ABSCISIC ACID-INSENSITIVE 3 is involved in brassinosteroid-mediated regulation of flowering in plants

Jeongeui Hong, Horim Lee, Jinsu Lee, Hyemin Kim, Hojin Ryu

ABSTRACT

ABSCISIC ACID-INSENSITIVE 3 (ABI3) is one of the essential transcription factors of ABSCISIC ACID (ABA) signaling, functioning in seed germination, early seedling development, and abiotic stress tolerance. A recent study showed that epigenetic repression of ABI3 by brassinosteroid (BR)-activated BR1 EMS SUPPRESSOR1 (BES1)-TOPELESS (TPL)-HISTONE DEACETYLASE 19 (HDA19) repressor complex is a critical event for promoting seed germination and early seedling development. However, other physiological roles of the repression of ABI3 and ABA responses by BES1-mediated BR signaling pathways remain elusive. Here, we show that BES1-mediated suppression of ABI3 promotes floral transition and ABI3 acts as a negative regulator for flowering. Ectopic expression of ABI3 specifically compromised the early flowering phenotype of bes1-D and induced severe late-flowering phenotypes in wild-type Arabidopsis and Solanum lycopersicum plants. Both spatiotemporal expression patterns and global transcriptome analysis of ABI3-overexpressing plants supported the biological roles of ABI3 in the negative regulation of floral transition and reproduction. Finally, we confirmed that the loss of function of ABI3 induced early-flowering phenotypes in both long- and short-day conditions. In conclusion, our data suggest that BES1-mediated regulation of ABI3 is important in the reproductive phase transition of plants.

1. Introduction

Flowering marks the phase transition from vegetative to reproductive growth in the plant life cycle. This developmental process is important not only for the reproductive success of plants, but also for maximizing the yields of agriculturally valuable crops. Numerous genetic and physiological studies in Arabidopsis thaliana have revealed that optimized flowering occurs through a complicated genetic regulatory program in which diverse internal and external signaling cues such as day length (photoperiod), low temperature (vernalization), developmental age, and endogenous plant hormones are integrated (Bouché et al., 2017; Conti, 2017). Moreover, various floral induction pathways ultimately induce the expression of central flowering integrators, FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) (Lee et al., 2000), which are repressed by FLOWERING LOCUS C (FLC) and SHORT VEGETATIVE PHASE (SVP) (Hartmann et al., 2000; Lee et al., 2000; Samach et al., 2000). The FT gene, which encodes a mobile protein, is expressed in the companion cells of the leaves and transported to the shoot apical meristem (SAM) through the phloem sieve elements to activate early targets including the floral meristem identity genes LEAFY (LFY) and APETALA1 (AP1), and the floral organ-related gene FRUITFULL (FUL) and SEPALLATA3 (SEP3) (Conti, 2017).

Plant hormones play multiple important roles in plant growth and development, including the regulation of flowering (Conti, 2017; Li et al., 2018a). While the essential roles of gibberellin (GA) in the major floral induction pathways have been well characterized (Conti, 2017), the regulation of other hormones, including abscisic acid (ABA), jasmonate (JA), brassinosteroid (BRs), and ethylene (ET), in reproductive phase transition remains elusive. GA signaling is largely mediated...
through the degradation of negative regulator DELLAs proteins. Interestingly, recent advances have shown the complicated behavior of plant hormones in flowering through crosstalk between the GA/DELLA module and other hormones such as ABA, JA, BR, and ET (Davière and Achard, 2016).

