SDG2 regulates Arabidopsis inflorescence architecture through SWR1-ERECTA signaling pathway

Highlights
- SDG2 genetically interacts with the SWR1-ERECTA pathway in inflorescence development
- SWR1-ERECTA pathway is required to enrich H2A.Z and H3K4me3 at auxin-related genes
- H2A.Z histone variant enrichment was regulated by SDG2
SUMMARY

Inflorescence architecture is diverse in flowering plants, and two determinants of inflorescence architecture are the inflorescence meristem and pedicel length. Although the ERECTA (ER) signaling pathway, in coordination with the SWR1 chromatin remodeling complex, regulates inflorescence architecture with subsequent effects on pedicel elongation, the mechanism underlying SWR1-ER signaling pathway regulation of inflorescence architecture remains unclear. This study determined that SDG2 genetically interacts with the SWR1-ER signaling pathways in regulating inflorescence architecture. Transcriptome results showed that auxin might potentially influence inflorescence growth mediated by SDG2 and SWR1-ER pathways. SWR1 and ER signaling are required to enrich H2A.Z histone variant and SDG2 regulated SDG2-mediated H3K4me3 histone modification at auxin-related genes and H2A.Z histone variant enrichment. Our study shows how the regulation of inflorescence architecture is mediated by SDG2 and SWR1-ER, which affects auxin hormone signaling pathways.

INTRODUCTION

Inflorescence architecture is determined by the Spatio-temporal arrangement of flowers on a stem, which directly affects seed production and is complex and delicate. In recent years, much attention has been drawn to plant inflorescence architecture, which is a key element to improving crop yield and grain quality. The inflorescence architecture depends on its branching pattern and the position of the flowers, in other words, when and where flowers are formed (Benlloch et al., 2007). The appearance of each inflorescence type varies depending on the arrangement of lateral meristems around the stem (phyllotaxy), the pattern of internode lengths, and additional variations on the three architectural themes (Prusinkiewicz et al., 2007).

In Arabidopsis, the shoot apical meristem (SAM) is a vegetative meristem that produces leaves and branches, is an open-raceme, where the flower formed from the flanks of the inflorescence SAM (Amasino and Michaels, 2010; Fernandez-Noahales et al., 2014; Huijser and Schmid, 2011).

Some genes involved in regulating plant inflorescence architecture have been reported in recent years. For example, Jatropha ADENOSINE KINASE 2 (JcADK2), ADENINE PHOSPHORIBOSYLTRANSFERASE 1 (JcAPT1) and CYTOKININ OXIDASE 3 (JcCKX3) which control inflorescence branching in Jatropha by regulating cytokinin metabolic pathway (Chen et al., 2019a). Panicle Morphology Mutant 1 (PMM1), a new allele of DWARF11 (D11), was identified that might have potential roles in affecting the differentiation of spikelet primordia and patterns of panicle branches in rice through BRs biosynthesis (Li et al., 2018). The transcription factors encoded by SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) gene family and the miR156-SPL module regulate plant growth and architecture. In Arabidopsis, miR156 regulated transcription factors AtSPL3, AtSPL4, and AtSPL5 show a high sequence identity and promoted the floral meristem identity transition (Cui et al., 2020; Prusinkiewicz et al., 2007; Wu and Poethig, 2006; Xu et al., 2016). Furthermore, miR156a influences inflorescence architecture in tomatoes by suppressing the SPL transcription factor gene family (Zhang et al., 2011). Further investigation indicated that SPL13 was the major SPL involved in miR156a-regulated tomato inflorescence architecture (Cui et al., 2020). A mitogen-activated protein kinase (MPK) cascade has previously been indicated to function downstream of the ER receptor in regulating localized cell proliferation in Arabidopsis (Meng et al., 2012). The evolutionarily conserved SWR1 complex plays a crucial role in several biological processes by catalyzing H2A.Z deposition in nucleosomes (Aslam et al., 2012).
Histone H3 lysine 4 trimethylation (H3K4me3) is a prominent histone methylation mark, acting as an active transcription mark in the epigenetic regulation of gene expression (Guo et al., 2010). The histone methylation is mediated by the histone lysine (K) methyltransferases (HKMTases) (Zhou et al., 2020), and many of HKMTases have been identified in plants and animals (Alvarez-Venegas and Avramova, 2002; Baumbusch et al., 2001; Guo et al., 2010; Springer et al., 2003; Zhao and Shen, 2004). Among these, HKMTases with a SET domain are called the SET domain group (SDG) proteins (Guo et al., 2010; Springer et al., 2003; Zhao and Shen, 2004), and only SDG proteins can work as HKMTases in plants and animals (Alvarez-Venegas and Avramova, 2002; Baumbusch et al., 2001; Guo et al., 2010; Springer et al., 2003; Zhao et al., 2005). For example, SDG721 and SDG705 encode TRITHORAX-like proteins, which appear to modulate H3K4 methylation levels. Loss of SDG721 and SDG705 function resulted in reduced panicle branching in rice (Jiang et al., 2018). SDG2, a member of a novel class of H3K4methyltransferases (Yun et al., 2012), has been reported to affect sporophyte and gametophyte development (Berr et al., 2010b) and is involved in the regulation of stem cell activity in Arabidopsis (Yao et al., 2013). Although the pleiotropic roles of SDG2 in Arabidopsis development are known, SDG2 function in the regulation of inflorescence architecture has remained largely unclear. In recent years it has been shown that H2A.Z deposition and histone methylation affects gene expression in a genome-wide manner in different organisms (Dai et al., 2018; Hu et al., 2013). Our previous data showed that the accumulation of anthocyanin in H2A.Z deposition-deficient mutants is associated with increased H3K4me3. This data revealed an antagonistic relationship between H2A.Z deposition and H3K4me3 in regulating the expression of anthocyanin biosynthesis genes. However, little is known about the roles of these two types of epigenetic regulation marks in the control of inflorescence architecture.

