JIPB Journal of Integrative Plant Biology

Research Article
https://doi.org/10.1111/jipb.13437

The GATA transcription factor TaGATA1 recruits demethylase TaELF6-A1 and enhances seed dormancy in wheat by directly regulating *TaABI5*

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ABSTRACT

Seed dormancy is an important agronomic trait in crops, and plants with low dormancy are prone to preharvest sprouting (PHS) under high-temperature and humid conditions. In this study, we report that the GATA transcription factor TaGATA1 is a positive regulator of seed dormancy by regulating *TaABI5* expression in wheat. Our results demonstrate that *TaGATA1* overexpression significantly enhances seed dormancy and increases resistance to PHS in wheat. Gene expression patterns, abscisic acid (ABA) response assay, and transcriptome analysis all indicate that *TaGATA1* functions through the ABA signaling pathway. The transcript abundance of *TaABI5*, an essential regulator in the ABA signaling pathway, is significantly elevated in plants overexpressing *TaGATA1*. Chromatin immunoprecipitation assay (ChIP) and transient expression analysis showed that TaGATA1 binds to the GATA motifs at the promoter of *TaABI5* and induces its expression. We also demonstrate that TaGATA1 physically interacts with the putative demethylase TaELF6-A1, the wheat orthologue of *Arabidopsis* ELF6. ChIP–qPCR analysis showed that H3K27me3 levels significantly decline at the *TaABI5* promoter in the *TaGATA1*-overexpression wheat line and that transient expression of TaELF6-A1 reduces methylation levels at the *TaABI5* promoter, increasing *TaABI5* expression. These findings reveal a new transcription module, including TaGATA1–TaELF6-A1–TaABI5, which contributes to seed dormancy through the ABA signaling pathway and epigenetic reprogramming at the target site. *TaGATA1* could be a candidate gene for improving PHS resistance.

Keywords: ABSCISIC ACID INSENSITIVE5 (ABI5), common wheat, demethylase, GATA transcription factor, preharvest sprouting, seed dormancy

Wei, X., Li, Y., Zhu, X., Liu, X., Ye, X., Zhou, M., and Zhang, Z. (2023). The GATA transcription factor TaGATA1 recruits demethylase TaELF6-A1 and enhances seed dormancy in wheat by directly regulating *TaABI5*. J. Integr. Plant Biol. **00:** 1–15.

INTRODUCTION

Seed dormancy is defined as the incapacity of an intact viable seed to complete germination under favorable conditions (Finch-Savage and Leubner-Metzger, 2006), and this trait was strongly selected during plant domestication (Rodríguez et al., 2015). Modern crop cultivars have a low seed dormancy, which guarantees uniform germination after sowing. However, a low dormancy puts crops at risk for preharvest sprouting (PHS) under high-humidity or rainy conditions. PHS can lead to severe yield losses and a decrease in seed quantity and will more dramatically affect global crop production as climate change increases the frequency of extreme weather events. Therefore, a deep understanding of seed dormancy is needed to understand plant ecology and improve crop management.

GATA-type zinc finger transcription factors are evolutionarily conserved factors that are named after the recognition of the conserved sequence WGATAR (W = T or A, R = G or A) at the promoter of their target genes (Lowry and Atchley, 2000). GATA family members were involved in several biological processes, including seed dormancy or germination. For example, BLUE MICROPYLAR END3 (BME3) promotes seed germination (Liu et al., 2005), while GNC and GNL suppress seed germination (Richter et al., 2010). *GATA12* acts as a downstream target of RGL2 and enforces primary seed dormancy (Ravindran et al., 2017). Although the role of the GATA transcription factor in seed dormancy or germination has been reported in model *Arabidopsis*, the molecular mechanisms underlying its functions remain largely unknown. In crops such as wheat, the role of members of the GATA family in seed dormancy and resistance to PHS remains unclear.

The phytohormones ABA and gibberellic acid (GA) function antagonistically to regulate the establishment of seed dormancy. ABA induces and maintains seed dormancy as the seeds mature, while GA releases dormancy and promotes germination. The ABA signaling pathway plays a crucial role in controlling seed dormancy (Gubler et al., 2005; Gao et al., 2012). ABI5 is a bZIP transcription factor and a crucial component of the ABA signaling pathway (Finkelstein and Lynch, 2000). *ABI5* is regulated at the transcriptional and epigenetic levels. At the transcription level, some genes have been identified that modulate seed dormancy or seed germination by positively regulating ABI5, such as *HY5*, *ABI4*, *SPATULA (SPT)*, *NUCLEAR FACTORY-Y C-RGA-LIKE2 (NF-YC-RGL2)*, *AGL21*, and *BBX19* (Chen et al., 2008; Bossi et al., 2009; Vaistij et al., 2013; Liu et al., 2016; Yu et al., 2017; Bai et al., 2019). Conversely, some genes have been identified that negatively regulate *ABI5* expression, such as *WRKY40*, *RAV1*, and *MYB7* (Shang et al., 2010; Feng et al., 2014; Kim et al., 2015), which lead to seed dormancy or germination.

ABI5 also undergoes epigenetic regulation. For example, a chromatin-remodeling factor of the SWI/SNF class, PICKLE, negatively regulates *ABI5* expression by depositing the histone methylation marks H3K9 and H3K27, which leads to a suppression of *ABI5* expression (Perruc et al., 2007). A member of the family of Regulator of Chromatin Condensation 1 (RCC1), *Sensitive to ABA1* (*SAB1*), suppresses *ABI5* expression by enforcing the H3K27me3 methylation state of its promoter (Ji et al., 2019). The demethylase JMJ17 removes H3K4 active marks at the *ABI5* promoter to suppress its expression by interacting with WRKY40 (Wang et al., 2021). Conversely, HOOKLESS1, a histone acetyltransferase, promotes *ABI5* expression by mediating H3 acetylation (Liao et al., 2016). Altogether, these multiple levels regulating *ABI5* expression enable plants to precisely regulate *ABI5* function, which controls subsequent responses to environmental conditions, including seed dormancy or germination. While significant progress has been made to better reveal *ABI5* regulation in recent years, a comprehensive understanding of the complex regulatory network of certain important crops is still needed.

Common wheat (*Triticum aestivum*, 2n = 6× = 42, AABBDD) is one of the most commonly cultivated crops worldwide and provides ~20% of all human calories. It contains a large (16 Gb) and complex allohexaploid genome (International Wheat Genome Sequencing, 2018). The PHS trait is complex and controlled by genetic and environmental factors. These factors make it difficult to identify PHS-resistant genes in wheat. Our previous study demonstrated that *TaGATA1* positively contributes to innate immunity to *Rhizoctonia cerealis* in wheat (Liu et al., 2020). In this study, we identify the role of *TaGATA1* in regulating seed dormancy and improving PHS resistance in wheat. We also analyze the underlying molecular mechanisms to identify the downstream target of *TaGATA1* during seed development.

We demonstrate that *TaGATA1* overexpression significantly increases seed dormancy and resistance to PHS in wheat. Biochemical assays indicate that TaGATA1 directly binds to the GATA motifs at the promoter of *TaABI5*, indicating that *TaGATA1* functions through the ABA signaling pathway. Interestingly, TaGATA1 can physically interact with a demethylase TaELF6-A1, an ELF6 orthologue in wheat. We further show that TaELF6-A1 is responsible for reduced H3K27me3 levels at the *TaABI5* promoter. We describe a novel molecular mechanism of *TaGATA1* that contributes to seed dormancy through direct regulation of *TaABI5* and show that *TaGATA1* is a candidate for improving PHS resistance in crops.

RESULTS

TaGATA1 overexpression enhances PHS resistance and seed dormancy in wheat

In previous work, we generated transgenic wheat plants overexpressing *TaGATA1*, which is driven by the ubiquitin promoter from maize (Liu et al., 2020). Based on data from wheat eFP and the Wheat Expression Browser, we observed that *TaGATA1* transcriptional levels gradually increased during seed development and peaked in mature seeds (Table S1) (Borrill et al., 2016; Ramírez-González et al., 2018). Therefore, we decided to investigate the role of *TaGATA1* in regulating wheat seed germination and performed a PHS assay. In this assay, we compared the sprouting rates of wild-type Yangmai16 (WT) spikes with three independent *TaGATA1*-overexpression lines (OEs). As shown in Figure 1A, the WT spikes showed visible sprouted kernels with a germination percentage of 33.28%, whereas three OE lines, L160, L163, and L165, showed significantly reduced germination percentages of 10.12%, 8.66%, and 7.42%, respectively (Figure 1A, B). These results indicated that *TaGATA1* overexpression enhanced resistance to PHS and indicated that *TaGATA1* could act as a negative regulator of seed germination in common wheat.

