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APETALA1 establishes determinate floral meristem through regulating cytokinins homeostasis in *Arabidopsis*

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In flowering plants, floral determinacy requires termination of stem cells in the center of floral meristems (FMs), and in the axils of outer floral organs, such as sepals. In the center of the FM, *AGAMOUS* together with other factors represses *WUSCHEL* expression to terminate stem cell activities.[1] In the outermost whorl, *APETALA1* (*AP1*) terminates sepal axil stem cell activities to suppress axillary secondary flower formation.[2-4] Recently, we reported that low cytokinin levels was required in *AP1*-expressing cells to suppress sepal axillary secondary flower formation. Furthermore, we showed that the MADS-box transcription factor encoded by floral homeotic gene *AP1* regulated cytokinins homeostasis by directly activating the cytokinin degradation gene *CYTOKININ OXIDASE/DEHYDROGENASE3* (*CKX3*), and suppressing the cytokinin biosynthetic gene *LONELY GUY1* (*LOG1*). Altogether, our experimental data provided a mechanistic explanation for how axillary meristem activity is suppressed in FMs.

The indeterminate growth inflorescence meristem (IM) produces floral meristems (FMs) during reproductive stage. In contract, determinate FMs produce flowers of a particular size and form by termination of stem cell divisions in the meristem. *AP1* activates floral organ identity genes to promote FM formation together with *LEAFY* and *CAULIFLOWER*.[3,5-7] After FM formation, *AP1* functions as a class A gene of the ABC model for floral organ identity determination to specify outer whorls of floral organs petals and sepals.[8] Inflorescence-like phenotypes in *ap1* flowers (**Fig. 1**) indicated that *AP1* also prevents the formation of flowers in the axils of sepals to establish determinate FMs.[2,4] But how *AP1* terminates sepal axil stem cell activities to suppress axillary flowers formation?

We have recently shown that elevated cytokinin signaling is required for leaf axil axillary meristem formation.[9] In flowers, we further showed that ectopic cytokinins promoted sepal axil stem cell activities to induce secondary flowers.[9,10] When we elevated cytokinin levels in flowers by expressing the *Arabidopsis adenosine phosphate-isopentenytransferase 8* (*IPT8*) gene from the *AP1* promoter in wild-type plants, we observed sepal axil secondary flowers that phenocopied *ap1* mutants. To understand if *AP1* maintain flower determinacy by regulating cytokinin homeostasis and/or signaling, we tested cytokinin signaling and cytokinin levels in wild-type and *ap1* flowers. To this end, *pTCS::GFP-ER*, a synthetic cytokinin signaling reporter, was introduced into the *ap1-1* background. We observed an elevated GFP signal in *ap1-1* flowers than in wild-type sibling flowers around the stages when secondary flowers initiate. Furthermore, we measured endogenous levels of 4 types of cytokinins, specifically zeatin riboside 5′-monophosphate, zeatin riboside, isopentenyladenine, and isopentenyladenine riboside, in young inflorescences. All four quantified cytokinins increased in *ap1* mutants. Conversely, disruption of the cytokinin signaling pathway by introducing cytokinin receptor mutations into the *ap1-1* mutant rescued the ectopic secondary flower phenotype.

Because *AP1* encodes a MADS domain transcription factor, it is possible that AP1 directly or indirectly regulates cytokinin biosynthesis and/or degradation. A scouting of recent genome-wide binding data for AP1 and *AP1* domain-specific expression data showed that a cytokinin biosynthetic gene *LONELY GUY1* (*LOG1*), and a cytokinin degradation gene *CYTOKININ OXIDASE/DEHYDROGENASE3* (*CKX3*) are both good AP1 downstream candidates.[11,12] Using chromatin immunoprecipitation assay and transient transfection assay in protoplasts, we found that AP1 directly bound to both *LOG1* and *CKX3* promoter regions containing CArG motifs, which are canonical MADS domain transcription