This study shows that SDG2 genetically interacts with the SWR1 and ER-MPK6-PREs pathways in regulating inflorescence architecture. Furthermore, transcriptome data indicate that auxin may potentially influence inflorescence growth mediated by SDG2 and SWR1-ER-PREs. SWR1 and ER signaling are required to enrich the H2A.Z histone variant and SDG2-mediated H3K4me3 histone modification at auxin-related genes. SDG2 also regulated enrichment of the H2A.Z histone variant. Our study reveals how H2A.Z, H3K4me3, and ER signaling mutually regulate inflorescence architecture by affecting auxin hormone signaling pathways.

RESULTS

**SDG2 is involved in the SWR1 and ER signaling pathway in regulating inflorescence architecture**

Previously we showed that the H3K4me3 level of PRE1 was altered in arp6 er-119 (Cai et al., 2017), and H2A.Z can coordinate with H3K4me3 to regulate gene’s expression (Cai et al., 2020; Dai et al., 2018). arp6 is a knockout line of Actin related protein 6 (ARP6) that encodes a subunit of SWR1 complex (Choi et al., 2005). To test if H3K4me3 also regulates inflorescence architecture, we first examined the inflorescence architecture of sdg2. SDG2 is a coenzyme that mediates H3K4me3 in Arabidopsis and sdg2 displays severely reduced H3K4me3 levels (Guo et al., 2010). sdg2 mutants show clustered inflorescence architecture phenotype (Figure 1A) is associated with a reduced pedicel length (Figures 1B–1D) and reduced cortex

al., 2019; Kumar, 2018). Our recent results showed that the basic helix-loop-helix (bHLH) transcription factor PACLOBUTRAZOL RESISTANCE1 (PRE1) acts downstream of ER-MPK signaling cascade regulating inflorescence architecture. In addition, the ATP-dependent chromatin remodeling complex SWR1 plays a crucial role in controlling the ER-MPK-PREs signaling pathway (Cai et al., 2017). Genetic interactions between ER signaling and the chromatin remodeling complex SWR1 in the control of inflorescence architecture were studied (Cai et al., 2017). Further investigation showed that HOMOLOG OF BEE2 INTERACTING WITH IBH1 (HBI1) functions downstream of the ER-MPK-PREs signaling pathway and regulates the inflorescence architecture via affecting the brassinosteroid (BR) biosynthesis and auxin signaling pathway (Cai et al., 2020). HBI1 directly binds to the promoters of the BR biosynthesis gene CYP85A2 and a series of auxin-related genes including ARF3 to promote their expression and regulate pedicel cortex cell proliferation and pedicel elongation. In turn, ARF3 can also bind to these auxin-related genes and CYP85A2 and activate their expression, consistent with the role of ARF3 as a master regulator that establishes a feedback loop of auxin signaling. The findings also show how inflorescence architecture regulation mediated by the SWR1-ER pathways involves the HBI1 regulatory hub, which affects both the BR and auxin hormone signaling pathways (Cai et al., 2020).
Figure 1. SDG2 Involvement in the regulation of inflorescence architecture and pedicel elongation in Arabidopsis

(A) Inflorescence stems apices of WT, arp6, er-119, arp6 er-119, sdg2, arp6 sdg2, er-119 sdg2, and arp6 er-119 sdg2 plants. Bars, 5mm. Fully open mature flowers and attached pedicels of the indicated genotype. Bar, 10mm.

(B) Lengths of mature pedicels of fully open flowers from the main stems of 4-to5-wk-old plants. Bars represent average values ± SD (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.

(C) Distribution of the internode length between two successive siliques. Ten internodes between the 1st and 11th siliques were analyzed for 10 pedicels per genotype.

(D) Longitudinal sections of mature pedicels from fully open flowers of WT, arp6, er-119, arp6 er-119, sdg2, arp6 sdg2, er-119 sdg2, and arp6 er-119 sdg2 plants. Co, cortex; Ep, epidermis. Bars, 10μm.

(E) Cell numbers in the longitudinal cortex file of mature pedicels from fully open flowers (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.

