15 mediate the promotion of seed germination by gibberellins in Arabidopsis thaliana. Mol. Plant **8:**482-485.

Uberti-Manassero, N.G., Viola, I.L., Welchen, E., and Gonzalez, D.H. (2013). TCP transcription factors: architectures of plant form. Biomol. Concepts **4:**111-127.

Wu, J.F., Tsai, H.L., Joanito, I., Wu, Y.C., Chang, C.W., Li, Y.H., Wang, Y., Hong, J.C., Chu, J.W., Hsu, C.P., and Wu, S.H. (2016). LWD-TCP complex activates the morning gene CCA1 in Arabidopsis. Nat. Commun. **7:**13181. doi: 10.1038/ncomms13181.

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FIGURE LEGEND

Figure 1. Regulation of flowering time by TCP transcription factors.

(A) Number of rosette leaves at flowering in wild-type (Col-0), *tcp* mutants and *35S:TCP15* plants.

(B) Number of days required for flowering in wild-type (Col-0), *tcp* mutants and *35S:TCP15* plants.

(C) β-glucuronidase (GUS) expression pattern in 10-day-old plants carrying a fusion of the *TCP15* promoter to the *uidA* (*gus*) reporter gene. The black bars represent 2 mm.

(D) Relative *SOC1* transcript levels in wild-type (Col-0), *tcp* mutants and *35S:TCP15* plants. Asterisks indicate significant differences with wild-type plants ($p < 0.05$) according to Student's *t* tests.

(E) Number of rosette leaves at flowering in wild-type (Col-0) plants, *soc1-2* mutants and plants that express *TCP15* from the *35SCaMV* promoter in a *soc1-2* background.

(F) ChIP experiment using either anti-RFP or antiIgG antibodies in plants that express TCP15-RFP (*35S:TCP15-RFP*). The location of the *SOC1* regions analyzed by qPCR are depicted in the scheme. *ACTIN* genes (ACT) were used as a control not targeted by TCP15.

(G) Number of rosette leaves at flowering in wild-type (Col-0), *soc1-2* and *soj8* plants, and in two homozygous lines obtained after crossing *soc1-2* with *soj8*.

(H) Relative *TCP3* and *TCP4* transcript levels in wild-type (Col-0), *tcp* and *soc1-2* mutants, and plants that express *TCP15* from the *35SCaMV* promoter in either a wild-type or a *soc1-2* background. Significant differences with wild-type ($p < 0.05$) were observed for all lines analyzed.

(I) miR319 levels in wild-type (Col-0), *tcp* and *soc1-2* mutants, and plants that express *TCP15* from the *35SCaMV* promoter in either a wild-type or a *soc1-2* background. The levels of an independent miRNA (miR171) were also analyzed to discard a general effect on miRNA metabolism. U6 snRNA was used as loading control. Band intensity was quantified using ImageJ software and the relative expression is given with wild-type (Col-0) as reference. The experiment was repeated twice with similar results.

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(J) Number of rosette leaves at flowering in wild-type (Col-0), *ga1* mutants and plants that express *TCP15-RFP* from the *35SCaMV* promoter in either a wild-type or a *ga1* background.

(K) Effect of GA treatment on flowering time in *35S:TCP15* and *tcp* mutant plants.

(L) Relative *SOC1* transcript levels in wild-type (Col-0) plants and *tcp* mutants either treated or not treated with 100 µM GA_3 24 h before total RNA was extracted. Asterisks indicate significant differences ($p < 0.05$) according to Student's *t* tests.

(M) A model of the role of TCPs in the regulation of SOC1-dependent flowering. Only components related to the results presented here are shown. The existence of additional GA- and CIN TCP-dependent pathways, as discussed in the text, is shown in dotted lines.

In **(A)**, **(B)**, **(E-G),** **(J)** and **(K)**, different letters indicate significant differences according to one-way ANOVA tests ($p < 0.05$). Error bars represent SE.