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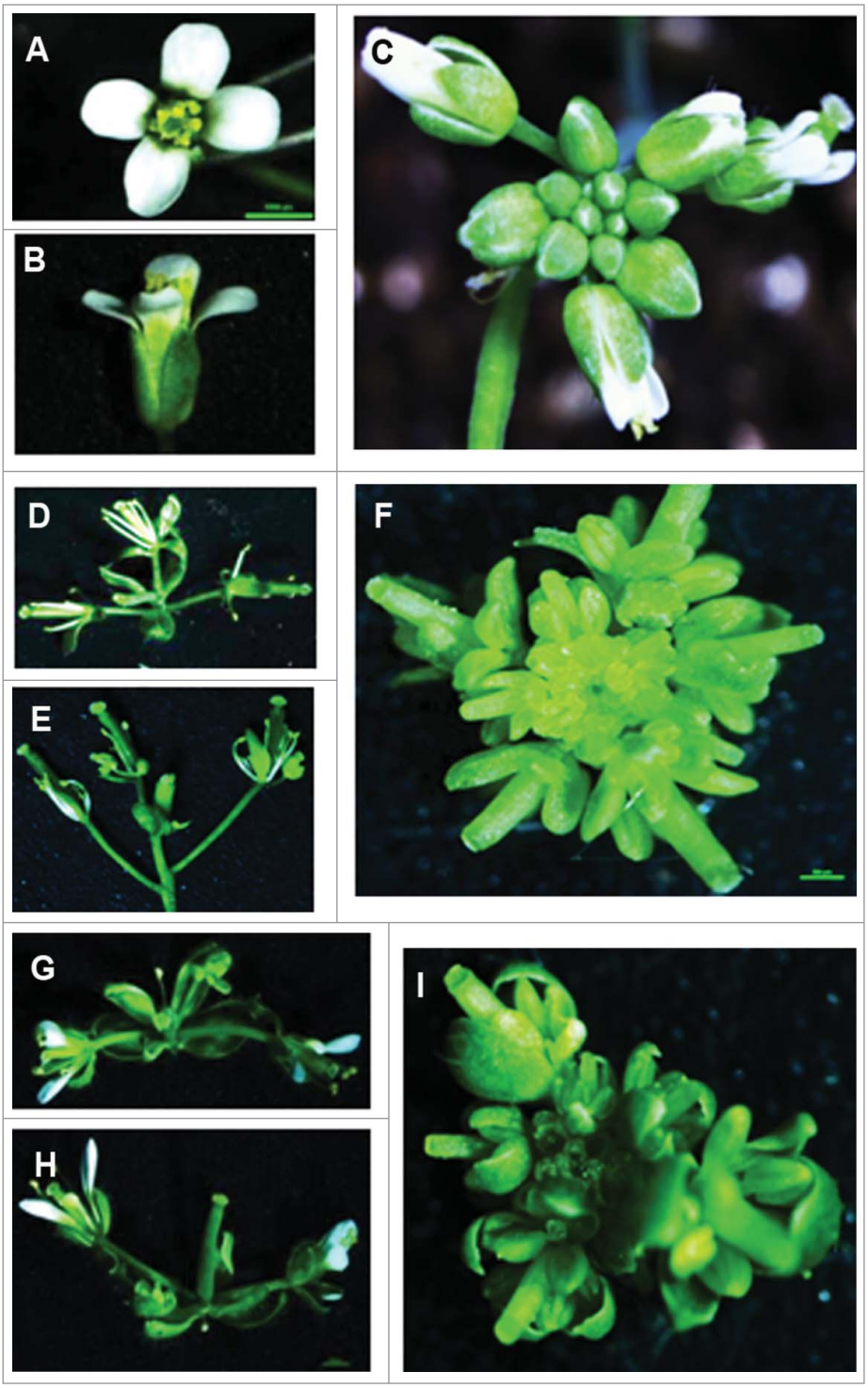