(F) Quantitative analysis of cortex cell length. Bars represent average values ± SD (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.
cell numbers compared with WT (Figures 1E and 1F). The cortex cell length in sdg2 mutant is comparable to WT (Figure 1G). These phenotypes are similar to those described for mutants of the SWR1-ER signaling pathway, which exhibited reduced pedicel length caused by defective cell proliferation in the pedicel cortex (Cai et al., 2017). We next crossed sdg2 with arp6, er-119 single and arp6 er-119 double mutants. arp6 sdg2, er-119 sdg2 double mutants showed significantly enhanced compact inflorescence architecture compared to arp6 and er-119 single mutants, respectively (Figure 1). In addition, arp6 er-119 sdg2 triple mutants showed significantly enhanced compact inflorescence architecture compared to arp6 er-119 double mutant (Figure 1). The compact inflorescence architecture in arp6 sdg2, er-119 sdg2 double mutants and arp6 er-119 sdg2 triple mutants were associated with short pedicel lengths (Figures 1B–1D), reduced pedicel cell number in the cortex and the pedicel epidermal cell length compared to WT, arp6, er-119, and sdg2 mutants, respectively (Figures 1E and 1F; S1). To investigate whether SDG2 acts downstream of SWR1-ER regulating inflorescence architecture, we crossed pSDG2:myc-SDG2 into arp6 er-119. In the F2 generation, individual arp6 er-119 plants carrying the pSDG2:myc-SDG2 transgene were obtained and all exhibited a partially rescued compact inflorescence (Figure 2A). The defect pedicel length could be partially rescued compared to arp6 er-119 (Figures 2B–2D) accompanied with significantly increased pedicel cortex cell number (Figures 2E–2G). These data suggested that SDG2 is involved in SWR1 and ER-dependent regulation of inflorescence architecture.

**SDG2 is involved in the PREs-controlled inflorescence architecture**

Previous studies have demonstrated that PREs transcription factors are involved in flower development and petiole/hypocotyl elongation in Arabidopsis (Shin et al., 2019). However, little is known about SDG2 and its effect on inflorescence architecture in Arabidopsis. Our data indicate that SDG2 is involved in SWR1-ER signaling pathway regulating inflorescence architecture (Figures 1 and 2). Our previous results have shown PREs shared overlapping functions downstream of SWR1-ER in regulating inflorescence architecture (Cai et al., 2017). Previous studies showed that the levels of H3K4me3 was a severe decrease in sdg2 mutant, and the PRE1 expression level was also a severe decrease in sdg2 mutant compared to WT (Guo et al., 2010). To determine whether the levels of H3K4me3 were regulated by SDG2 and SWR1-ER pathway, we assessed the levels of H3K4me3 by Western blotting using proteins extracted from WT, sdg2, and arp6 er-119 sdg2 floral buds. The analysis revealed a severe decrease in the cellular level of H3K4me3 in sdg2 single mutant and a more severe decrease in arp6 er-119 sdg2 triple mutant (Figure 3A). The enrichment of H3K4me3 in PREs transcription start site (TSS) was significantly reduced in er-119 and sdg2 and reduced even further in er-119 sdg2, arp6 er-119, arp6 sdg2 and arp6 er-119 sdg2 compared to WT and arp6 (Figure 3B). These results indicated that SDG2-mediated H3K4me3 was regulated by the SWR1-ER pathway and PREs were activated by SWR1-ER and SDG2 pathway. To investigate whether SDG2 also interacts genetically with PREs in regulating inflorescence architecture, we first performed qRT-PCR to compare PREs transcript levels in WT, arp6, er-119, sdg2, er-119 sdg2, arp6 er-119, arp6 sdg2, and arp6 er-119 sdg2 mutants. The PREs levels were significantly reduced in er-119 and sdg2 and reduced even further in er-119 sdg2, arp6 er-119, arp6 sdg2, and arp6 er-119 sdg2 compared to WT and arp6 (Figure 3C). Next, we crossed sdg2-1 with pre-amIR. Similar to er-119 sdg2 and arp6 er-119 sdg2 plants, the sdg2 pre-amIR mutant showed further reduced pedicels and internode lengths and more compact inflorescences than the sdg2 and pre-amIR single mutants (Figures 4A–4D), reduced pedicel cell number in the cortex and the pedicel epidermal cell length (Figures 4E–4G and S1). Taken together, these results suggest that SDG2 interacts genetically with PREs in regulating inflorescence architecture by promoting cell proliferation in the pedicel.