To further investigate whether differences in PHS were due to different degrees of wheat seed dormancy between WT and transgenic lines, we performed an after-ripening experiment and measured the germination percentage of wheat seeds at various time intervals during dry storage of

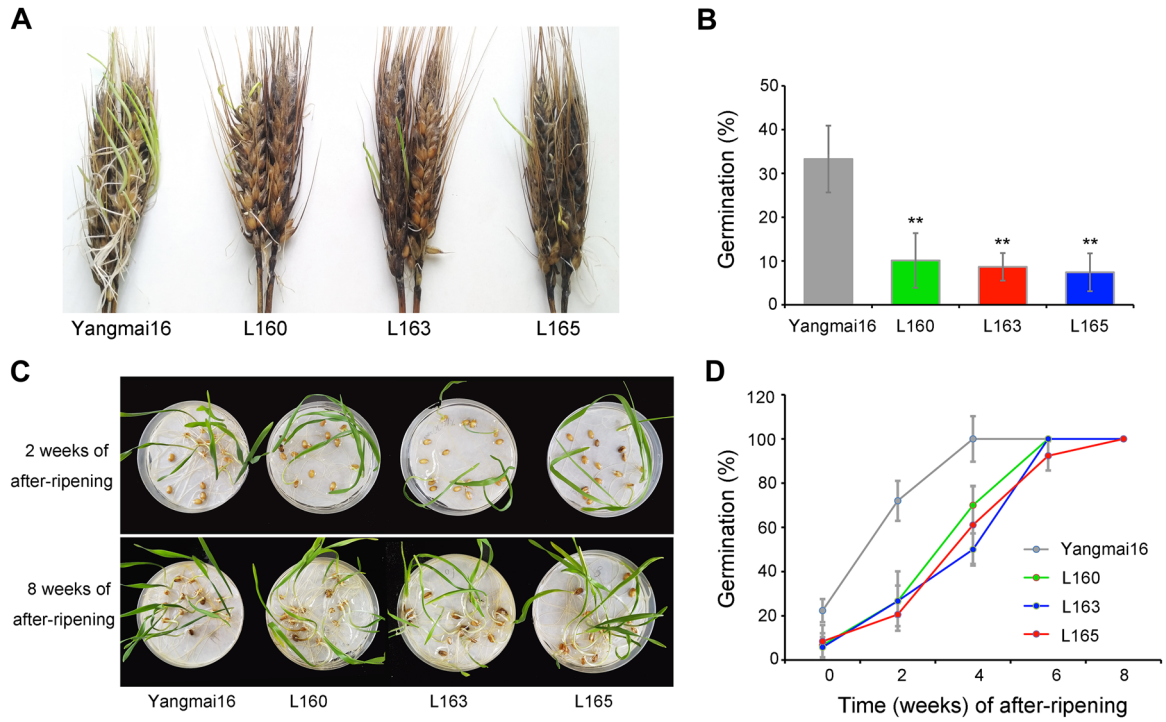

Figure 1. *TaGATA1* overexpression enhances resistance to preharvest sprouting (PHS) and seed dormancy in wheat

(**A**) PHS phenotypes of the Yangmai16, *TaGATA1*-overexpression (OE) lines L160, L163, and L165. Photographs were taken after plants were maintained in a moist chamber for 7 d at 22°C. Bar = 1 cm. (**B**) Germination percentage of visible sprouted kernels of Yangmai16 and OE lines L160, L163, and L165, were analyzed. The experiments were performed three times, and each replicate evaluated 10 spikes from different plants of each OE line or Yangmai16. Error bars indicate means ± *SE* ($n = 3$, three biological replicates). Significant differences were calculated using Student's *t*-test. **, $P < 0.01$. (**C**) Germination phenotypes of seeds of Yangmai16, and OE lines L160, L163, and L165 at 2 weeks of after-ripening (upper row) and 8 weeks of after-ripening (lower lane). Photographs were taken after 7 d of imbibition. Bar = 1 cm. (**D**) Germination percentage after different periods of after-ripening of Yangmai16, and OE lines L160, L163, and L165. The experiments were performed with three biological replicates, and each replicate evaluated 45–50 seeds from each OE line or Yangmai16. Error bar indicates means ± *SD* ($n = 3$, three biological replicates).

seeds. The WT and three *TaGATA1*-overexpression wheat lines were grown under standard conditions (22°C 16 h light/16°C 8 h dark cycles), and we assessed the germination percentage of seeds that germinated 8 d after imbibition (Figure 1C, D). The WT seeds showed 22.34% germination, while three transgenic lines had significantly low germination percentages of 6.67%, 5.77%, and 8.42%. After 2 weeks (w) of after-ripening, the WT seeds reached 72.0% germination, and 4 weeks of after-ripening was required for complete germination. In contrast, an after-ripening time of 6 weeks was required for OE lines for >90% seed germination. These results indicate that the seeds of *TaGATA1*-overexpression lines had a deeper dormancy compared with WT and required a longer after-ripening time to release dormancy. This indicates that *TaGATA1* positively regulates seed dormancy in wheat.

TaGATA1 positively regulates ABA-suppressed seed germination

As seed dormancy is antagonistically controlled by the phytohormone ABA and GA and our previous results demonstrated that the *TaGATA1* promoter contains several ABA-responsive and GA-responsive elements (Liu et al., 2020), we investigated how *TaGATA1* responded to ABA and GA and examined the transcriptional responses of *TaGATA1* to ABA and GA in WT wheat seedlings using qPCR. Our results showed that *TaGATA1* transcript levels were significantly upregulated by ABA, with dramatic induction, especially after 6 h of treatment. We also found that GA gradually suppressed *TaGATA1* transcription (Figure 2A). We then investigated whether *TaGATA1* was involved in the ABA regulation of seed germination. Therefore, we analyzed the germination responses of *TaGATA1*-overexpression lines to ABA treatment. As shown in Figure 2B,C, ABA treatment suppressed the germination of wheat seeds in both the WT and OE lines compared with the control. After ABA treatment, three transgenic lines showed a significantly lower germination percentage compared with the WT. These results indicated that *TaGATA1* overexpression significantly increases the ABA hypersensitivity of seed germination in transgenic plants, suggesting that *TaGATA1* is positively correlated with ABA.

Genome-wide transcriptome analysis reveals *TaGATA1* primarily regulated the ABA signaling pathway

To investigate the signaling networks regulated by *TaGATA1* during wheat seed development, we performed

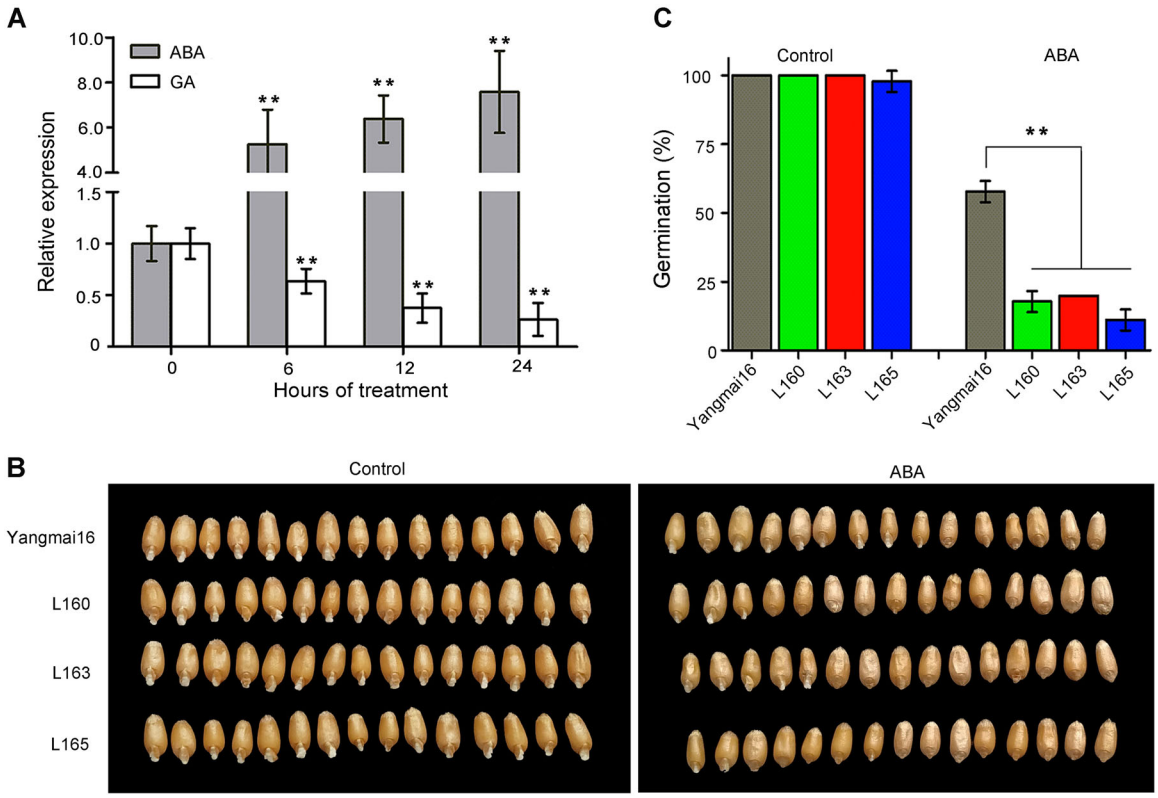

Figure 2. *TaGATA1* modulates ABA responses in wheat

(**A**) qRT-PCR analysis of *TaGATA1* transcripts in seedlings at the two-leaf stage after treatment with abscisic acid (ABA) or gibberellic acid (GA). The transcripts levels were normalized to *TaACTIN*. The experiments were performed with three biological replicates. Error bar indicates means ± SD from three biological replicates. **, Student's *t*-test, $P < 0.01$. (**B**) Photographs showing seeds of Yangmai16, TaGATA1-overexpression (OE) lines L160, L163, and L165 wheat after 24 h of imbibition. The seeds were incubated in distilled water with or without 50 μM ABA. (**C**) Germination percentage of Yangmai16, OE lines L160, L163, and L165 plants were analyzed. The germination of seeds was counted after 1 d of imbibition. The experiments were performed three times, with each replicate containing >35 seeds. Error bar indicates means ± *SE* ($n = 3$, three biological replicates). Significant differences were based on Student's *t*-test. **, $P < 0.01$.