BRs are a group of plant steroid hormones that have a variety of functions, mainly regulating photomorphogenesis, cell growth, and organ formation (Clouse et al., 1996). The involvement of BR in the floral induction network was discovered by observation of the late-flowering phenotype of BR-related loss-of-function mutants (Li et al., 2010). For example, BR biosynthetic mutants, including det2, dwf4, and cd, and BR-signaling mutant brl1 showed a delayed flowering time (Aziroz et al., 1998; Domagalska et al., 2007; Hong et al., 2018), indicating that endogenous BR levels and BR signal transduction affect flowering time. Although BR-signaling-defective brl1 mutants showed strongly enhanced FLC expression with the conditional genetic background of dominant FRIGIDA (FRI) allele or autonomous pathway gene mutation through increased histone H3 acetylation at the FLC chromatin (Domagalska et al., 2007), how BR signaling contributes to the genetic network for floral induction needs to be further defined. BRASSINOOSTEROID INSENSITIVE 1 (BR11)/BRASSINAZOLE RESISTANT 1 ASSOCIATED RECEPTOR KINASE 1 (BAK1) co-receptor complex-initiated signaling cues eventually reach the major transcription factors, BR11 EMS SUPPRESSOR 1 (BES1) and BRASSINAZOLE RESISTANT 1 (BZR1) (Li et al., 2018a). Despite high similarity in amino acid sequence and phosphorylation-dependent regulation of transcriptional activity, the specific DNA binding motifs and different interaction partners of BES1 and BZR1 lead to distinct, transcriptional networks during plant growth and development (Sun et al., 2010). Recent studies reported controversial results regarding the physiological roles of BR in flowering. Interestingly, BR signaling activated gain-of-function mutants such as bar1-D and overexpression of constitutively active BES1-L isoform displaying late- and early-flowering phenotypes, respectively (Jiang et al., 2015; Zhang et al., 2013) Because of the positive role of BR levels and BR signaling in the floral induction pathway, as revealed by phenotypic analysis of loss-of-function mutants, it is suggested that BR-signaling-mediated flowering is probably relayed through BES1 in complex BR signaling networks. However, the extremely late-flowering phenotype of gain-of-function bar1-D1D was explained by its direct modulation of FLD and FLC expression (Li et al., 2018b; Zhang et al., 2013). However, constitutively activated BES1-L isoform showed early flowering (Jiang et al., 2015), suggesting another developmental regulatory layer in BR-mediated floral transition.

Recent findings have shown that BES1–TPL–HDA19 co-repressor complex-mediated epigenetic repression of AB13 is one of the critical mechanisms of antagonistic crosstalk between BR and ABA in early seedling development (Ryu et al., 2014). ABI3, a B3 domain transcription factor, plays essential roles in the ABA-mediated regulation of seed dormancy and young seedling development. However, the expression patterns of ABI3 in vegetative stages and the early-flowering phenotype of the abi3-4 mutant suggest other developmental roles beyond seed dormancy and early seedling development. However, the expression patterns of ABI3 in vegetative stages and the early-flowering phenotype of the abi3-4 mutant suggest other developmental roles beyond seed dormancy and early seedling development (Kurup et al., 2000). Consistent with this, other ABA-related transcription factors, ABA INSENSITIVE 4 (ABI4) and ABA INSENSITIVE 5 (ABI5), have also been reported to act as negative regulators of flowering (Shu et al., 2015). These findings imply that the BES1-mediated antagonistic interactions of ABA and BR signaling pathways play diverse roles in plant growth and development.

We here demonstrate that ABI3 attenuates the BES1-induced early-flowering phenotype. Importantly, a BES1-mediated BR signaling pathway was shown to be positively involved in the floral transition, but its functions were diminished by the overexpression of ABI3, but not ABI5. In addition, global genome-wide transcriptome analysis of plants overexpressing ABI3 showed the increased expression of floral repressor FLC clade members such as FLC and MADS AFFECTING FLOWERING 5 (MAF5) and decreased expression of floral activators/ integrators such as FT and SOC1. Therefore, our data reveal that the positive roles of BRs in the reproductive phase transition occur via BES1-mediated repression of ABI3.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana ecotype Col-0, Ler, and Solanum lycopersicum cultivars Micro-Tom (MT) were used as wild-type controls as the genetic backgrounds of transgenic lines. Arabidopsis seeds were germinated on solid media (pH 5.7–5.8) containing 1/2 Gamborg B5 salts (Duchefa, Netherlands), 1% sucrose and 0.8% plant-agar and all plants were grown in a greenhouse under long-day conditions (16-h light/8-h dark cycles) and short-day conditions (14-h light/10-h dark cycles) at 20–22°C. The mutant seeds were ordered from ABRC stock center. All plants were grown in a greenhouse at 20–22°C under a long-day conditions with 60% humidity.