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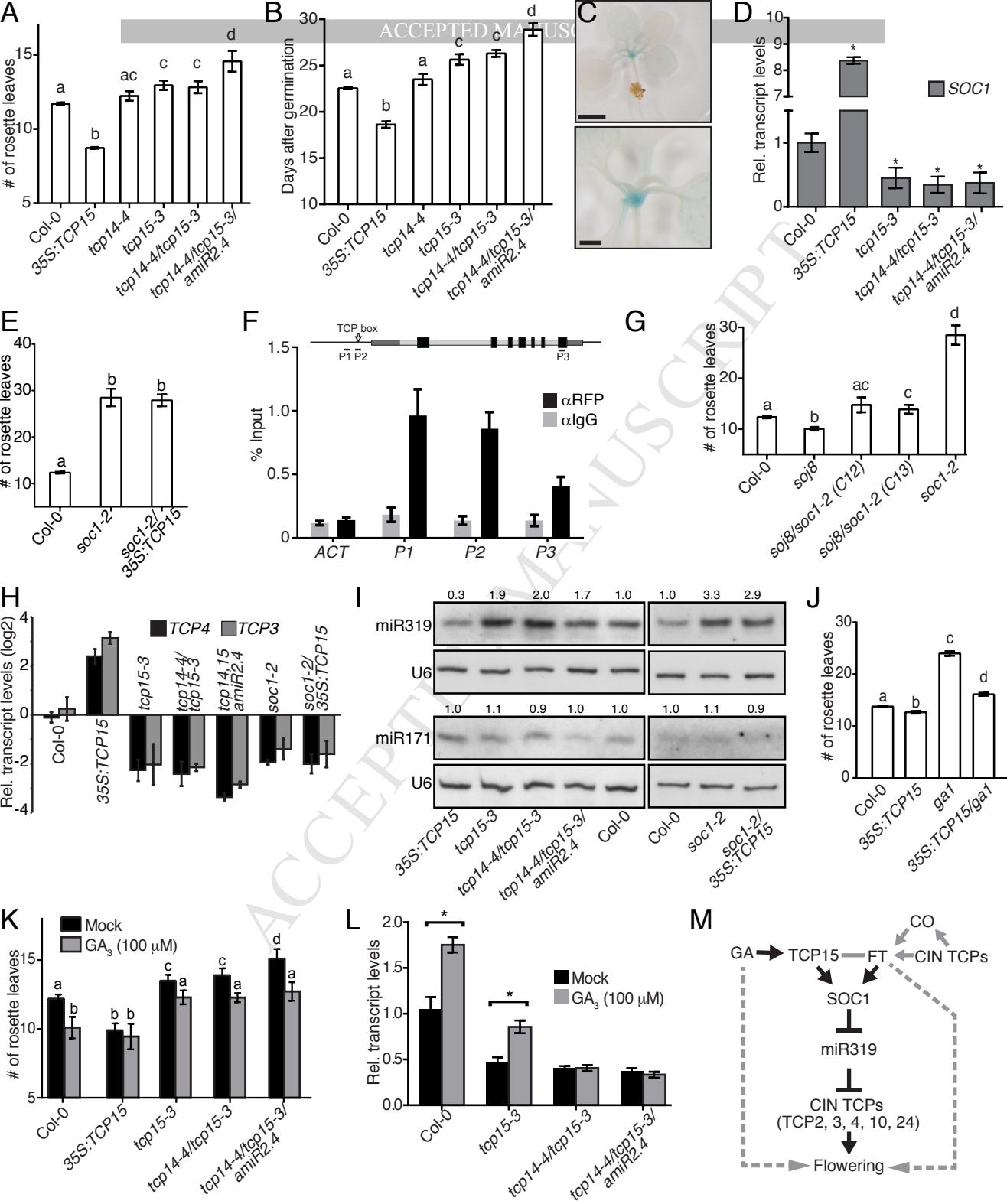
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