

SHORT COMMUNICATION



AUXIN RESPONSE FACTOR3 plays distinct role during early flower development

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ABSTRACT

AUXIN RESPONSE FACTOR3 (ARF3), one of the auxin response factors family of transcription factors, is well characterized by its functions in polarity identification and organ patterning. We recently demonstrated that ARF3 plays important roles in floral meristem (FM) maintenance and termination by regulating cytokinin biosynthesis and signaling. However, the relationship of its multiple roles in differently developmental stage is still unclear. Here, we present data that ARF3 plays distinct roles during early flower development that are different from its roles in organ polarity determination and patterning. Thus, our findings shed light on the functional diversity of one specific transcription factor in plant development.

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Auxins, one classical plant hormone, are well-reported as a regulator in controlling many aspects of plant growth and development, such as embryogenesis, organogenesis, tropic growth and organ architecture.^{1,2} Many genes are involved in the biosynthesis, transporter, signal transduction and metabolism to mediate the function of auxin.^{3,4} AUXIN RESPONSE FACTORS (ARFs) are group of transcription factors to transduce the auxin signal by interacting with Aux/IAAs proteins.^{1,5} Each ARF has own distinct expression patterns and functions in various developmental processes.⁶ ARF3, also known as ETTIN (ETT), is well characterized by its roles in organ polarity, gynoecium patterning, self-incompatibility and *de novo* organ regeneration.^{7–10} Recently, we found that ARF3 is important for the shoot apical meristem (SAM) maintenance as well as floral meristem (FM) maintenance and programmed termination.¹¹ Further molecular mechanism analysis revealed that ARF3 mediated the functions of AGAMOUS (AG) and auxin to repressing cytokinin biosynthesis and signaling and then, to regulate the expression of cell-cycle genes and WUSCHEL (WUS), a critical gene for stem cells maintenance.¹² However, the relationship of the role of ARF3 in meristem activity and its other roles in plant development is unclear.

Previous studies showed that ARF3 physically interacts with KANADI (KAN) protein family to determine organ asymmetry including leaf polarity and gynoecium patterning.^{13–15} KAN proteins are group of nuclear-localized proteins in the GARP family of putative transcription factors required for the organ polarity identity.¹³ KAN1 and KAN2 were found to be involved in the establishment of polarity of most lateral organs including leaf and flower organs such as sepal, petal, stamen and carpel.¹⁶

Moreover, KAN1 acts as a transcriptional repressor to regulate auxin biosynthesis, transport and signaling.¹⁷ Given that the functional complexes duo to the physical interaction between ARF3 and KANs are essential for the integument development and polarity determination,¹⁵ we are curious to know the correlation of the functions of ARF3 in FM maintenance and polarity determination during the early flower development since we detected that the expression of some genes involved in polarity were down-regulated after ARF3 induction.¹² Although the developmental defect of gynoecia patterning is serious in *arf3* mutant compared with *Ler*, the other floral 7organs such as sepal, petal and stamen are produced normally without organ patterning defects (Figures 1A and 1B), even in *arf3 arf4* double mutant,¹⁴ indicating that the function of ARF3 in meristem maintenance and termination is distinct from its role in organ polarity of gynoecium establishment during early flower development (stages 1–6) when the organ identity and patterning of sepal, petal and stamen are determined. This is consistent with the findings that the distribution of ARF3 protein is symmetric even through the RNA distribution of ARF3 is complex in SAM and early FM meristem.^{11,18} We further introduced the *kan1 kan2* mutants into *ag-10* background by crossing to examine the functions of KAN1 and KAN2 in FM meristem activity. While the flowers of *kan1 kan2* double mutant showed serious floral organ polarity defect in all floral organs (Figure 1C),¹⁶ the FM of *kan1 kan2* was properly terminated after the generation of carpel gynoecium (Figure 1C). Compared with *ag-10* flower and *ag-10 arf3* flowers with FM determinacy defect showing bulged gynoecium (Figures 1D and 1E), the *ag-10 kan1 kan2* flowers displayed normal FM determinacy phenotype (Figure 1F), indicating that