2.2. Transgenic plants and immunoblotting assay

To generate transgenic plants overexpressing HA-tagged ABI3 in the Col-0 background, genomic DNA fragment was cloned into pCB302ES containing the 3S promoter and HA tag sequences as described previously (Ryu et al., 2014). Electroporation was used to introduce the gABI3-PCB302ES vector into Agrobacterium tumefaciens strain GV3101. All transgenic lines were generated by Agrobacterium-mediated floral dipping methods. For ABI3-overexpressing plants in Micro-tom (MT), genomic DNA fragment was cloned into pBI121 containing the 3S promoter and FLAG tag sequences. A single, well-formed colony from the bacterial selection plate was transferred to 4 ml of LB selective medium containing 50 mg/L kanamycin and cultured in a shaking incubator at 28°C for 16 h to reach an OD600 of 0.6–0.7. Cotyledon segments of tomato seedlings grown for 10 days were transformed by dipping into Agrobacterium suspension containing 100 μM of acetosyringone. The transformed cells were selected and regenerated by previously reported (Ryu et al., 2014). Transgene expression was verified by immunoblotting. Total proteins from seedlings were extracted with protein extraction buffer (50 mM Tris-HCl (pH 7.5), 75 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, 1 × protease inhibitor cocktail (Roche), and 1% Triton X-100). Total protein (3–20 μg) was separated by SDS–PAGE (10% polyacrylamide), transferred to a polyvinylidene difluoride membrane and immunodetected using 1/2000 dilution of peroxidase-conjugated high-affinity anti-HA (Roche), anti-FLAG (Sigma) and anti-actin (MP Biomedicals, catalogue number 69100) antibodies.

2.3. RNA extraction and qRT-PCR

Total RNAs were extracted from seedlings using a Total RNA extraction kit (Intron Biotechnology, Korea) according to the manufacturer’s instructions. Total RNA concentration and quality were measured using a K5600 Micro-spectrophotometer (Shanghai Biotechnol Co., China). A first-strand synthesis kit (Enzymics, Korea) with oligo (dt) primers was used for cDNA synthesis from 1 μg of total RNA. The cDNA was then used for real-time quantitative PCR with a Quanto Studio 3 (Applied Biosystems, USA) instrument using SYBR Green Real-time PCR Master Mix (Applied Biosystems) or semi-quantitative RT-PCR for GUS and ABI3 expression. Primers are listed in Supplementary Table 3. Threshold cycle (Ct) values were used to calculate 2−△△Ct for expression analysis, where ΔΔCt for treated plants was determined as follows: (Ct target gene - Ct actin gene) - control plant (Ct target gene - Ct actin gene) (Livak and Schmittgen, 2001).
2.4. Flowering-time experiment

Plants in a growth greenhouse and chamber under LD or SD conditions were examined. In this study, flowering time was scored as the days from germination to flowering and the number of total rosette leaves at bolting, according to the protocol of Mai et al. (2011). At maturity, the fluorescence internode number and height were determined. More than 10 plants were examined for each genotype.

2.5. Histochemical GUS staining and paraffin sections

To investigate GUS expression patterns during vegetative growth, 3, 6, 9, and 18-day-old pABI3-GUS seedlings were used. The seedlings were stained with GUS-staining buffer (100 mM Sodium Dihydrogen Phosphate Monohydrate, 10 mM Ethylenediaminetetraacetic acid disodium salt dehydrate, 0.5 mM Potassium ferricyanide, 0.5 mM Potassium ferrocyanide, 0.1% Triton X-100 and 1 mM X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide)) for 16 h at 37°C. The hand cut section of around SAM from the GUS stained seedlings were prepared. For paraffin sectioning, tissues were dehydrated and embedded in paraffin. The samples were prepared into 10-15-μm-thick sections and mounted onto slides.