a comparative transcriptome analysis between the *TaGATA1*-overexpression line L165 and WT seeds at 28 d post anthesis (DPA). Our analyses showed that 2,736 genes were significantly differentially expressed between these two genotype plants (Table S2). Of the differential expression genes (DEGs), 2,005 genes were upregulated, and 731 genes were downregulated. Gene Ontology (GO) analysis showed that DEGs were enriched in several biological processes, including beta-amylase activity (GO: 0016161), nutrient reservoir activity (GO: 0045735), and photosynthesis (GO: 0015979). (Table S3). We further analyzed DEGs that were related to phytohormones (Table S4) and found that 13 genes were related to the ABA signaling pathway, including 12 upregulated genes and one downregulated gene, and six genes were related to the GA signaling pathway containing three upregulated and three downregulated genes, and only one gene was involved in the jasmonic acid signaling pathway. Of these upregulated ABA signaling-related genes, 10 were annotated as ABSCISIC ACID-INSENSITIVE 5 (TraesCS3B03G1007500, TraesCS3A03G0880900, TraesCS3A03G0880800, TraesCS3A03G0881000, TraesCS3B03G1006500, TraesCS3A03G0880700, TraesCS3A03G0880400, TraesCS3B03G1006600, TraesCS3B03G1006800, and TraesCS3D03G0808000), and two were annotated as abscisic acid (ABA) receptors (TraesCS7A03G0875800, TraesCS7D03G0780400). The downregulated gene was annotated as ABA 8′-hydroxylase 3 (TraesCS5A03G0601100). These results suggested that *TaGATA1* was primarily involved in ABA signaling pathways.

TaGATA1 has similar expression patterns as *TaABI5*

The ABA hypersensitive phenotypes of the *TaGATA1*-overexpression lines and the results of the transcriptome analysis indicated that *TaGATA1* functions in ABA signaling. To identify how *TaGATA1* overexpression affects the ABA signaling pathway, we investigated the transcriptional changes in genes involved in the ABA signaling pathway using qPCR. According to previous research (Izydorczyk et al., 2018) and the transcriptome results, *TaNCED1* is representative of the gene corresponding to ABA synthesis. The gene

corresponding to ABA metabolism is *TaCYP707A1*, while *TaPYL5*, *TaABI3*, and *TaABI5* were selected to detect the ABA signaling pathway. Among the genes annotated as *TaABI5*, TraesCS3B03G1007500 was selected to investigate further because it showed the strongest upregulation in the OE line compared with WT plants in the comparative transcriptome.

Our results demonstrated that the total transcriptional levels of *TaGATA1* (including endogenous *TaGATA1* transcripts and transgenic introduced transcripts) from the three OE lines were higher in seeds at 28 DPA compared with WT. The total transcription abundances in L160, L163, and L165 were 1.73, 2.60, and 2.16-fold higher, respectively, than in WT. The transcripts of the *TaABI3* and *TaABI5* from three OE lines were significantly increased compared with the WT, whereas *TaNCED1*, *TaCYP707A1*, and *TaPYL5* showed no apparent change between the WT and OE lines. In the germinated embryo at 12 h after imbibition (HAI), we observed similar results as found in the developing grains. In the geminated embryos, however, *TaABI3* and *TaABI5* exhibited a much higher induction compared with the developing grains, indicating that *TaGATA1* was involved in ABA signaling and positively regulated the downstream ABA signaling genes *TaABI3* and *TaABI5*.

Based on these results, we further investigated the transcription pattern of *TaGATA1* in grains during seed development, in dry seeds, and in imbibed seeds of WT Yangmai16. As shown in Figure 3C, *TaGATA1* transcripts gradually increased during grain development. In particular, the transcripts dramatically accumulated at 28 DPA and peaked in the dry seed. After imbibition, its transcription gradually decreased. These results indicated that *TaGATA1* expression was positively correlated with the induction and maintenance of seed dormancy in wheat.

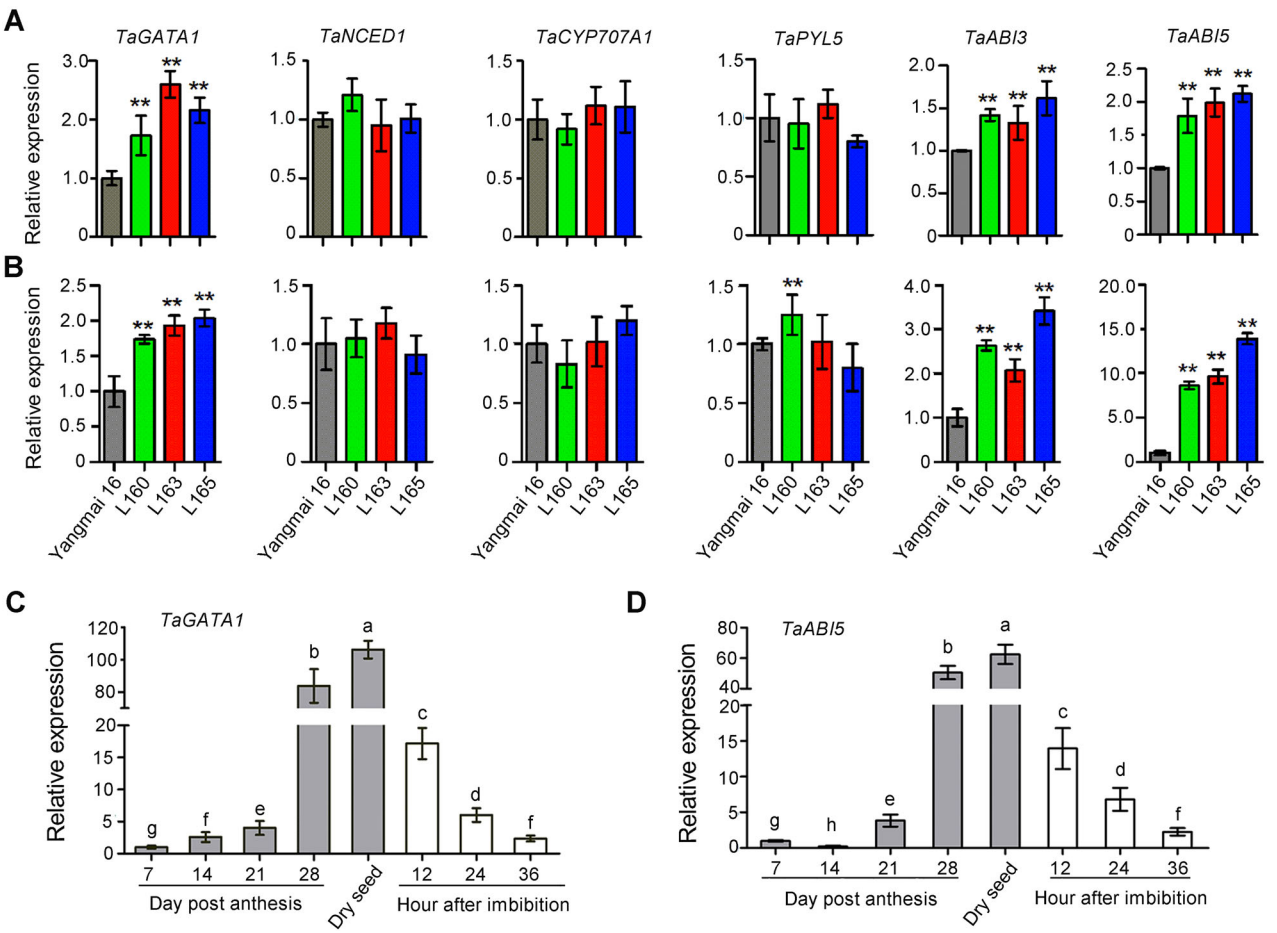

Figure 3. Expression of *TaGATA1* and abscisic acid (ABA)-responsive genes in developing seeds and germinated embryos of Yangmai16 and OE lines wheat

(**A**, **B**) qRT-PCR analysis of transcripts from developing seeds 28-d post anthesis (DPA) (**A**) and germinated embryos 12 h after imbibition (HAI) (**B**). The transcripts levels were normalized to *TaACTIN*. The experiments contain three biological replicates. Error bars indicate means ± *SD* from three biological replicates. **, Student's *t*-test, $P < 0.01$. (**C**, **D**) qRT-PCR analysis of *TaGATA1* transcripts (**C**) and *TaABI5* transcripts (**D**) at different developmental stages of seeds and the indicated time-point after imbibition. Transcript levels were normalized to *TaACTIN*. The experiments contain three biological replicates. Error bars indicate means ± *SD* from three biological replicates. **, Different letters represent statistically significant differences ($P < 0.05$) as determined using one-way ANOVA analysis followed by Tukey's test.