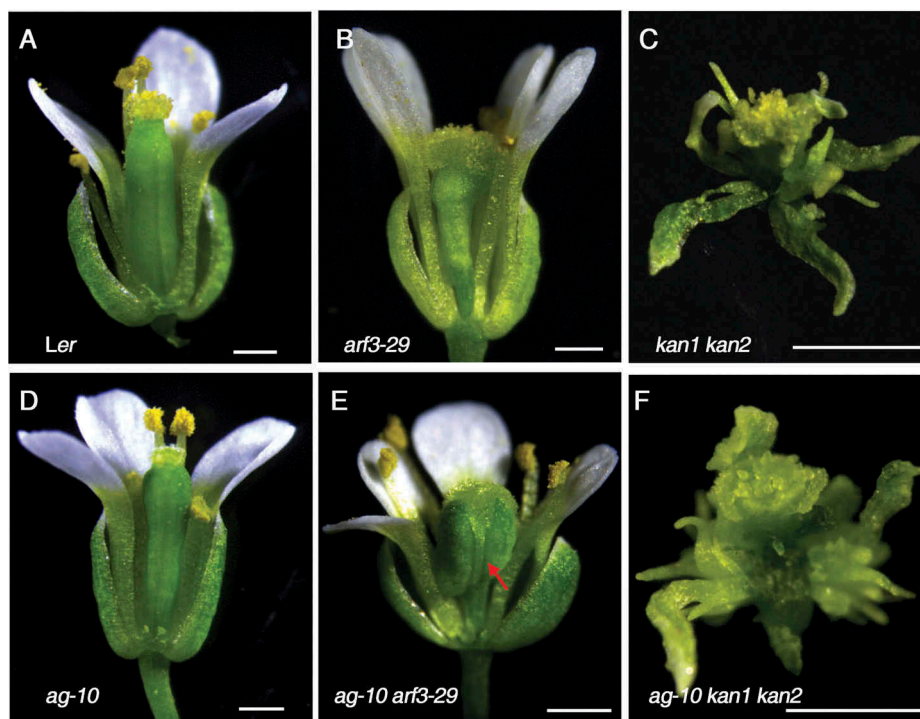


Figure 1. Floral meristem (FM) determinacy and organ polarity phenotype of *Ler* (A), *arf3-29* (B), *kan1 kan2* (C), *ag-10* (D), *ag-10 arf3-29* (E) and *ag-10kan1 kan2* (F). *ag-10 arf3-29* displayed FM indeterminacy phenotype showing bulged gynoecium (marked by red arrow). *kan1 kan2* and *ag-10 kan1 kan2* flowers showed normal FM determinacy and serious organ polarity defect in all floral organs, while *arf3-29* only show organ polarity defect in gynoecium. One or two sepal, petals and stamens were removed for better view. Scale bars, 0.5mm.

the KNAs-ARF3 complex is not responsible for the FM determinacy, In the other words, the function of *ARF3* in organ patterning is irrelevant to its function in FM determinacy.

To globally view the function of *ARF3* in regulating gene expression during early flower development, we performed RNA-seq analysis using *ARF3:ARF3-GR arf3-29* under the dexamethasone (DEX) treatment since *ARF3-GR* induction with DEX treatment could fully rescue the developmental defects of *arf3-29*.¹² Compared to the plants treated by DMSO as control, the DEX-treated plant harbored 1881 up-regulated genes and 1259 down-regulated genes,¹² indicating that *ARF3* plays dual roles in regulating gene expression in early flower development, which is in line with the finding in

recent report.¹⁹ In that study, the *ett-3* mutant was used for RNA-seq analysis to examine the function of *ARF3* in gynoecium development.¹⁹ Using same standards (Fold Change >2, FDR (false discovery rate)<0.01), we analyzed the global gene expression in *ett-3* vs. WT and isolated 377 down-regulated and 361 up-regulated genes in *ett-3* compared to WT (Supplemental data 1 and 2). Further analysis revealed that there are only 63 genes overlapped between the up-regulated genes in DEX-treated *ARF3:ARF3-GR arf3-29* plant and the down-regulated genes in *ett-3* mutant (those genes are *ARF3*-activated genes) and 10 genes overlapped between the down-regulated genes in DEX-treated *ARF3:ARF3-GR arf3-29* plant and the up-regulated genes in *ett-3* mutant (those genes are

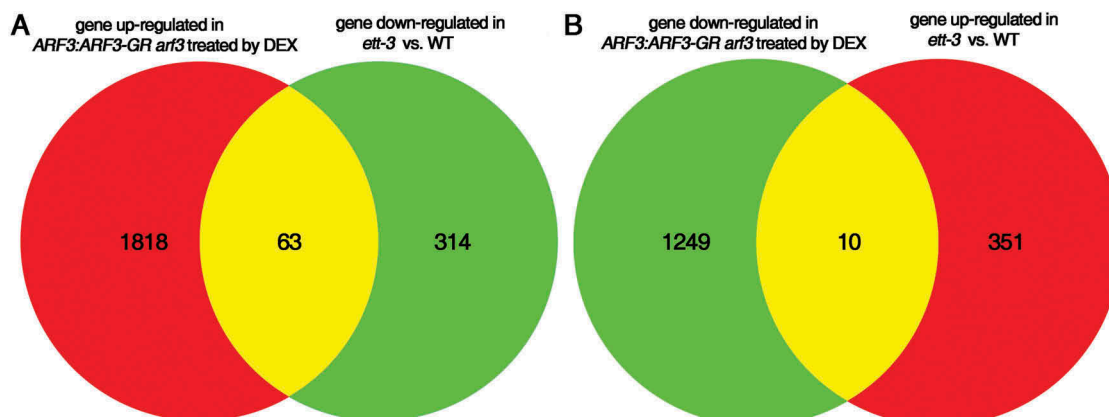


Figure 2. RNA-seq analysis of *ARF3* regulated genes in floral meristem development and gynoecium development. *ARF3* up-regulated genes (A) and down-regulated genes (B) in early flower development and gynoecium development were analyzed. The numbers of altered genes are indicated in the pie chart.