2.6. RNA-seq transcriptome and data analysis

All transcriptomic analyses were based on RNA-seq data (NCBI accession number is SRP142568). Differential gene expression analysis was based on previously published protocols (Trapnell et al., 2012). Functional hierarchical enrichment of DEGs was analysed by gene set enrichment toolkit (Yi et al., 2013) Plant GSEA, http://structuralbiology.cau.edu.cn/PlantGSEA/), GO Analysis Toolkit, and Agrigō (Du et al., 2010) (http://bioinfo.cau.edu.cn/agrigō/) were used in further analysis of DEGs. The lists of GO terms were clustering by REVIGO (Supek et al., 2011) (http://revigo.irb.hr/). Data are expressed as means ± standard error of mean. The expression values were calculated by log2 (FPKM) and were presented in heat map using XLSTAT software. Statistical differences were assessed by student’s t-test or one-way ANOVA. The significance of differences between the means was assessed using a p-value of < 0.05. All analyses were performed using PRISM 6 software (ver. 6.01, GraphPad Software Inc.).

3. Results

3.1. BES1-mediated BR signaling is involved in the promotion of flowering

To investigate the relationship between BR signaling and floral transition, we first compared flowering time phenotype of BR bio-synthetic or signaling mutants, including, det2, dwf1, and bri1-116 (Fig. 1A and B). Our recent study revealed that an activation tagging mutant dwf4-D containing high levels of endogenous BR showed a longer petiole and a slightly early-flowering phenotype, but a bri1-301 mutant displayed a late flowering (Hong et al., 2018). Consistently, both BR-biosynthesis-defective mutants, such as det2 and dwf1, and a BR-signaling defective bri1-116 mutant showed dwarfish, a shorter petiole, and a late flowering time (Fig. 1A and B). Oddly, the BR biosynthetic mutants including det2 and dwf1 showed fewer rosette leaves at flowering than Col-0, which suggests that non-canonical BR signaling pathways may be involved in the regulation of leaf development (Fig. 1B). To test whether canonical BR signaling pathways are directly involved in the promotion of floral transition, we determined the flowering time of BR-activated gain-of-function mutants bai1-D (En-2 background) and bhz1-D (Col-0 background) compared with that of wild-type (WT) plants such as En-2 and Col-0, respectively. Consistent with previous reports (Zhang et al., 2013; Jiang et al., 2015), bai1-1D showed delayed floral transition, but bai1-D showed the early-flowering phenotype under long-day (LD) conditions (Fig. 1C).

3.2. Overexpression of ABI3 but not ABI5 restores the early-flowering phenotype of bai1-D

Previously, we reported that BR signaling is integrated into the inhibitory mechanism of ABA-mediated early seedling development by epigenetically suppressing ABI3 and ABI5 via the BES1–TPL–HDA19 repressor complex (Ryu et al., 2014). For instance, we observed that the overexpression of ABI3 or ABI5 completely restored the suppressed ABA responses of bai1-D in seed germination and root growth inhibition (Ryu et al., 2014). There was also evidence that ABI3 might be involved in flowering (Kurup et al., 2000), and previous studies showed that FUS3, a B3 domain transcription factor family member having similar roles to ABI3, acts on flowering (Duong et al., 2017). To elucidate whether BR signaling promotes flowering time via BES1-mediated downstream regulators such as ABI3 and ABI5 through decreasing ABA responses (Ryu et al., 2014), we retested 3SS:ABI3-HA and 3SS:ABI5-HA transgenic lines in the bai1-D mutant background, which were generated in our previous work (Ryu et al., 2014). Intriguingly, we found that most lines overexpressing ABI3 but not ABI5 complemented the early-flowering phenotype of bai1-D, indicated by both bolting date and rosette leaf number (referred to as bai1-D/3SS:ABI3-HA and bai1-D/3SS:ABI5-HA, respectively) (Fig. 2A–C). The bai1-D/3SS:ABI3-HA #5 T3 homozygote line, which showed the highest level of ABI3 protein as previously shown by Ryu et al. (2014), did not induce any floral organs (Fig. 2A). Despite the roles of ABI3 and ABI5 in BR-mediated ABA responses in early seedling development (Ryu et al., 2014), only ABI3 exhibited an effect in regulating flowering time in the bai1-D mutant background (Fig. 2B and C). Furthermore, similar effects of exogenous ABA to repression of flowering in both bai1-D and bai1-D/3SS:ABI5-HA lines were observed (Supplementary Fig. 1A). Since ABI5 acts as a downstream target of ABI3 to promote ABA responses (Ryu et al., 2003), we reconfirmed that the ABI5 expression level was highly enriched in both bai1-D/3SS:ABI3-HA #3 and bai1-D/3SS:ABI5-HA #18 lines (Supplementary Fig. 1B), suggesting that ABI3, but not ABI5, plays important negative roles in flowering. Taken together, these results indicate that BR-signaling-mediated regulation of floral induction is presumably divergent from the ABI5-mediated ABA response.