**Transcriptome data reveals potential roles of phytohormones in inflorescence growth mediated by the SWR1-SDG2-PREs pathway**

To further explore the potential mechanism of inflorescence growth regulated by SWR1-SDG2-PREs pathway, we analyze transcriptome data of WT, arp6 sdg2, pre-amIR inflorescence by RNA-seq, and the results showed that there were 448 commonly down-regulated and 98 commonly up-regulated genes in arp6 sdg2 and pre-amIR compared to WT (Figures 5A and 5B). Gene ontology (GO) enrichment analysis showed that the down-regulated genes in arp6 sdg2 and pre-amIR compared to WT were enriched in chemical stimulus, abiotic stimulus, organic substance and hormone stimulus (Figure 5C). The upregulated genes in arp6 sdg2 and pre-amIR compared to WT were enriched in metabolic and biosynthetic processes (Figure 5D). Previous research supports that phytohormones are linked to plant development, particularly concerning pedicel elongation and inflorescences architecture. We previously demonstrated that auxin plays a role in inflorescence growth mediated by SWR1-ER-PREs signaling pathway (Cai et al., 2017).
**Figure 2.** SDG2 is involved in SWR1-ER signaling pathway in regulating inflorescence architecture

(A) Inflorescence stem apices of WT, arp6, er-119, pSDG2:myc-SDG2, arp6 er-119, and arp6 er-119 pSDG2:myc-SDG2 plants. Bars, 5mm.

(B) Fully open mature flowers and attached pedicels of the indicated genotype. Bar, 10mm.

(C) Lengths of mature pedicels of fully open flowers from the main stems of 4-to-5-wk-old plants. Bars represent average values ± SD (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.

(D) Distribution of the internode length between two successive siliques. Ten internodes between the 1st and 11th siliques were analyzed for 10 pedicels per genotype.

(E) Longitudinal sections of mature pedicels from fully open flowers of WT, pSDG2:myc-SDG2, arp6 er-119, and arp6 er-119 pSDG2:myc-SDG2 plants. Co, cortex; Ep, epidermis. Bars, 10μm.

(F) Cell numbers in the longitudinal cortex file of mature pedicels from fully open flowers (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.

(G) Quantitative analysis of cortex cell length. Bars represent average values ± SD (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.
Considering the enrichment of hormone-regulated genes among the down-regulated genes, we generated an expression heatmap of these genes in *arp6* *sdg2* and pre-amiR compared to WT. Auxin-related genes with significantly reduced *arp6* *sdg2* and pre-amiR mutants include auxin-induced SAURs, ARGOS, auxin influx carrier IAA5, auxin efflux carriers PIN3, and auxin transporter PIL5, and GA/-BR-regulated genes (Figure 5E). To verify the transcriptome, we performed qRT-PCR analysis and could verify the
Figure 4. SDG2 is involved in the PREs-controlled inflorescence architecture

(A) Inflorescence stems apices of WT, pre-amiR, sdg2, sdg2 pre-amiR plants. Bars, 5 mm.
(B) Fully open mature flowers and attached pedicels of the indicated genotype. Bar, 10 mm.
(C) Lengths of mature pedicels of fully open flowers from the main stems of 4-to5-week-old plants. Bars represent average values ± SD (n = 10 pedicels per genotype). Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.
(D) Distribution of the internode length between two successive siliques. Ten internodes between the 1st and 11th siliques were analyzed for 10 pedicels per genotype.
(E) Longitudinal sections of mature pedicels from fully open flowers of WT, pre-amiR, sdg2, sdg2 pre-amiR plants. Co, cortex; Ep, epidermis. Bars, 10μm.
H2A.Z deposition at auxin-related genes were altered in arp6 er-119, arp6 sdg2 and arp6 er-119 sdg2

H2A.Z deposition has a conserved role in the various cellular processes through its function in chromatin structure and dynamics in eukaryotes (Kumar, 2018). The replacement of H2A by H2A.Z in nucleosomes depends on ATP-dependent chromatin remodeling complex SWR1 (Mizuguchi et al., 2004). In preceding results, we found that the relative expression level of auxin-related genes was significantly decreased in arp6 sdg2 and pre-amiR compared to WT. To further clarify how H2A.Z deposition affects SDG2 function in inflorescence architecture via SWR1-SDG2-PREs signaling pathway. We performed chromatin immunoprecipitation (ChiP) experiment using an H2A.Z antibody of WT, arp6, sdg2, er-119, er-119 sdg2, arp6 er-119, arp6 sdg2, and arp6 er-119 sdg2 floral buds. We detected an enrichment of H2A.Z in the region of TSS and the ±1 nucleosome of auxin-related genes. The results showed that the enrichment of H2A.Z in the region of TSS and ±1 nucleosome of PIN3, PIL5, SAUR66, IAA14, GH3.17, IAA19, EXPA1, and ARL was significantly decreased in arp6 mutant and was further depleted in arp6 er-119, arp6 sdg2 double mutants, and arp6 er-119 sdg2 triple mutant compared with arp6 mutant and WT. No decrease of H2A.Z deposition in the region of TSS and ±1 nucleosome could be detected in sdg2, er-119, and er-119 sdg2 mutants (Figures 6A–6H). These results indicated that the deposition of H2A.Z on auxin-related gene loci depends on ARP6 but not ER or SDG2. In Arabidopsis, AT4G07700 is widely believed to contain H2A.Z-free nucleosomes. To confirm whether the lack of H2A.Z signal in these assays corresponds with the absence of H2A.Z, we detected the H2A.Z deposition in AT4G07700. In WT and mutants, the deposition of H2A.Z was not detected in all assayed regions of AT4G07700 (Figure 6I). In addition, we also detected H2A.Z deposition in TSS and ±1 nucleosome regions of AtHSP70, which is known as H2A.Z positive control gene, and found a decrease of H2A.Z in arp6, arp6 er-119 and arp6 sdg2 floral buds compared with WT (Figure 6J). To ensure the accuracy of ChiP-qPCR assays by using H2A.Z antibody, we further sequence the ChiP-qPCR products. The results showed that the sequences of the ChiP-qPCR products were the same as the expected sequences (Figure S2). These indicated that the ChiP-qPCR used is accurate.