Figure 1. Sepal axil secondary flower phenotype in *ap1* mutants. Vertical view of a wild type flower in (**A**), lateral view of a wild type flower in (**B**), and top view of a wild type inflorescence in (**C**). Vertical view of an *ap1-1* flower in (**D**), lateral view of an *ap1-1* flower in (**E**), and top view of an *ap1-1* inflorescence in (**F**). Vertical view of an *ap1-4* flower in (**G**), lateral view of an *ap1-4* flower in (**H**), and top view of an *ap1-4* inflorescence in (**I**). Note ectopic sepal axil secondary flowers in *ap1* mutants.

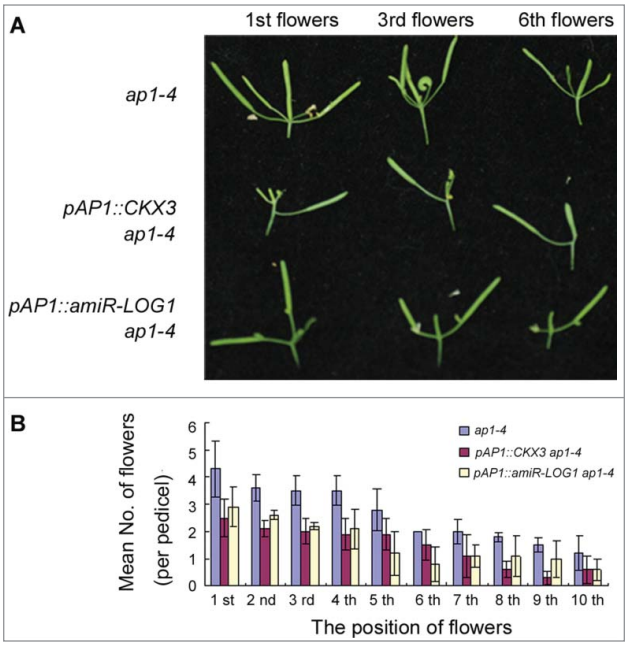

Figure 2. Partial rescue of the *ap1* axil flower phenotype by local manipulation of *CKX3* and *LOG1* expression. (**A**) Siliques phenotypes of *ap1-4*, *pAP1::CKX3 ap1-4* and silencing *pAP1::LOG1 ap1-4*. Siliques were taken at the same position of inflorescence. (**B**) Mean number of flowers (per pedicel) of *ap1-4*, *pAP1::CKX3 ap1-4* and *pAP1::amiR-LOG1 ap1-4* plants. Blue represents wild-type, purple represents *pAP1::CKX3 ap1-4* and white represents *pAP1::amiR-LOG1 ap1-4*.

factors. Chemical inducible AP1-glucocorticoid-receptor fusion also confirmed that AP1 regulation of *LOG1* and *CKX3* expression did not require protein synthesis, and showed that AP1 suppressed *LOG1* expression but activated *CKX3* expression. We further altered *LOG1* and *CKX3* expression in an *ap1* background to short-circuit AP1 regulation. To this end, we suppressed *LOG1* expression in the *AP1* expressing domain using an *LOG1*-specific artificial miRNA, or over expressed *CKX3* under the *AP1* promoter in the *ap1-4* mutant. In transgenic *ap1-4* mutant lines containing either an *pAP1::amiR-LOG1* or an *pAP1::CKX3* transgene, we found partial rescue of the sepal axil secondary flower phenotype (**Fig. 2**). Thus, the AP1 regulation of *LOG1* and *CKX3* expression is relevant to sepal axil secondary flower formation.

Transformation of floral meristems into inflorescence meristem has profound impact on seeds yield. The conversion of a single flower into a dichasium or a pleiochasium inflorescence is seen in many plant species, including cauliflower and broccoli. The cytokinin-mediated AP1 regulation is likely conserved in these vegetable crops to control their inflorescence architecture.[6]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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