3.3. Ectopic expression of ABI3 produces the late-flowering phenotype

Studies on the biological roles of ABI3 in plants have mainly focused on the regulation of seed desiccation, early seedling development, and stress responses. However, our results clearly indicated that the high expression level of ABI3 rescued the early-flowering phenotype of bai1-D in the reproductive phase (Fig. 2), suggesting that ABI3 plays additional roles beyond the early developmental processes in plants. To test this, we examined the spatiotemporal expression patterns of ABI3 during various developmental stages of Arabidopsis seedlings using a pABI3-GUS transgenic line (Rohde et al., 1999) (Supplementary Fig. 2). Similar to the findings in a previous report (Rohde et al., 1999), expression was observed throughout early stage seedlings (3 DAG, days after germination) and its expression was continuously detected in several limited tissues, such as hypocotyl, cotyledons, and shoot apex, over 18 DAG (Supplementary Fig. 2A). GUS and endogenous ABI3 expression level were further confirmed by a semi-quantitative reverse transcriptase PCR analysis (Supplementary Fig. 2B). To further analyze the expression patterns of ABI3 in the shoot apical meristem (SAM) region in more detail, we performed paraffin sectioning and whole-tissue clearing using pABI3-GUS seedlings (Supplementary Fig. 2C). ABI3 promoter-driven GUS activity was found in the SAM at 3 DAG but not after 8 DAG (Supplementary Fig. 2C). These results suggest that the biological functions of ABI3 are not restricted to early development in plants.

To determine whether ABI3 directly regulates flowering regardless of BR signaling, we generated 3SS:ABI3-HA transgenic plants in the wild-type Col-0 background. Interestingly, we observed significantly
shifted distributions of late-flowering phenotypes of 35S:ABI3-HA T1 transgenic lines (Supplementary Figs. 3A and B). In addition, about 12% of them did not flower until 80 DAG. We selected three independent homozygous 35S:ABI3-HA lines (Fig. 3A) showing late-flowering phenotypes with ectopic expression levels of ABI3-HA proteins (Fig. 3 and Supplementary Fig. 3C). For example, the 35S:ABI3-HA #4 line containing the highest accumulation of ABI3 proteins displayed the most severe late-flowering phenotypes in terms of bolting date (51.0 ± 0.8 DAG in 35S:ABI3-HA #4, 21.4 ± 0.4 DAG in Col-0, \( P < 0.01 \)) and rosette leaf number (39.4 ± 1.4 DAG in 35S:ABI3-HA #4, 8.3 ± 0.3 DAG in Col-0, \( P < 0.01 \)), compared with those of WT Col-0 (Fig. 3 and Supplementary Fig. 3C). Moreover, the number of inflorescence internodes (cauline leaf), which is another indicator of flowering time (Pouteau and Albertini, 2011), was also increased in all 35S:ABI3-HA lines (Supplementary Fig. 3D), exhibiting delay of the phase transition in reproductive development. Oddly, a loss-of-function abi3-8 mutant allele in the Col-0 background rarely differed in flowering time from WT plants (Fig. 3C and Supplementary Fig. 3C). However, the abi3-8 mutant showed significant reductions in both the number of inflorescence internodes and height, but all 35S:ABI3-HA lines showed an increased number of internodes relative to WT plants (Supplementary Fig. 3D). Consistently, a loss-of-function abi3-4 strong