Accumulated evidence indicates that ABI5 is a master regulator of the ABA signaling pathway, which regulates seed dormancy. Therefore, to determine the relationship between *TaGATA1* and *TaABI5* in seed dormancy, we examined transcriptional changes in *TaABI5* in the same materials as those used to examine the *TaGATA1* expression pattern. *TaABI5* exhibited an expression pattern similar to that of *TaGATA1* (Figure 3D). *TaABI5* also significantly increased at 28 DPA and showed an expression peak in dry seeds and then decreased after imbibition, indicating that *TaABI5* expression was positively correlated with *TaGATA1*. These studies suggest that *TaGATA1* could directly regulate *TaABI5* expression during seed development.

TaGATA1 directly regulates *TaABI5* by binding its promoter

Based on the induced expression of *TaABI5* in *TaGATA1*-overexpression lines and the similar expression patterns between *TaGATA1* and *TaABI5*, we speculated that *TaABI5* could be a direct regulatory target of *TaGATA1*. To test this hypothesis, we first obtained the promoter sequence of *TaABI5* (TraesCS3B03G1007500) from Yangmai16 (Figure S1). The promoter sequence contains 3338 nucleotides upstream of the start codon ATG of the coding sequence. Careful sequence analysis of the *TaABI5* promoter region showed that GATA elements were primarily enriched in three regions located at –2926 to –2660 nucleotides (F1), –2508 to –2128 nucleotides (F2), and –304 to –1 (F3) nucleotides at the promoter (Figure 4A). We then detected and quantified *TaGATA1* accumulation in these three regions using ChIP–qPCR. Due to the technical difficulties encountered while performing ChIP assays in mature seeds, the immature seeds were used at 21 DPA of the *TaGATA1*-overexpression line L165. Compared with the WT, a significant accumulation of TaGATA1 was observed at the distal promoter (F2), and a slight but insignificant enrichment was detected in the F1 region. No significant enrichment of TaGATA1 was detected in the proximal promoter region (F3). These results indicate that TaGATA1 can bind to the *TaABI5* promoter. As expected, *TaACTIN* (the control), was not detected as an enrichment of TaGATA1 (Figure 4B).

To evaluate whether TaGATA1 could directly bind to the GATA elements of the *TaABI5* promoter, we performed a yeast-one-hybrid assay. The GATA motif enrichment regions were constructed to drive the reporter gene *His* expression in yeast and found that the yeast contained the F2 region-driven His plasmid and pAD-TaGATA1 plasmid, which grew normally in SD supplement medium (Figure 4C). To confirm the direct regulation of TaGATA1 to *TaABI5*, we cloned differential regions of the *TaABI5* promoter into the pGreenII0800 vector for the subsequent dual-luciferase reporter assay and LUC activity assay. In the constructed vectors, the inserted DNA sequence P1 contains F1 and F2 regions, while P2 contains the F3 region. As expected, in the presence of TaGATA1, a relatively strong luminescence signal indicating considerable luciferase activity was detected in *Agrobacterium*-infiltrated P1::LUC tobacco leaves (Figure 4D, E), while the tobacco leaves containing P2::LUC also displayed a significantly strong luminescence signal compared with that containing the empty LUC vector (Figure 4E). Together, the results presented above demonstrated that *TaGATA1* regulated *TaABI5* expression by directly binding to its promoter.

TaGATA1 physically interacted with TaELF6-A1

ABI5 functions as a central regulator of ABA-mediated seed dormancy. Previous results have demonstrated that epigenetic regulation plays a critical role in suppressing ABI5 expression when seeds germinate (Perruc et al., 2007; Ji et al., 2019; Wang et al., 2021). Histone methylation (H3K9 and H3K27) of the *ABI5* promoter suppresses its transcription and enables subsequent seed germination, while *ABI5* transcript levels accumulate dramatically during seed dormancy. These contrasting expression patterns led us to investigate whether demethylase removed the repressive marks in the ABI5 promoter. We first examined the chromatin modification status at the *TaABI5* promoter from a public database and found that there were always various histone-modified marks, including H3K27me3, at different stages of development (Li et al., 2019). This suggests that epigenetic regulation could play a critical role in *TaABI5* expression in wheat. We next identified a gene encoding the Jumonji C (JmjC) domain-containing protein from wheat. This protein has the highest amino acid sequence identity with ELF6 from *Arabidopsis thaliana* (Crevillén et al., 2014) and has a close phylogenetic relationship with ELF6 and JMJ705 in maize (Li et al., 2013) (Figure S2). Therefore, this gene was named TaELF6-A1 (TraesCS4A02G416800). TaELF6-A1 and the homologous TraesCS4B02G317400 and TraesCS4D02G314000 have high levels of sequence identity at amino acid levels (Figure S3), and during grain development they display similar expression patterns and transcriptional levels (Table S5). Therefore, TraesCS4A02G416800 was selected for further analysis. In the CS genome, TraesCS4A02G416800 contains seven exons and six introns. The putative protein from Yangmai16 contains the JmjC domain at the N-terminus and the zinc finger domain at the C-terminus (Figure S3). The Wheat eFP browser data showed that *TaELF6-A1* expression is highly enriched in ripening seeds (Table S5). Consistent with these data, our expression analysis also showed that *TaELF6-A1* transcription gradually accumulated during seed development, and that transcription levels peaked in dry seeds (Figure S4). This indicates that the putative demethylase TaELF6-A1 could function during seed dormancy.

Transcription factors can recruit demethylase through physical interaction to regulate target gene expression (Cheng et al., 2018; Hung et al., 2021; Wang et al., 2021). The overlapping expression pattern between *TaGATA1* and *TaELF6-A1* during seed development prompted us to investigate whether TaGATA1 directly interacted with the putative