The Pol II, H3K4me3 and H3K27me3 levels of auxin-related genes were altered in arp6 er-119, er-119 sdg2, arp6 sdg2 and arp6 er-119 sdg2

Polymerase II (Pol II) is required for active transcription, and previous research indicated that Pol II enrichment was widely affected by H2A.Z deposition (Dai et al., 2018). Next, we wanted to analyze if the detected decrease of H2A.Z deposition in arp6 er-119, er-119 sdg2, and arp6 sdg2 double mutants and arp6 er-119 sdg2 triple mutant leads to an enrich Pol II association in those genes. We performed a ChiP assay using a Pol II antibody. Pol II enrichment was not altered at PIN3, PIL5, SAUR66, IAA14, GH3.17, IAA19, EXPA1, and ARL in arp6 and sdg2 mutants compared to WT (Figure 7). However, the Pol II enrichment was significantly reduced at PIN3, PIL5, SAUR66, IAA14, GH3.17, IAA19, EXPA1, and ARL in the er-119 single mutant, arp6 er-119 and arp6 sdg2 double mutants, and the Pol II enrichment was more significantly reduced at PIN3, PIL5, SAUR66, IAA14, GH3.17, IAA19, EXPA1, and ARL in arp6 er-119 sdg2 triple mutant compared to WT, arp6 and sdg2 single mutants (Figure 7). To ensure the accuracy of ChiP-qPCR assays by using Pol II antibody, we further sequence the ChiP-qPCR products. The results showed that the sequences of the ChiP-qPCR products were the same as expected sequences (Figure S2). These indicated that the ChiP-qPCR results are reliable.

Histone methylation plays a broad and important role in transcription regulation. Actively transcribed genes contain H3K4me3, whereas developmentally repressed genes are enriched for H3K27me3 (Berr et al., 2010a, 2010b; Li et al., 2013). H2A.Z is widely known to mediate the different histone modifications through affecting the MLL and PRC2 complex activity (Hu et al., 2013). To investigate the transcriptional
Figure 5. Gene expression profiling of arp6 sdg2 and pre-amiR inflorescences compared WT revealed a set of commonly regulated genes

(A and B) Venn diagrams show the number of downregulated and upregulated. Genes in both arp6 sdg2 and pre-amiR compared with WT.

(C and D) The top 10 Gene Ontology (GO) terms (ranked by p value) for the commonly regulated genes. Downregulated and upregulated genes in both arp6 sdg2 and pre-amiR compared with WT.

(E) Heatmap showing the down regulation of the indicated genes in arp6 sdg2 and pre-amiR compared with WT. from RNA-sequencing analysis. The average of three replicates is shown to demonstrate consistency. The scale represents log10-transformed expression values, and the genes are grouped according to functional classification. FPKM, fragments per kilobase of exon model per million reads mapped.

(F) Quantitative real-time PCR expression analysis of auxin-related genes in pedicel tissues of WT and arp6, sdg2, pre-amiR, and arp6 sdg2 mutants. Values are means ± SD from three biological replicates. Each biological replicate represents three technical repeats. Different letters above the columns indicate significant differences at p < 0.05, as determined by one-way ANOVA.
regulation of auxin-related genes via H3K4me3 or H3K27me3 Histone methylation in SWR1-SDG2-PRE1 signaling pathway, we detected the enrichment of H3K4me3 active maker and H3K27me3 repressive maker in auxin-related genes in WT, *arp6*, *er-119*, and *sdg2* single mutants, *er-119 sdg2*, *arp6 er-119*, *arp6 sdg2* double mutants and *arp6 er-119 sdg2* triple mutant through using ChIP assays. The results showed

**Figure 6.** H2A.Z deposition at auxin-related genes were altered in *arp6 er-119, arp6 sdg2*, and *arp6 er-119 sdg2*. (A–J) Diagram of PIN3, PILS5, SAUR66, IAA14, IAA19, GH3.17, EXPA1, ARL, Atg07700, and AthSP70 genes with exons indicated as black boxes and the promoter indicated as the region before ATG indicator. The transcription start site (TSS) is shown as a black indicator line. PCR primer sets are shown as black bars below the diagram. Primer set numbers to correspond to the numbers on the x axis of the graphs in Figures 5, 6, 7, 8, and 9. Different letters above columns indicate significant differences at were p < 0.05, as determined by one-way ANOVA.
that H3K4me3 enrichment in most of the auxin-related genes was significantly decreased in sdg2 single mutant and er-119, arp6, and arp6 er-119 sdg2 double mutants, and arp6 er-119 sdg2 triple mutant compared with WT, arp6 and er-119 single mutants (Figure 8). In contrast to the performance of H3K4me3 reduced enrichment, a significantly increased enrichment of H3K27me3 in the region of TSS and G1 nucleosome of these auxin-related genes was detected in er-119, arp6 er-119, and arp6 sdg2 flower buds. Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.