Fig. 1. BR signaling pathway positively affects flowering time via BES1
(A) Flowering phenotypes of Col-0, bri1-116, det2, and dwf1 plants under LD at 20 DAG. Bar indicates 1 cm. (B) Days to bolting and leaf number at flowering of Col-0, bri1-116, det2, and dwf1 plants grown under LD (n ≥ 10). Each box is located between the upper and the lower quartiles and the whiskers represent the lowest or highest data point within the 1.5 interquartile range of the lower or upper quartile. The thick horizontal lines in the boxes represent the mean and dots represent all value (P < 0.05; one-way ANOVA). (C) Days to bolting and leaf number at flowering of En-2, best1-D, Col-0, and br1-1D plants grown under LD (n = 14). The details of the box plots are the same as those in Fig. 1B (*P < 0.05, **P < 0.01, Student’s t-test).

Fig. 2. Overexpression of ABI3, but not ABI5, restored early-flowering phenotypes of best1-D.
(A) Flowering phenotypes of best1-D and representative transgenic complementation lines; best1-D 35S:ABI5-HA #12, best1-D 35S:ABI3-HA #4, #5, and #6 plants under LD. (B, C) Flowering times of En-2, best1-D best1-D 35S:ABI5-HA (#12, #18), and best1-D 35S:ABI3-HA (#3, #4, #6) plants under LD (n = 14). Days to bolting (B) and leaf number at flowering (C) in En-2, best1-D, and best1-D ABI3- or ABI5-overexpressing lines. Each box is located between the upper and the lower quartiles and the whiskers represent the lowest or highest data point within the 1.5 interquartile range of the lower or upper quartile. The thick horizontal lines in the boxes represent the mean and dots represent all value (P < 0.05; one-way ANOVA).
mutant allele in the Ler background displayed early-flowering phenotypes under both LD and SD condition as previously reported (Fig. 3C and D and Supplementary Fig. 3C; Kurup et al., 2000). To further confirm the biological role of ABI3 in floral induction using other plant species, we overexpressed the Arabidopsis ABI3 (AtABI3) gene in microtom (MT) tomato (Martí et al., 2006). Similar to Arabidopsis, MT plants overexpressing AtABI3 exhibited significant delay in the transition from vegetative to reproductive phase, producing more vegetative leaves before the first inflorescence emerged (Vicente et al., 2015) (Supplementary Fig. 4).

3.4. Ectopic expression of ABI3 induces both ABA-responsive genes and negative regulators of flowering

To evaluate transcriptional regulatory networks mediated by ABI3 encoding a B3 domain TF in plant development, genome-wide transcriptome analyses using WT Col-0 and ABI3-overexpressing seedlings (referred to as 35S:ABI3-HA #4) were carried out by an RNA-seq method (Fig. 4 and Supplementary Fig. 5A). We found 693 differentially expressed genes (DEGs, p-value < 0.05 and fold change ≥ 1.5) in 35S:ABI3-HA #4 compared with the levels in Col-0 from the RNA-seq results (Supplementary Fig. 5A). Gene Ontology (GO) analysis was performed with the Plant GSEA database (Yi et al., 2013) to predict the major biological functions of isolated DEGs (Supplementary Figs. 5B and 6). As expected, most DEGs were significantly enriched in ABA-related terms including responses to stress, response to ABA, lipid metabolism, seed germination, and seed development (Supplementary Figs. 6 and 7). Moreover, flowering-related GO terms including reproduction, reproductive process, and reproductive development were also revealed to be significantly enriched in 35S:ABI3-HA #4 plants (Supplementary Fig. 6C, and Supplementary Table 2). To investigate the regulatory effects of ABI3 on floral induction in more detail, we visualized the expression patterns of the reproduction-related genes (Fig. 4B, Supplementary Fig. 7 and Supplementary Table 3). Interestingly, major flowering-time-related regulatory genes such as FLC, COL1, MAF5, and SOC1 were differentially enriched in reproduction-related GO terms and these expression patterns were correlated with the late-flowering phenotype of overexpression of ABI4 (Fig. 4B). Interestingly, ABI4 and ABI5 functioning as negative regulators of flowering through the induction of FLC (Shu et al., 2015) were highly expressed in 35S:ABI3-HA #4 plants (Fig. 4B). The different expression patterns of these flowering-time-related genes were subjected to quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis (Fig. 4C and D). Consistent with the RNA-seq results, the expression of flowering-promoting genes, including SOC1, FT, CO, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL15), AP1, and PIF4, was significantly reduced in the 35S:ABI3-HA #4 plants (Fig. 4C and D), whereas we found the induced expression of floral repressors such as FLC and MAF5 (Fig. 4C). These results strongly suggest that ABI3 plays critical roles in not only early seedling development and stress.
tolerance, but also modulation of spatiotemporal flowering-time gene regulatory networks through negative regulation of the phase transition from vegetative to reproductive stages.