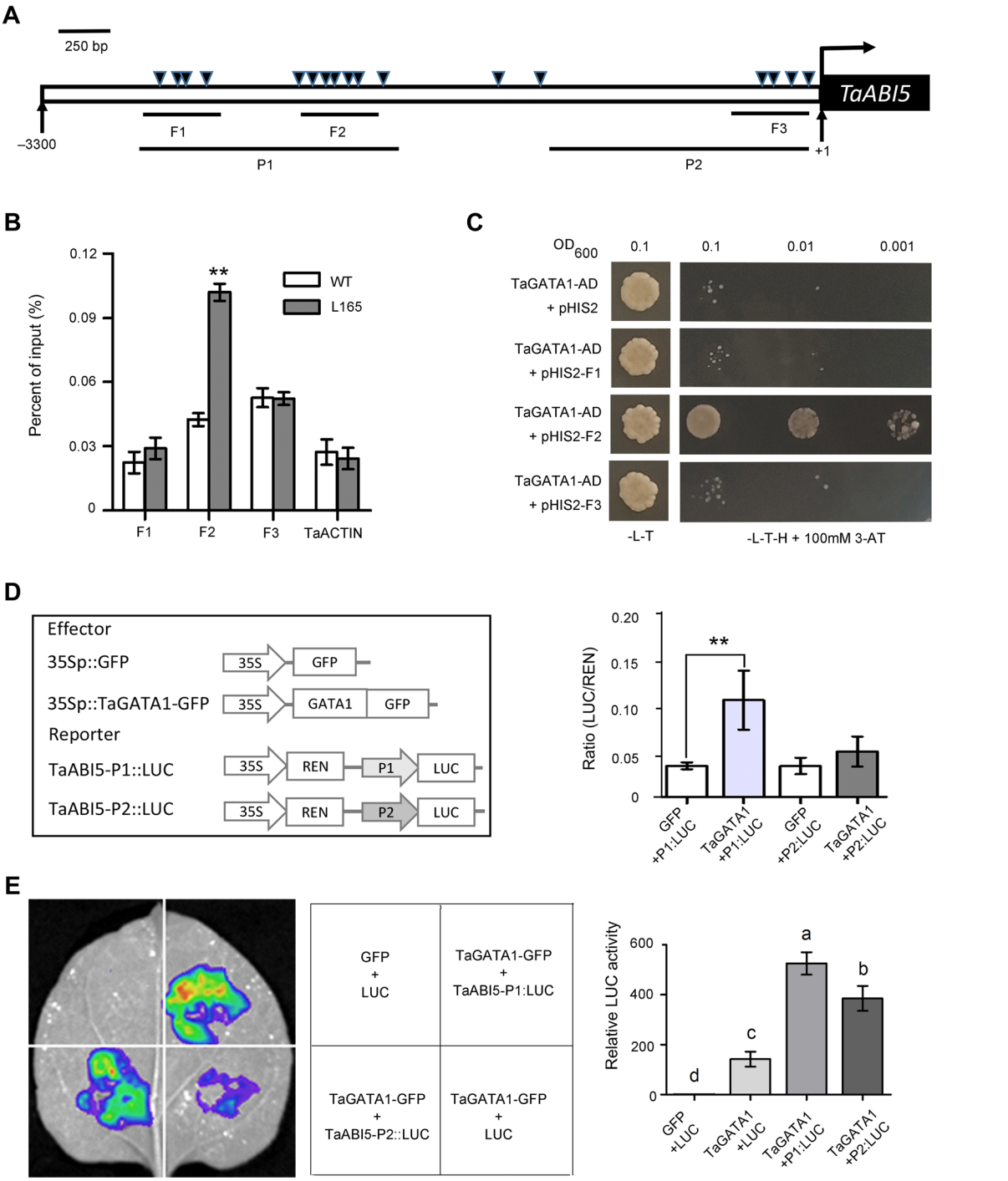

Figure 4. TaGATA1 directly binds to the *TaABI5* promoter

(**A**) Schematic representation of the *TaABI5* promoter. The triangles indicate the position of the GATA motifs. Three fragments, named F1, F2, and F3, were used for the yeast-one-hybrid assay and chromatin immunoprecipitation–qPCR (ChIP–qPCR) detection. Two fragments, named P1 and P2, were used for the LUC activity assay. Bar = 250 bp. (**B**) ChIP–qPCR assay shows that TaGATA1 binds to the F2 fragment of the *TaABI5* promoter. Immunoprecipitation was performed using developing seeds 21 d post anthesis from the *TaGATA1*-overexpression line L165 and wild-type (WT) Yangmai16 with anti-MYC antibody. The values are shown as percentages of the input. The ChIP–qPCR assay was performed with three biological replicates. One of the biological replicates is shown here, whereas the other two replicates are shown in Supporting information Figure S5. Error bars indicate means ± *SD* of three technical replicates. **, Student's *t*-test, $P < 0.01$. (**C**) Yeast-one-hybrid assay showing that TaGATA1 binds to the F2 fragment of the *TaABI5* promoter. −L−T indicates −Leu/−Trp medium, −L−T−H + 100 mM 3-amino-1, 2, 4-triazole (3-AT) indicates 100 mM 3-AT was added in −Leu/−Trp/−His medium. (**D**) Transient expression assay shows that TaGATA1 primarily binds to the P1 fragment of the *TaABI5* promoter. Firefly LUC activity was normalized to REN activity (as a control). Schematic diagrams of various constructs used in the assay are shown in the left panel. Values are means ± *SD* from three biological replicates. **, Student's *t*-test, $P < 0.01$. (**E**) LUC activity assay showing that TaGATA1 mainly binds to the P1 fragment of the *TaABI5* promoter. Values are means ± *SD* from three biological replicates. Different letters represent statistically significant differences ($P < 0.05$) as determined using one-way ANOVA analysis followed by Tukey's test.

TaELF6-A1 to regulate *TaABI5* expression. Therefore, we performed yeast-two-hybrid (Y2H), split-luciferase complementation imaging (LCI) and bimolecular fluorescence complementation (BiFC) assays to determine the physical interaction between TaGATA1 and TaELF6-A1. In Y2H assays, the yeast cells of the AH109 strain co-transformed with pGBDKT7-TaELF6-A1 and pGADKT7-TaGATA1 and could grow on defective medium (SD−Leu−Trp−His−Ade), while negative yeast cells did not grow. This suggests that TaELF6-A1 and TaGATA1 can interact in yeast cells (Figure 5A). In LCI assays, the strong luminescence signal was detected in *N. benthamiana* leaves co-infiltrated with *nLUC-TaGATA1* and *cLUC-TaELF6-A1* constructs, indicating that TaGATA1 can interact with TaELF6-A1 in plant cells. Probably as a result of the high expression level of infiltrated genes, the weak signal was also observed in control leaves (Figure 5B). We confirmed the interaction between TaGATA1and TaELF6-A1 in BiFC assays: yellow fluorescence was observed in the nuclei of tobacco cells coexpressing the constructs TaELF6-A1-YFPc and TaGATA1-YFPn, indicating that TaELF6-A1 interacts with TaGATA1 to reconstitute the YFP. However, no fluorescence signal was detected in the combination of TaELF6-A1-YFPc and YFPn, or the combination of YFPc and TaGATA1-YFPn (Figure 5C). Altogether, these findings indicate that TaGATA1 interacts with TaELF6-A1.

TaELF6-A1 expression is associated with H3K27me3 removal at the *TaABI5* promoter

As *Arabidopsis* ELF6 has histone demethylase activity that is specific for H3K27me2 and H3K27me3 (Crevillén et al., 2014) and TaELF6-A1 interacts with TaGATA1 *in vivo* (Figure 5), we investigated whether TaELF6-A1 and TaGATA1 functioned together to regulate *TaABI5* by removing H3K27me3 marks. Therefore, we evaluated the H3K27me3 levels at the promoter of *TaABI5* using ChIP followed by quantitative PCR (ChIP–qPCR). For this assay, we used immature seeds from the transgenic OE line L165, which are the same materials used to detect TaGATA1 enrichment at the *TaABI5* promoter. Three promoter regions, which were annotated in the public database as H3K27me3 deposition, were selected for the qPCR analysis (Figure 6A). ChIP–qPCR analysis indicates that the OE line L165 has decreased H3K27me3 levels at all three fragments at the *TaABI5* promoter (Figure 6B), suggesting that TaGATA1 could recruit TaELF6-A1 to the *TaABI5* promoter to remove H3K27me3 marks.

To further verify the function of TaEFL6 in removing H3K27me3 marks at the *TaABI5* promoter, we transiently expressed the TaEFL6-A1-GFP fusion protein in WT Yangmai 16 leaf protoplasts. ChIP–qPCR results demonstrated that the transient overexpression of TaEFL6-A1-GFP leads to decreased H3K27me3 levels in the three regions at the *TaABI5* promoter compared with the control (transient expression of GFP), while the transcriptional levels of *TaABI5* in TaELF6-A1-overexpression protoplasts were significantly higher than in the control (Figure 6C). We then transiently introduced the TaELF6-A1-GFP fusion expression vector into the *TaGATA1*-overexpression wheat L165 protoplast and performed ChIP–qPCR. The results demonstrate that in all three tested regions of the *TaABI5* promoter, the H3K27me3 levels further decreased due to TaEFL6-A1-GFP overexpression compared with the control and the *TaABI5* transcripts in TaELF6-A1-GFP-expressed protoplasts increased more than in the control (Figure 6D). These results indicated that TaELF6-A1 was responsible for decreasing H3K27me3 levels at the *TaABI5* promoters and for the high expression of *TaABI5*.

DISCUSSION

Seed dormancy controls germination timing, which enables plants to adapt to their living environment and maximize the survival of their offspring. In agricultural fields, seeds with prolonged dormancy can affect germination uniformity, while PHS can easily occur in those without adequate dormancy prior to harvest. Wheat is an important staple crop, in which PHS causes considerable yield and quality losses. Wheat has a complex and large genome, and PHS traits are primarily controlled by genetic factors with a substantial environmental influence. These factors make it difficult to identify PHS-resistant genes in wheat. In this study, we reported the role of a GATA-type transcription factor, TaGATA1, in regulating wheat seed dormancy and its underlying mechanisms. Our results indicated that *TaGATA1* induces the establishment of seed dormancy by modulating the ABA signaling pathway. We identified *TaABI5*, a master regulator of the ABA signaling pathway, as a direct regulatory target of *TaGATA1*. TaGATA1 interacts with TaELF6-A1, a demethylase that contains a JMJ domain, and targets the promoter of *TaABI5* to remove the repressive marks H3K27me3.

It has been shown that GATA transcription factors regulate several aspects of the developmental processes of *Arabidopsis*, including chlorophyll synthesis, chloroplast and stomata development, senescence, light signaling, and seed germination (Bi et al., 2005; Naito et al., 2007, Richter et al., 2010; Hudson et al., 2011; Chiang et al., 2012; Richter et al., 2013; Ranftl et al., 2016; Zubo et al., 2018). The data in wheat eFP and Wheat Expression Browser (Table S1) demonstrate that *TaGATA1* primarily accumulates in mature seeds (Borrill et al., 2016; Ramírez-González et al., 2018). This prompted us to investigate the potential role of *TaGATA1* in seed dormancy. Our findings clearly demonstrate that *TaGATA1* is a positive regulator of seed dormancy, as demonstrated by the PHS test and the after-ripening assay (Figure 1).