Figure 7. The Pol II level at auxin-related genes were altered in arp6 er-119, arp6 sdg2, and arp6 er-119 sdg2
(A–H) ChIP analysis for the enrichment of Pol II at auxin-related genes in WT, arp6, er-119, sdg2, er-119 sdg2, arp6 er-119, and arp6 sdg2 flower buds.
sdg2, arp6 er-119, and arp6 sdg2 double mutants and arp6 er-119 sdg2 triple mutant compared to WT, arp6, sdg2, and er-119 single mutants were correlated with the decreased enrichment of activation maker H3K4me3 and the increased enrichment of repression maker H3K27me3 in auxin-related genes. To ensure the accuracy of ChIP-qPCR assays by using H3K4me3 and H3K27me3 antibodies, we further sequence the ChIP-qPCR products. The results showed that the sequences of the ChIP-qPCR products were the same as expected sequences (Figure S2). These indicated that the ChIP-qPCR results are reliable.

**SDG2 binds to auxin-related genes are involved in SWR1-ER signaling pathway**

We previously showed that the SDG2 mediated H3K4me3 influences inflorescence architecture by interfering with the SWR1-ER signaling pathway. To further investigate whether SDG2 binding to auxin-related genes is
dependent on the SWR1-ER signaling pathway, we expressed pSDG2: myc-SDG2 in arp6 er-119 and performed ChIP-qPCR experiments using an anti-myc antibody. We found that SDG2 binds to the promoter and gene body regions of auxin-related genes which were significantly reduced in arp6 er-119 (Figure 10).

These results support further that SDG2 and SWR1-ER signaling pathway regulates the expression of auxin-related genes and that the binding of SDG2 to the auxin-related genes depends on the SWR1-ER signaling pathway.

DISCUSSION

SDG2 coordinates with SWR1-ER signaling pathway in regulating inflorescence architecture

The histone lysine methyltransferase domain containing SDG2 was reported to be broadly expressed during plant growth (Guo et al., 2010) and mutants displayed defects in sporophytic and gametophytic
development (Berr et al., 2010b). SDG2 is involved in gamete mitotic cell cycle progression and pollen vegetative cell function (Pinon et al., 2017). Although SDG2 had been reported to play a role in the regulation of vegetative growth and reproductive growth, including root development and gametophytic development (Yao et al., 2013), SDG2-mediated H3K4me3 deposition and function in inflorescence architecture is still poorly studied. SWR1 and H2A.Z cooperate with histone modifications to regulate gene expression on a genome-wide scale (Carter et al., 2018; Dai et al., 2018). H2A.Z associates with H3K4me3 at promoters to activate gene expression but represses the activities of enhancers by inhibiting H3K4me3 (Dai et al., 2018). In addition, the relationship between H2A.Z and H3K4me3 in plant-specific processes has also been revealed. For example, H2A.Z regulates vegetative phase change by facilitating the enrichment of H3K4me3 marks that promote the expression of the key microRNA genes MIR156A and MIR156C (Xu et al., 2018). In contrast, H2A.Z and H3K4me3 play antagonistic roles in regulating anthocyanin accumulation during plant responses to drought and high-light stress (Cai et al., 2019). In this study, we showed that SDG2 is involved in the SWR1-ER signaling pathway, which has been reported to be involved in the regulation of inflorescence architecture (Cai et al., 2017, 2020). The sdg2 single mutant had no obvious inflorescence phenotype, but the double mutants or triple mutants of sdg2, arp6 and er-119 had a compact inflorescence phenotype, which could be partially rescued by pSDG2: myc-SDG2 expression. It is reported that the expression level and H3K4me3 level of PRE1 was severely reduced in sdg2 (Guo et al., 2010). Most importantly, our previous data showed that PRE1 and its homologous, PRE2, PRE5, and PRE6 are direct downstream components of the ER and SWR1 complex, regulating inflorescence development (Cai et al., 2017). In addition, the more compact inflorescence and further reduced pedicels visible in sdg2 pre-amiR mutants indicated that SDG2 genetically interacted with PREs in regulating inflorescence architecture. These results indicated that SDG2 functions may act downstream of the SWR1-ER signaling pathway through affecting the expression of PREs in Arabidopsis inflorescence architecture or may act in parallel pathway with SWR1-ER signaling pathway regulating the Arabidopsis

Figure 10. SDG2 binds to auxin-related genes is involved in SWR1-ER signaling pathway
ChIP analysis for the enrichment of SDG2 at auxin-related genes in WT and arp6 er-119 flower buds. Different letters above columns indicate significant difference at p < 0.05, as determined by one-way ANOVA.
inflorescence architecture (Figure 11). Our work, therefore, identified a new role for SDG2 in the growth and development of Arabidopsis, as SDG2 coordinates with SWR1-ER signaling pathway in regulating inflorescence architecture (Figure 11).