4. Discussion

The control of flowering time to ensure its optimization is essential for the success of plant reproduction. Numerous studies have revealed the central regulatory networks that are key for the developmental transition from vegetative to reproductive phases. Plant hormones play important roles in various stages of development and various physiological responses including flowering (Conti, 2017). BRs are well characterized as regulators of growth throughout the entire plant developmental process, and recent studies have shown the possibility that they play biological roles in flowering (Li et al., 2018a). In this study, we revealed that ABI3 plays a negative role in flowering via BES1-mediated signaling to promote the reproductive phase transition.

Recently, it has been reported that BR plays positive roles in GA responses through the BES1-induced activation of GA biosynthesis genes (Unterholzner et al., 2015). Interestingly, the endogenous levels of bioactive GA were significantly reduced in the BR signaling mutant bri1-301, which exhibited broad growth defects in germination, hypocotyl length, and flowering time, whereas overexpression of the GA biosynthetic gene GA20ox1 enabled recovery from various growth defects including the late-flowering phenotype of the bri1-301 mutant (Unterholzner et al., 2015). These results suggest that BES1-mediated BR signaling could promote floral induction through increasing GA biosynthesis. Our data also suggest that BR-signaling-mediated

Fig. 4. Ectopic expression of ABI3-induced ABA signaling and flowering-related genes
(A, B) Heat map of ABA response- (A) and flowering-related DEGs (B), Green, upregulation; red, downregulation; black, intermediate. (C, D) Expression levels of flowering-related genes were determined by real-time qRT-PCR. Validation of the expression levels of the flowering-related DEGs presented in Fig. 4C (C) and the expression levels of positive regulators of flowering, including FT, AP1, CO, and PIF4 (D), in Col-0 and 35S:ABI3-HA #4 plants at 8 DAG (n = 3). Error bars indicate S.E.M. (*P < 0.05, **P < 0.01, Student’s t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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promotion of flowering time probably occurs in a BES1-dependent manner, but not one involving BZR1 (Fig. 1). Unlike the positive role of BES1 in the regulation of floral transition, several recent studies have reported the negative role of BZR1 in floral transition by surveying the extremely late-flowering phenotype of the gain-of-function mutant bvr1-1D through the direct regulation of FLD and FLC (Li et al., 2018b; Zhang et al., 2013). Consistent with this, our investigation of different flowering times of bes-1D and bvr1-1D clearly supported the opposite roles of the two transcription factors, at least in the reproductive phase transition (Fig. 1C). Because BR-defective and BR signaling mutants show delayed flowering time (Hong et al., 2018; Li et al., 2010) and BES1 is reported to interact with both EARLY FLOWERING 6 (ELF6) and RELATIVE OF EARLY FLOWERING 6 (REF6) regulating H3K27me3 status on the chromatin of the FLC locus (Yu et al., 2008), it is suggested that BRI1-initiated BR signaling pathways are integrated into BES1-modulated transcriptional networks for accelerating the floral transition.