Several GATA members in *Arabidopsis*, including *GNC*, *GNL*, *BME*, and *GATA12*, have been shown to regulate seed germination or dormancy through an unknown mechanism (Liu et al., 2005; Richter et al., 2010; Ravindran et al., 2017). *GNC* and *GNL* were identified as important transcription targets of the GA signaling pathway, and GATA12 was negatively regulated by GA. ABA signaling plays a key role in inducing and maintaining seed dormancy (Gubler et al.,

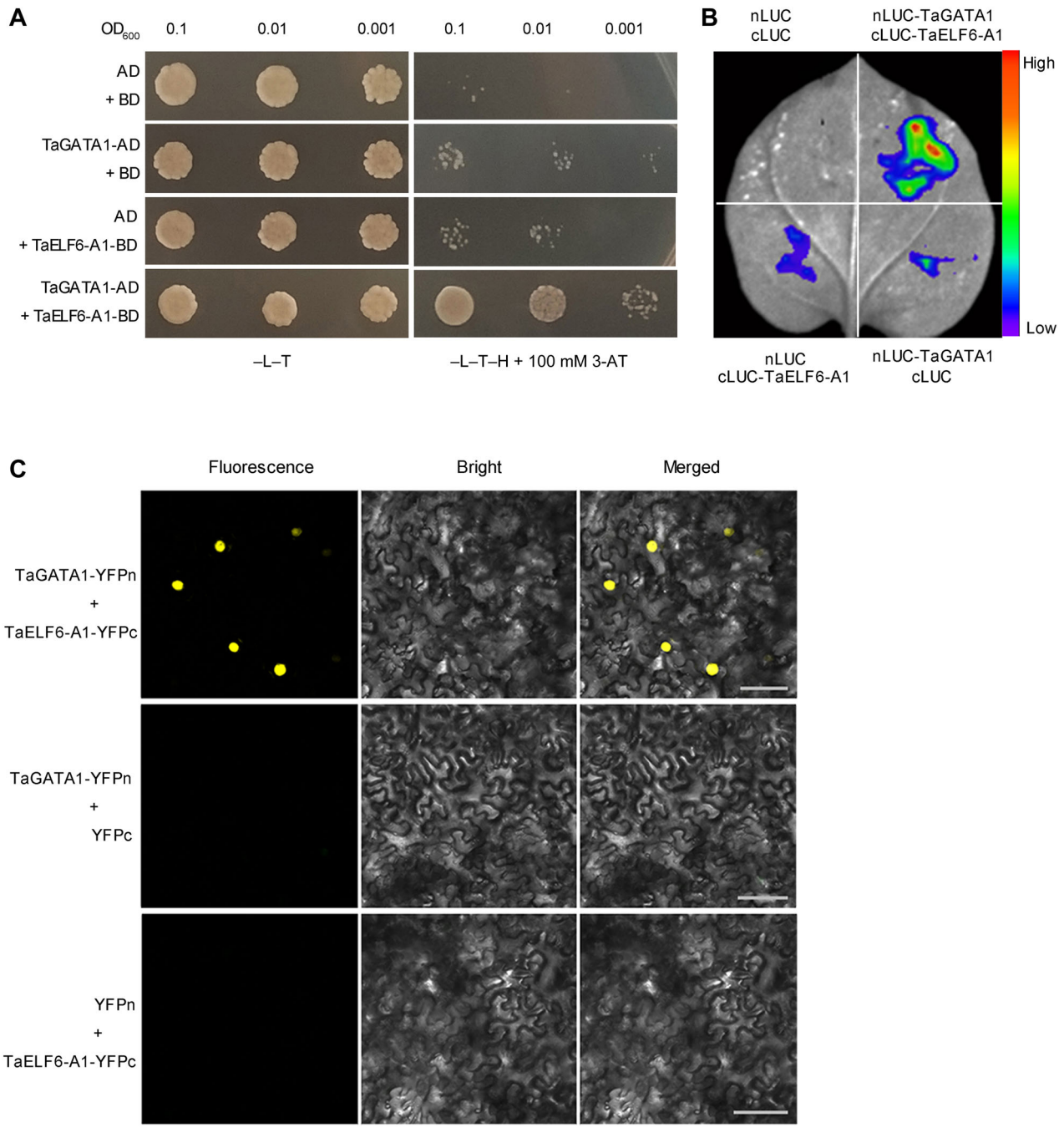

Figure 5. TaGATA1 interacts with TaELF6-A1

(**A**) Yeast-two-hybrid assay showing that TaGATA1 interacts with TaELF6-A1 in yeast. –L–T indicates –Leu/–Trp medium, –L–T–H + 100 mM 3-AT indicates that 100 mM 3-AT was added to the –Leu/–Trp/–His medium. (**B**) Luciferase (LUC) complementation imaging (LCI) assay showing that TaGATA1 can interact with TaELF6-A1 in *N. benthamiana*. Strong luminescence signals were detected with the co-infiltration of TaGATA1 and TaELF6-A1. (**C**) Bimolecular fluorescence complementation (BiFC) assay shows that TaGATA1 interacts with TaELF6-A1 in epidermal cells of *N. benthamiana* leaves. YFP signal is shown as imaged by laser-scanning confocal microscopy. The interaction of TaGATA1-YFPn and TaELF6-A1-YFPc is shown as a yellow signal. Bars = 50 μm.

2005). In this study, we found that *TaGATA1* functions through the ABA signaling pathway. First, *TaGATA1* expression was induced by ABA (Figure 2A). Second, *TaGATA1* overexpression increased ABA sensitivity in wheat seeds (Figure 2B, C). Compared with WT, the germination percentage of *TaGATA1*-overexpression transgenic seeds was lower when treated with ABA. Third, transcriptome analysis of *TaGATA1*-overexpression wheat lines showed that the genes involved in ABA signaling were significantly affected (Table S4). Our results clearly demonstrated that *TaGATA1* can regulate seed dormancy in wheat by modulating ABA signaling.

ABI5 is a key component of the ABA signaling pathway (Lopez-Molina et al., 2001; Carles et al., 2002). Our results indicated that *TaGATA1* acts as an inducer of *TaABI5* in wheat. In this study, we provide extensive evidence and demonstrate that TaGATA1 directly targets the *TaABI5* promoter and consequently induces its expression. First, *TaABI5* expression is upregulated in developing seeds, and *TaGATA1* overexpression induces *TaABI5* expression (Figure 3A). Second, *TaABI5* shows a pattern of expression similar to *TaGATA1* in developing seeds and germinated seeds of wheat (Figure 3B). Third, the comparative transcriptome analysis revealed that several genes annotated as ABI5 were upregulated in OE plants. Fourth, the *TaABI5* promoter contains several GATA motifs (Figures 4A, S1). Fifth, the ChIP–qPCR results showed that TaGATA1 is primarily enriched for chromatin in the distal region of the promoter of *TaABI5*. The transient reporter gene expression assay showed that the distal region mainly contributed to transcriptional activation and these observations are consistent with our ChIP–qPCR data. These results suggested that *TaGATA1* is a direct regulator of *TaABI5* and identified a new mechanism by which TaGATA1 positively modulates seed dormancy.

To date, several major QTLs and genes for PHS resistance have been cloned into wheat. For example, *Myb10-D* confers PHS-3D resistance to PHS by positively regulating *NCED* transcription to promote ABA synthesis (Lang et al., 2021); *MOTHER OF FT AND TFL1* (TaMFT1) confers TaPHS1 resistance, and its knockout and overexpression assays showed that TaMFT1 is a positive regulator of ABA sensitivity (Nakamura et al., 2011); *TaMKK3* (mitogen-activated kinase kinase 3) was identified as a candidate gene of *Phs1-4A* and enhanced dormancy by positively modifying ABA responsiveness (Torada et al., 2016); *TaVp1*, an *ABA Insensitive 3* (*ABI3*) homologue of wheat, was shown to positively affect seed dormancy by increasing embryo ABA sensitivity (Nakamura and Toyama, 2001). In this study, *TaGATA1* enhances seed dormancy by directly regulating *TaABI5* and increasing ABA sensitivity in seeds. Taken together, *TaGATA1* and the above genes confer PHS resistance by modulating the ABA signaling pathway. *TaGATA1* could be a promising gene target in crop improvement for PHS resistance. In future research, mining *TaGATA1* alleles for PHS resistance from genetic resource collections and developing functional markers could improve PHS resistance breeding through mark-assisted selection in wheat. Additionally, genome editing techniques such as