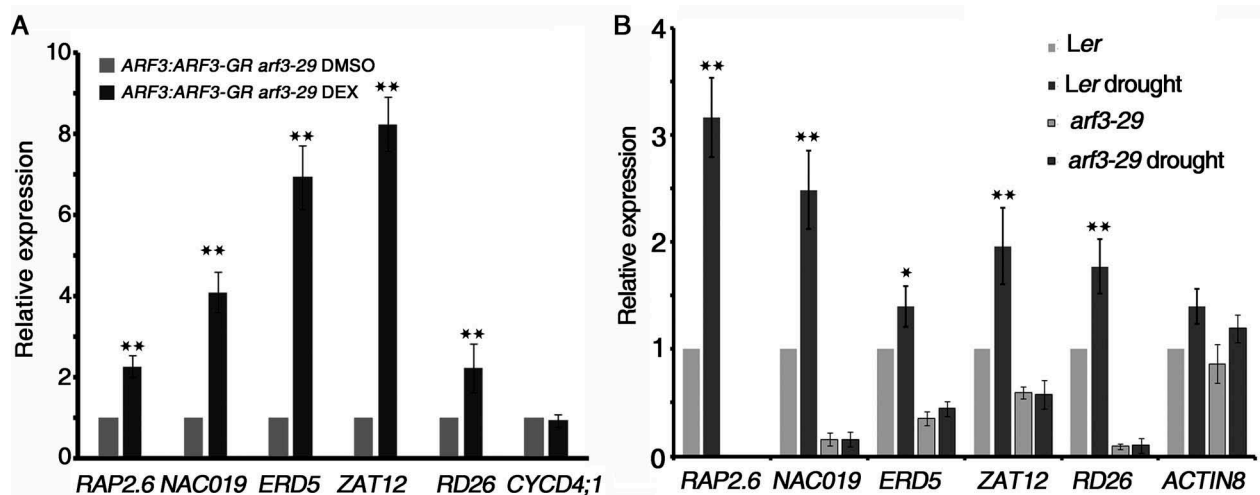


Figure 3. *ARF3* mediates the drought stress response during early flower development. (A) The expression of drought stress related genes after *ARF3* induction measured by real-time RT-PCR. (B) The gene expression of indicated genes in *Ler* and *arf3-29* under the drought stress condition. Three biological replicates were performed. Error bars represent SD from three biological repeats. * $P < 0.05$ and ** $P < 0.01$.

ARF3-repressed genes) (Figures 2A and 2B), indicating that *ARF3* plays dramatically different roles during early flower development and gynoecium development.

While the GO terms related developmental processes were enriched in the *ARF3*-repressed genes, GO terms related response to stimuli, particularly to environmental stimuli such as heat, salt, light, and insects, were highly enriched among the *ARF3*-activated genes.¹² We then proposed that *ARF3* may exert activating effects on the different sets of stress-response genes to increase the stress resistance of FM during early flower development. To test the assumption, we treated the *arf3-29* mutant with drought along with WT. One week old seedlings of mutant and WT were transferred from MS to pots containing a well-watered vermiculite and humus mix, and grown for one more week. Thereafter, water was withheld for two weeks to simulate drought stress treatment as previously described.²⁰ The inflorescences containing stage 6 and younger flowers were collected for realtime-PCR analysis. The genes related to drought stress response such as *RAP 2.6*, *NAC019*, *ERD5*, *ZAT12* and *RD26* were up-regulated in DEX-treated *ARF3:ARF3-GR arf3-29* plant under normal condition (Figure 3A). Under the drought stress, those genes were up-regulated in WT showing their functions in drought stress response (Figure 3B). However, the expression of those genes were decreased in *arf3-29* mutant and remained in very lower expression level even under the drought stress condition, indicating that *ARF3* is important for those genes expression responding to drought stress during early flower development (Figure 3B). Thus, the present study, particularly combined with our recent findings,^{11,12} revealed that *ARF3* plays distinct roles during early flower development.

Abbreviations

AG	AGAMOUS
ARF3	AUXIN RESPONSE FACTOR3
DEX	dexamethasone
ETT	ETTIN
FDR	false discovery rate

FM	Floral Meristem
KAN1	KANADI1
KAN2	KANADI2
SAM	Shoot Apical Meristem
WUS	WUSCHEL

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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