SDG2 regulates inflorescence architecture through potential roles of auxin

The regulation of plant inflorescence architecture is mediated by many factors, such as flowering time genes, LSH1 family genes, and interaction between the auxin pathways (Teo et al., 2014). Auxin biosynthesis, transporter and response process are involved in floral meristem formation and inflorescence architecture; biosynthesis mediating by the YUC genes is essential for the formation of floral organs and vascular tissues (Cheng et al., 2006; Teo et al., 2014). Enhanced branching and decreased inflorescence branch angles were observed in ful-7 mutants, SMALL AUXIN UP-REGULATED RNA 10 (SAUR10) was discovered that was repressed by MADS-domain factor FRUITFULL (FUL) in stems and inflorescence branches of Arabidopsis (Bemer et al., 2017). BIK1 and ER play opposing roles in leaf morphogenesis and inflorescence architecture. BIK1 is required to maintain an appropriate auxin response during leaf margin morphogenesis (Chen et al., 2019b). Our recent publication indicated that HBI1 acted downstream of SWR1 and ER-MPK6 signaling pathway via binding to the promoter of brassinosteroid (BR) biosynthesis gene CYP85A2 and auxin-related genes in regulating the inflorescence architecture, which revealed that auxin and BR signal regulating the inflorescence architecture synergistically (Cai et al., 2020). Here we showed that SDG2 is involved in the PREs-controlled inflorescence architecture and may act in a parallel pathway to the SWR1-ER signaling pathway. Our recent work concluded that PREs function
downstream of SWR1-ER signaling pathway (Cai et al., 2017). Besides, previous research showed that PRE1 was significantly down-regulated in sdg2 (Guo et al., 2010). These results contribute that SDG2 is required for the normal expression of PREs and may act upstream of PREs. Furthermore, the bHLH transcription factor genes PRE1 were widely known as being involved in plant growth and development through the plant hormone pathway (Boeglin et al., 2016; Du et al., 2016; Guo et al., 2017). Our present work discovered that Pol II, H3K4me3, and H3K27me3 levels of auxin-related genes were altered in SWR1-ER and SDG2 signaling pathways. We could infer that SDG2 is associated with the SWR1-ER signaling pathway in regulating inflorescence architecture through mediating the expression of PREs and auxin-related genes. In addition, the SWR1-ER signaling pathway is required for the binding of SDG2 to auxin-related genes. Taken together, SDG2 was indicated that being involved in SWR1-ER signaling pathway through potential roles of auxin in regulating inflorescence architecture, one of the functions of SDG2 underlying the development of inflorescence architecture was postulated.

Limitations of the study
In this study, we determined that SDG2 genetically interacts with the SWR1-ER signaling pathways in regulating inflorescence architecture. Transcriptome results showed that auxin may play potential roles in inflorescence growth mediated by SDG2 and SWR1-ER pathway. SWR1 and ER signaling are required to enrich H2A.Z histone variant and SDG2-mediated H3K4me3 histone modification at auxin related genes and H2A.Z histone variant enrichment was also regulated by SDG2. However, little is known about the roles of other epigenetic regulation marks or transcription factors in the control of inflorescence architecture. Additional studies are required to elucidate whether other epigenetics factors or transcription factors are involved in SWR1-ER signaling pathways in regulating inflorescence architecture.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103236.

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AUTHOR CONTRIBUTIONS

L.L. cloned the gene, generated the transgenic lines, and performed phenotypic and genetic analysis. M.C., Y.H., and J.Q. conducted ChIP analyses. W.Z., X.X., and F.C. performed qPCR experiments and analyzed the data. H.C. and Y.Q. revised the manuscript. H.C. and Y.Q. designed the research and wrote the manuscript.
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DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Experimental models: Organisms | Arabidopsis: arp6 | (Cai et al., 2017) |
| | Arabidopsis: er-119 | (Cai et al., 2017) |
| | Arabidopsis: sdg2 | This paper |
| | Arabidopsis: arp6 er-119 | (Cai et al., 2017) |
| | Arabidopsis: arp6 sdg2 | This paper |
| | Arabidopsis: er-119 sdg2 | This paper |
| | Arabidopsis: arp6 er-119 sdg2 | This paper |
| | Arabidopsis: pSDG2:myc-SDG2 | (Guo et al., 2010) |
| | Arabidopsis: pre-amiR | (Cai et al., 2017) |
| | Arabidopsis: sdg2pre-amiR | This paper |
| Antibodies | Rabbit monoclonal anti-MYC | This paper |
| | H2A.Z polyclonal antibody | R. Deal' Lab |
| | H3K27me3 polyclonal antibody | This paper |
| | H3K4me3 polyclonal antibody | This paper |
| | Pol II monoclonal antibody | This paper |
| Chemicals, peptides, and recombinant proteins | Total RNA Kit II | Omega |
| | Qiagen RNeasy kit | Qiagen |