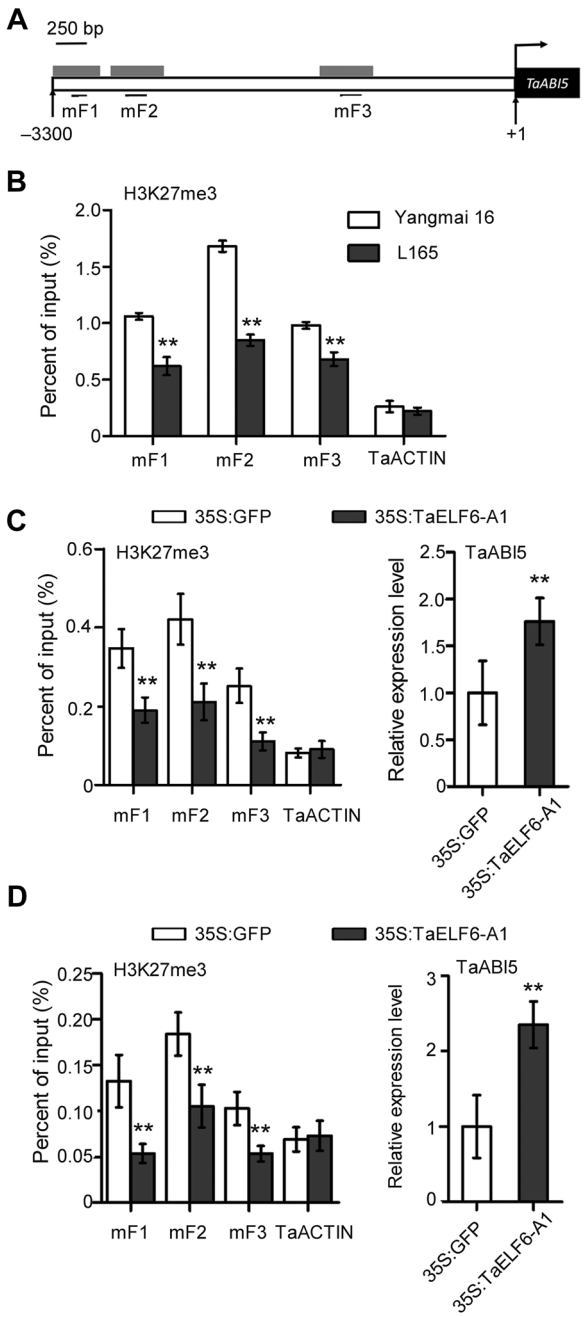

Figure 6. TaELF6-A1 regulates H3K27me3 levels at the promoter of *TaABI5*

(**A**) Schematic representation of the promoter of *TaABI5*. The black box on the promoter indicates the deposition of H3K27me3 marks. Three fragments, named mF1, mF2, and mF3, were used for detection in ChIP–qPCR of H3K27me3. Bars = 250 bp. (**B**) ChIP–qPCR analysis shows that the H3K27me3 level at the *TaABI5* promoter was lower in the *TaGATA1*-overexpression line L165 than in Yangmai16. Immunoprecipitation was performed using developing seeds 21 d post anthesis from the *TaGATA1*-overexpression line L165 and Yangmai16 with anti-H3K27me3 antibody. The values are shown as percentages of the input. The ChIP–qPCR assay was performed with three biological replicates. Error bars indicate means ± SD from three biological replicates. **, Student's *t*-test, $P < 0.01$. (**C**, **D**) ChIP–qPCR analysis of H3K27me3 levels and RT-qPCR analysis of *TaABI5* transcripts in wheat protoplasts from Yangmai16 (**C**) and OE line L165 plants (**D**). The cells were collected 24 h after transient transformation with TaELF6-A1 or GFP. Immunoprecipitation was performed using an anti-H3K27me3 antibody, and the values are shown as percentages of the input. The *TaABI5* transcripts were normalized to *TaACTIN*. The experiments were performed with three biological replicates. Error bar indicates means ± *SD* from three biological replicates. **, Student's *t*-test, $P < 0.01$.

CRISPR-Cas are powerful tools for creating targeted genetic variation (Eshed and Lippman, 2019). Therefore, creating new alleles of *TaGATA1* with desired transcription levels could accelerate the breeding of crops with PHS resistance.

DNA methylation plays an important role in seed dormancy in *Arabidopsis* and wheat (Gao et al., 2012; Kawakatsu et al., 2017). ABI5 expression is also subject to epigenetic regulation in *Arabidopsis*. For example, when *Arabidopsis* seeds germinate, the ABI5 promoter is modified by the addition of repressive marks (H3K9 and H3K27), resulting in its transcriptional repression. These modifications are carried out by PICKLE, a chromatin-remodeling factor of the SWI/SNF class (Perruc et al., 2007), and SAB1, a member of the Regulator of Chromatin-Condensition1 (RCC1) family (Ji et al., 2019). The repressive H3K27 modification in the *TaABI5* promoter has also been observed in wheat (Li et al., 2019). It has been reported that a demethylation pathway is activated in *Arabidopsis* during late seed development (Kawakatsu et al., 2017). This all enables the successful identification of TaELF6-A1, a wheat orthologue of *Arabidopsis* ELF6. ELF6 has shown demethylation activity in removing H3K27me3 (Crevillén et al., 2014). In the TaGATA1-GATA element-binding assay, the results indicate TaGATA1 can bind to both P1 (containing F1 and F2 fragment) and P2 fragments (including F3 fragment) (Figure 4D and E), while TaGATA1 only binds to F2 fragment in the yeast-one-hybrid assay (Figure 4C). These results imply that there could be additional factors, such as demethylases, involved in target recognition *in planta*. In the present research, we clearly demonstrated that *TaELF6-A1* expression was associated with the removal of repressive marks H3K27me3 at the *TaABI5* promoter. In the Yangmai16 protoplast, transient expression of *TaELF6-A1* reduces H3K27me3 levels at the *TaABI5* promoter. The level of H3K27me3 at the *TaABI5* promoter further decreases when *TaELF6-A1* is transiently expressed in the protoplast of *TaGATA1*-overexpression plants. These results and the interaction between TaGATA1 and TaELF6-A1 indicate that TaGATA1 could recruit TaELF6-A1 to transcriptionally activate its target *TaABI5*. More recently, H3K27 demethylase REF6 was reported to directly target DNA by its own DNA-binding domain (Li et al., 2016). However, the DNA-binding capacity of TaELF6-A1 has not been determined. In model plants, such as *Arabidopsis* and rice, it has been reported that the demethylases interactions with DNA-binding transcription factors ensure their efficient and precise targeting of a specific set of genes (Cheng et al., 2018; Hung et al., 2021). Therefore, future research should assess the biological roles of TaELF6-A1 in seed dormancy and its target sites at the genome-wide level under the *TaGATA1*-mutant background.

This study proposed a working model in which TaGATA1 positively regulates *TaABI5* expression by directly binding GATA motifs at its promoter, which contributes to seed dormancy in wheat. This model also depicts the recruitment of TaELF6-A1 by TaGATA1 through physical interaction, resulting in the removal of H3K27me3 repressive marks in the *TaABI5* promoter (Figure 7). Therefore, we show a novel molecular mechanism underlying the contribution of *TaGATA1* to seed dormancy. This indicates that *TaGATA1* could be a useful gene for improving PHS resistance in plants.

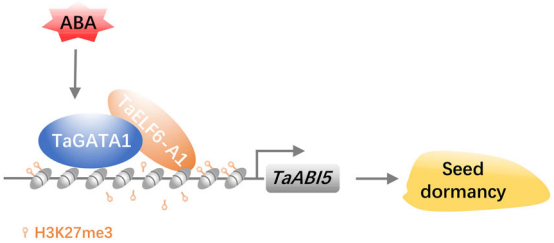

Figure 7. A working model of TaGATA1 and TaELF6-A1 regulated *TaABI5* expression to enforce seed dormancy in wheat
The phytohormone ABA induces the expression of the transcription factor TaGATA1. TaGATA1 directly binds to the promoter of TaABI5 and in addition recruits TaELF6-A1 to remove the gene repression mark (H3K27me3) to regulate TaABI5 expression and thereby improve seed dormancy.

MATERIALS AND METHODS

Plant materials and growth conditions

Ubi::myc-TaGATA1 transgenic overexpression Yangmai16 lines (OE) were obtained in our previous study (Liu et al., 2020). Homozygous T3 transgenic plants were used in this study. Wheat plants were grown in an experimental field at the Institute of Crop Sciences, CAAS, Beijing, under natural growth conditions. The immature grains at 7, 14, 21, and 28 d post anthesis (DPA) and dry seeds (air dried for a week after seeds physiologically mature) were collected for dissection. The seeds with completely released dormancy (stored at room temperature for at least 8 weeks) were sown in Petri dishes for germination and collected to extract the total RNA at 12, 24, and 36 HAI. The *Nicotiana benthamiana* plants used for dual-luciferase reporter assay were grown under 16 h, 28°C/8 h, 22°C, and light/dark conditions, and 3-week-old plants were used in this study. For the gene response assay to the ABA or GA treatment, the 5 mM ABA or 10 mM GA_3 solutions were sprayed on the wheat leaves at the two-leaf stage, water treatment was used as a control, and the leaves were collected at the indicated time intervals for dissection.