The antagonistic functions of BR and GA in ABA responses during plant growth and development have been extensively explored (Conti, 2017; Ryu et al., 2014). For instance, BR-induced BES1–TPL–HDA19 co-repressor complex is specifically involved in the epigenetic repression of ABI3, and its regulation is critical for ABA signaling in early seedling development (Ryu et al., 2014). To date, studies of the physiological functions of ABI3 have mainly focused on seed development, germination, and response to stress. However, BES1 expression was not found to be restricted to the period during seed development or stress conditions, but was extended until the late vegetative growth stage (Supplementary Fig. 2). Moreover, the accumulation of ABI3 or its paralogous FUS3 protein in the aip2-1 mutant, which exhibits impaired E3 ubiquitin ligase-mediated protein degradation of ABI3 and FUS3, resulted in a late-flowering phenotype (Duong et al., 2017). These results support the possibility of ABI3 playing other biological roles in flowering beyond seed dormancy and early seedling development. Consistent with this, a loss-of-function abi3-4 mutant displayed early flowering, whereas overexpression of ABI3 restored the early-flowering phenotype of bes1-D and markedly delayed flowering time in wild-type Col-0 (Figs. 2 and 3, and Supplementary Fig. 3). Notably, it has been shown that ABI3 is required for the expression of downstream ABI3 for ABA responses (Lopez-Molina et al., 2002). However, the overexpression of ABI3 in the bes1-D background could not rescue the early-flowering phenotype (Fig. 2). Because plants overexpressing ABI3 decreased the ABA insensitivity of bes1-D, it is indicated that ABI3-mediated negative regulation of the floral transition would be independent of ABI5 activity, at least under higher-BR-response conditions. These findings were further supported by a global genome-wide transcriptome analysis using plants overexpressing ABI3. GSEA and GO enrichment analysis of DEGs revealed the high correlation of primary stress and ABA responses with the overexpression of ABI3 (Fig. 4B). In addition, diverse GO terms related to reproduction and flowering were also highly enriched (Fig. 4B, Supplementary Figs. 5, 6 and Supplementary Table 1). We confirmed the upregulation of negative flowering regulators, such as FLC and MAF5, but the lower expression patterns of SOC1, FT, and PIF4, which act as floral stimulators (Fig. 4B–D and Supplementary Tables 2 and 3). Recent findings have shown that VIN3-LIKE 2 (VIL2) promotes flowering in the noninductive photoperiod through epigenetic repression at MAF5 chromatin, in which VIL2 maintains the status of H3K9me2 and H3K27me3 through physical interaction with polycomb repression complex 2 (PRC2) (Kim and Sung, 2010). It has also been reported that PRC1 RING-finger protein AtrRING1A regulates floral transition through the repression of MAF4 and MAF5 expression by affecting H3K27me3 via interaction with the PRC2 complex, which in turn activates the expression of FT and SOC1 to promote flowering (Shen et al., 2014). Though associated with the transition from seed maturation to germination and seedling development, it is clear from the work of Qiesta et al. (2016) that the B3 domain transcriptional repressors VAL1 and VAL2 are also required for the epigenetic repression of the FLC locus. It should be noted that the expression of ABI3 along with other B3 domain genes FUS3 and LEC2 is upregulated in val1 plants; however, the repression of these genes by VAL1 is, apparently, indirect (Chen et al., 2018). However, it is possible that repression of ABI3 by VAL-medium epigenetic repression to promote floral transition during vernalization. Taken together, these findings support the negative roles of ABI3 in flowering, in which its biological functions are linked to BES1-mediated floral transition.

Recently, the critical roles of BRs in crosstalk with other plant hormone pathways and diverse developmental plasticity such as morphogenic changes in response to light and elevated temperature have been reported (Martins et al., 2017; Wang et al., 2012). For example, prolonged high temperature induced thermomorphogenesis including cell elongation and early flowering through the activation of PIF4 and BR signaling pathways (Ibañez et al., 2018). Our data demonstrated the novel function of ABI3 in BR-mediated floral transition with repressed PIF4 expression. Therefore, it is supposed that BES1-TPL–HDA19-mediated repression of ABI3 expression appears to be involved in the environmentally-responsive regulation of plant developmental transitions such as flowering. More detailed studies of the interactions of BR and ABA through ABI3-controlled transcriptional networks should be performed.

Author's contribution

J.H., H.L., J.L., H.K., and H.R. performed experiments. J.H., H.L., and H.R. designed experiments and analysed the data. J.H., H.L., and H.R. wrote the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.03.022.

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