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hanyang Cai (caihanyang123@163.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The original RNA-seq data is available at European Nucleotide Archive (ENA) under accession number PRJEB47720.
- No original code was produced in this study.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant materials and growth conditions

The Arabidopsis thaliana Columbia (Col-0) ecotype was used in this study. The other Arabidopsis mutant lines were described as follows: arp6 (Garlic_599_G03), sdg2 (SALK_021008), pSDG2:myc-SDG2 (Guo et al., 2010), pre-amiR (Oh et al., 2012), er-119 (Cai et al., 2017). After vernalizing at 4°C for 36-48 hours, all the described wild-type and mutant seeds were planted and grown in soil at 22°C under 16 h-light/8 h-dark photoperiod conditions.
**METHOD DETAILS**

**Phenotype characterization and histological sections**

To analysis the phenotype of inflorescence, the inflorescence and signal flower were placed on 1/2 MS medium, followed by observation under a Leica (M205 FA) microscope. Fixing of Pedicel tissue sample was performed as described previously (Cai et al., 2020) and dehydrated through a graded series of ethanol (30, 50, 70, 80, 90, 95 and 100%). The dehydrated sample was infiltrated with Eponate 812 resin (TED Pella, Inc.), followed by embedding with Eponate 812 resin (TED Pella, Inc.) and polymerization process in an incubator 40°C for 12h, 60°C for 18-36h. The Leica (RM2255) microtome was used to cut 1.5-2μm sections, followed by observation under Olympus (BX63) microscope. The number of cells in a middle longitudinal cortex row was counted, and this number was used to calculate the total number and average length of cells in the cortex row of each pedicel. The number of cells was counted using 15–20 sectioned pedicels for each genotype. Cell length was measured directly on the photographic images of plastic sections (Cai et al., 2017).

**Chromatin immunoprecipitation**

For each chromatin immunoprecipitation (ChIP) experiment, 1.5 g of floral bud tissue at stages 11–14 was used. Floral buds were formaldehyde cross-linked as described (Bowler et al., 2004). Cross linked chromatin was fragmented with 0.2 units of micrococcal nuclease (Sigma) in 1 ml of MNase digestion buffer (10 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM-mercaptoethanol, 0.1% NP40, 1 mM CaCl2, and 1 9 protease inhibitor cocktail, Roche). Digestion was stopped using 5 mM EDTA. ChIP was performed using an H2A.Z polyclonal antibody (from R. Deal, Emory University), or polyclonal antibody against H3K27me3 (Millipore, 07- 449), a polyclonal antibody against H3K4me3 (Millipore, 07-473), or a monoclonal antibody against the RNA polymerase II CTD repeat YSPTSPS (Abcam ab817). Relative enrichment of associated DNA fragments was analyzed by qPCR. All oligonucleotide sequences used in the ChIP experiments are given in Table S1. Each ChIP experiment was repeated three times, and the data represent the average of three biological replicates (Cai et al., 2017, 2020).

**Quantitative real-time RT-PCR**

RNA was isolated from pedicel tissues of developing flowers at stages 11–14. Total RNA was extracted using the Omega Total RNA Kit II and reverse transcribed into cDNA using the TransScript All-in-One transcription kit. For quantitative RT-PCR, 50 ng cDNA was used to detect the transcript levels of marker genes, and HK2 was used as an internal control. Quantitative real-time (qRT)-PCR was performed with specific primers (Table S1) on the Bio-Rad real-time PCR system. Data were analyzed and presented as the normalized relative expression level (2^ΔΔCT) of the respective genes in different samples (Cai et al., 2017).

**RNA-sequencing and data analysis**

RNA was extracted from inflorescence with flower buds younger than stage 14 (Smyth et al., 1990) using the Qiagen RNeasy kit. We were using the Qiagen RNeasy kit. One microgram of RNA from each sample for three independent biological replicates was used for Illumina Sequencing, performed as previously described (Zhao et al., 2014). We performed data analysis as previously described and the Arabidopsis thaliana genome from TAIR 10 as reference (Cai et al., 2020).

**Western Blot**

Proteins were separated by electrophoresis in 15% SDS-polyacrylamide gels and then transferred to PVDF membrane in CAPS/methanol buffer. Primary antibodies specific for anti-H3K4me3 (Millipore, 07-473).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All t-test analysis was conducted using Excel, and the ANOVA analysis was conducted using SPSS software. To determine statistical significance, we employed independent t-tests with two-tail distribution between two groups and one-way ANOVA Turkey’s test among various genotypes. A value of p < 0.05 was considered to be statistically significant.