PHS and seed dormancy assay

PHS assay was performed according to the methods described by Lang et al. (2021). This assay was performed three times. No less than 10 heads from different plants of each OE lines or WT Yangmai 16 were contained in each assay. Whole wheat spikes were harvested at physiological maturity, which is characterized by loss of the green color on the ear, after which they were air dried for a week at room temperature until seed moisture was <10% and immediately used to test the sprouting rates. The air-dried spikes were

immersed in distilled water for 3 h and then placed in a growth chamber with saturating moisture in the dark for 7 d. After 7 d, a photograph was taken of the ears and the seeds were threshed from the ears. The germinated and ungerminated seeds were counted, and the germination percentages (the germinated seeds/total assayed seeds) were calculated. For the seed dormancy test, seeds were harvested at physiological maturity and stored at room temperature during the after-ripening (AR) process. At different times during AR, germination tests were performed using 45–50 seeds (15–20 seeds per Petri dish). The seeds were sown on Petri dishes containing filter paper and 5 ml of distilled water at 20°C for 7 d. After 7 d, the germinated seeds were photographed and the germination percentage was calculated. All assays were performed with at least three biological replicates.

For ABA response assays, the WT and OE wheat seeds with complete dormancy release were imbibed in water with 0 (control) or 50 μM ABA for 24 h. The germinated seeds were then counted, and the germination percentages were calculated.

RNA extraction, cDNA synthesis, and real-time qPCR

Total RNA of wheat leaves and protoplasts was extracted using TRIzol reagent (Invitrogen). Total RNA from developing seeds and germinated seeds were extracted using a MiniBEST Plant RNA Extraction Kit (Takara, Japan), according to the manufacturer's instructions. cDNA was synthesized using M-MLV Reverse Transcriptase with 2 μg of total RNA as a template (Takara, Japan). qPCR was performed using a SYBR Green kit (Takara, Japan) according to the manufacturer's instructions. At least three biological replicates, each with at least two technical replicates for each sample, were used for RT-qPCR. Relative expression levels of target genes were normalized to *TaACTIN*. All primers used in the RT-qPCR are listed in Table S6.

Plasmid construction

All DNA constructs used in this work were prepared following standard molecular biology protocols, and all plasmids were verified by DNA Sanger sequencing. For the yeast-one-hybrid assay, the *TaABI5* promoter fragments F1 (−2926 to −2660), F2 (−2508 to −2128), and F3 (−304 to −1) were cloned into the pHis2 vector. *TaGATA1*-ORF was cloned into pGADT7 to generate TaGATA1-AD. For the yeast-two-hybrid assay, *TaELF6-A1*-ORF was cloned into pGBKT7 to generate TaELF6-A1-BD. For the BiFC, the coding sequence of *TaGATA1* was cloned into pSYNE to generate TaGATA1-YFPn, and *TaEFL6-A1* was cloned into pSYCE to generate TaEFL6-A1-YFPc. For the LCI assay, TaGATA1 was fused to nLUC and TaELF6-A1 was ligated to cLUC to generate nLUC-TaGATA1 and cLUC-TaELF6-A1, respectively. *TaGATA1* was cloned into the pCAMBIA1302 vector to generate the TaGATA1-GFP fusion protein. For the dual LUC assay, the *TaABI5* promoter fragments P1 (−2926 to −2128) and P2 (−812 to −1) were cloned into pGreen0800-LUC. For the protoplast transient expression assay, *TaELF6-A1* was cloned into pJET163-hGFP to generate GFP fusion proteins.

Yeast-one-hybrid assay and yeast-two-hybrid assay

The yeast-one-hybrid assay was performed according to the manufacturer's instructions for the Matchmaker Gold Yeast-one-hybrid system (Clontech). The TaGATA1-AD construct and pHis2 constructs with different promoter fragments (P1, P2, or P3) were introduced into the AH109 yeast strain and selected on SD−Leu−Trp−His plates supplemented with 100 mM 3-amino-1,2,4-triazole (3-AT) to screen for protein–DNA interactions.

The yeast-two-hybrid assay was performed according to the manufacturer's instructions for the Matchmaker GAL4-based two-hybrid system (Clontech). The TaGATA1-AD and TaEFL6-1-BD constructs were transformed into the AH109 yeast strain and selected on SD−Leu−Trp−His−Ade plates.

Dual-luciferase reporter assay and LUC assay

The TaGATA1-GFP construct and pGreen0800-LUC containing different promoter fragments (P1 or P2) were transformed into the *Agrobacterium* strain GV3101. *Agrobacterium* sp. was cultured in the appreciated culture for 12 h and resuspended in infiltration buffer (10 mM $MgCl_2$ and 40 μM acetosyringone [AS]) to a final OD_{600} of 0.6. Before infiltration, *Agrobacterium* sp. was incubated at 28°C for 3 h. Tobacco plants were cultured under a 16 h light/8 h dark for 48 h after infiltration. A portion of the leaf samples was harvested for the dual LUC assay using Beetle Luciferin (Promega), and another portion was used to observe the LUC image using a low-light-cooled charge-coupled device (CCD) imaging apparatus (NightSHADE LB985).

Bimolecular fluorescence complementation assay

BiFC assays were performed as previously described with some modifications (Gehl et al., 2009). The *A. tumefaciens* strain GV3101 was transfected with DNA vectors (TaGATA1-YFPn, TaEFL6-A1-YFPc, or the empty vectors). The obtained GV3101 was adjusted to equal concentration and volume in the infiltration medium (pH 5.6; 40 μM AS and 10 mM $MgCl_2$), incubated at room temperature for 2 h, and co-infiltrated into 4-week-old *N. benthamiana* leaves. Each combination of constructs was infiltrated in different leaves. The tobacco plants were grown for 3 d, after which the lower epidermis of the leaves was subjected to confocal microscopy to detect the YFP signal.

Firefly LCI assay

The LCI assays for protein interaction detection were performed in *N. benthamiana* leaves. The nLUC-TaGATA1, cLUC-TaELF6-A1, and empty vectors were transformed into the *Agrobacterium* strain GV3101. *Agrobacterium* infiltration and tobacco plant culture were performed following the method for the dual-luciferase reporter assay as mentioned previously. The infiltrated leaves were analyzed using a low-light-cooled CCD imaging apparatus (NightSHADE LB985).

ChIP

The ChIP assay was performed according to previously described methods with slight modifications (Bowler et al., 2004). Briefly, 1 g (~50 grains) of immature grains of the *TaGATA1*-overexpression line L165 and WT Yangmai16 at 21 DPA were cross-linked with 1% (v/v) formaldehyde. The isolated chromatin complex was sonicated into fragments with lengths of 200–700 bp and used for immunoprecipitation with commercially available anti-MYC (Merk, A7470) or anti-H3K27me3 (Abcam, ab6002). The ChIP assays were performed in wheat leaf protoplasts according to previously described methods (Xiong et al., 2013). The p35S:6MYC-TaELF6-A1 construct was transformed into WT or OE leaf protoplasts using the PEG-mediated method. The chromatin complex was immunoprecipitated using an anti-H3K27me3 antibody (Abcom, ab6002). After reversing the cross-links, the precipitated DNA was recovered. The enrichment of particular DNA fragments in the *TaABI5* promoters was analyzed by qPCR. Three biological replicates were performed, and the data from each replicate were calibrated by input. The results are shown as a percentage of the input. The primers used in this assay are listed in Table S6.

RNA-Seq

The developing seeds of WT and OE line L165 wheat at 28 DPA were harvested for RNA sequencing. At least 15 seeds from each genotype were pooled for total RNA extraction. The cDNA library was constructed using a NEBNext Ultra RNA Library Prep Kit for Illumina and sequenced on the Illumina NovaSeq. 6000 system. After screening and trimming, clean reads were mapped to the wheat cultivar CS reference genome IWGSC v2.1 and annotations v2.1. DESeq. 2 was used to determine the DEGs, which were determined with a fold change >2 and *p*-value <0.05.

Transient expression

Wheat leaf protoplasts were isolated according to the method used by Yoo et al. (2007). p35S:TaELF6-A1 was transformed into wheat leaf protoplasts via PEG-mediated methods. Total RNA was isolated using TRIzol reagent (Invitrogen).

Statistical analysis

Statistical differences between the two samples were determined using two-tailed, unpaired Student's *t*-test with equal variance in Microsoft Excel. Multiple comparisons with equal standard deviations were analyzed using one-way ANOVA followed by Tukey's test in GraphPad Prism V5 software.

Accession numbers

Sequence data from this article can be found in GenBank under the following accession numbers: *TaELF6-A1* (OL581719), *TaABI5* (OL581720).

ACKNOWLEDGEMENTS

We thank Lipu Du (ICS, CAAS) for their help with wheat transformation. This work was funded by grants from the National Natural Science Foundation of China (31671692 and 32072005).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Xuening Wei designed and performed the experiments, and wrote the manuscript. Yuyan Li, Xiuliang Zhu, Xin Liu, Xingguo Ye, and Miaoping Zhou performed the experiments. Zengyan Zhang revised the manuscript. No conflict of interest was declared. All authors read and approved of its content.

Edited by: Pengcheng Wang, Shanghai Center for Plant Stress Biology, CAS, China

Received Oct. 24, 2022; **Accepted** Dec. 13, 2022; **Published** Dec. 19, 2022

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SUPPORTING INFORMATION

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