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Genome-wide association study reveals that *GmJAZ8* is involved in the germination of soybean seeds

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Abstract

Soybean (*Glycine max* L.) is one of the major oil and economic crops for human beings. Seed germination is a critical stage in soybean's growth cycle and plays a crucial role in determining soybean yield, yet only few genes controlling the rate of seed germination have been characterized in soybean. Here, we performed a genome-wide association study (GWAS) and identified a quantitative trait locus (QTL) for the rate of seed germination on chromosome 8. By using transcriptomic analysis, cross-species homology alignment, and haplotype-based selection analysis, we identified *GmJAZ8* (*Glyma.08G096500)* as a key candidate gene regulating soybean's germination rate. We further confirmed that *GmJAZ8* accelerated the rate of seed germination by regulating the transcriptional levels of genes involved in abscisic acid and gibberellin signaling. Thus *GmJAZ8* might be useful for improving the rate of seed germination in breeding soybean.

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Introduction

Seed germination represents the first and most critical stage of the plant life cycle, playing a decisive role in crop yield[1,2]. However, soybean (*Glycine max* L.) seeds are short-lived and highly susceptible to deterioration because of their high protein and oil content, leading to significantly reduced seed vigor and germination rates[3]. Previous studies have found a strong positive correlation between soybean's germination rate and yield[4]. In practical production, high-germination soybean seeds could enhance the uniformity of field emergence, reduce the amount of seed sown, lower agricultural planting costs, and ultimately increase yields[5]. Therefore, identifying the key genes regulating soybean's germination and elucidating their molecular mechanisms is vital for advancing high-yield soybean breeding programs.

The transition of crop seeds from dormancy to germination is determined by the balance between the levels of abscisic acid (ABA) and gibberellin (GA) within the seeds. A lower ABA/GA ratio is required for seed germination[6]. These hormones act antagonistically, mutually and negatively regulating each other's biosynthetic pathways[7]. Reducing the expression levels of ABA biosynthesis genes (such as 9-cis-epoxycarotenoid dioxygenases [*NCEDs*]) and GA metabolic genes (such as *GA2OXs*, gibberellin 2-oxidases), while increasing the expression levels of the ABA metabolic gene *CYP707As* (cytochrome P450 707As) and GA biosynthetic genes (such as *GA20OXs*, gibberellin 20-oxidases), can lower the ABA/GA ratio, thereby enhancing the rate of seed germination[8,9]. ABI5 (abscisic acid-insensitive 5) is one of the master regulators in ABA signaling that negatively regulates seed germination, which regulates ABA and GA metabolic and signaling genes to control ABA and GA levels, thereby repressing seed germination[10]. The DOG1 (Delay of Germination 1) protein is another major regulator of seed dormancy, which interacts with ABA signaling and delays seed germination[11,12].

Jasmonate-Zinc-finger expressed in inflorescence meristem (ZIM) domain (JAZ) proteins, as components of jasmonic acid (JA) signaling, participate in various growth and developmental processes of plants[13−15]. Studies have demonstrated that JAZ proteins interact with ABI3/ABI5 and suppress their transcriptional activity, thereby affecting seed germination in wheat (*Triticum aestivum*), rice (*Oryza sativa*), and *Arabidopsis thaliana*[16−19]. The soybean genome encodes 28 JAZ proteins (Wm82.a4.v1), with some JAZ repressors involved in plant development and abiotic stress responses. For instance, JAZ1/2 interacts with Avh94 to negatively regulate soybean's resistance to *Phytophthora* infection through the jasmonate signaling pathway[20]. GmJAZ3 directly interacts with the jasmonate signaling transcription factor GmMYC2a, suppressing its transcriptional activation of *GmCKX3-4*. Simultaneously, GmJAZ3 interacts with both GmRR18a and GmMYC2a to inhibit their activation of the cytokinin oxidase gene *GmCKX* for controlling seed size and weight[21]. *GmJAZ8* may mediate soybean's resistance to soybean cyst nematodes through jasmonate signaling processes[22]. However, the role of JAZs in soybean seed germination remains to be elucidated.

This study provides important insights into the role of *GmJAZ8* in soybean seed germination.

Materials and methods

Plant materials

A set of 277 soybean accessions (with 164 improved varieties and 113 landraces)[23] used for a genome-wide association study (GWAS) was planted at the Yangdu experimental station of Zhejiang Academy of Agricultural Sciences during autumn in 2023. Detailed information regarding these soybean accessions is provided in Supplementary Table S1. Field planting was performed according to a randomized complete block design, which used a plot with a single row and three replicates. Seeds were collected at the fully ripe stage. *Arabidopsis* (Col0 and *overexpression-GmJAZ8*) was grown under uniform conditions in a climate-controlled growth chamber with a 16-h light/8-h dark cycle at 22 °C.

Seed germination

Before germination, chlorine gas (5 mL HCl + 95 mL sodium hypochlorite) was used to sterilize the seeds and reduce the risk of microbial contamination. Twenty healthy soybean seeds were sown on a 0.9% agar medium, and 6 mL of sterile water was then added. The soybean seeds were incubated at 25 °C under dark conditions for 48 h after imbibition (HAI). *Arabidopsis* seeds were incubated for 2 d at 22 °C under a 12-h light/12-h dark cycle after 3 d of cold treatment. The germinated seeds (embryonic axis penetrating the seed coat) were counted to obtain the germination rate (GR) from 0 to 48 HAI. Three biological replications were performed.

Genome-wide association study

Single-nucleotide polymorphisms (SNPs) derived by resequencing 277 soybean accessions were used to conduct a GWAS for seed germination. In total, 89,125,094 high-quality SNPs with a missing rate of < 0.1 or a minor allele frequency of > 0.05 were used for the GWAS. The threshold for a significant association was set to 1/*n* (*n* is the number of SNPs, $p < 1.122 \times 10^{-8}$ or $-\log10(p) > 7.95$). The general linear model (GLM) and the mixed linear model (MLM) were used to analyze the association with seed germination. Manhattan plots were generated by the R package rMVP.

RNA extraction, cDNA, and quantitative real-time polymerase chain reaction

Total RNA was extracted from dry seeds utilizing Vazyme's FastPure® Universal Plant Total RNA Isolation Kit (RC411-01) following the manufacturer's protocol, and three biological replicates were performed for each experiment. Reverse transcription was carried out using a cDNA synthesis kit. The cDNA sequence was subsequently used as the template for quantitative real-time polymerase chain reaction (qRT-PCR). qRT-PCR was performed using a LightCycler 480 with SYBR Green I Master Mix. Gene expression was normalized to the expression of *AtTUBLIN*. The relevant primers used are listed in Supplementary Table S2.

Phylogenetic analysis

Duplicates and orthologs with high similarity to *GmJAZ8* from soybean and other representative species (rice and *Arabidopsis*) were obtained from Phytozome 13 (https://phytozome-next.jgi.doe.gov/blast-search). The aligned sequences were used to construct phylogenetic trees in MEGA5 software. The bootstrap value was set to 1,000 replications.

Vector construction and transformation

To construct the *GmJAZ8* (*Glyma.08G096500*) overexpression vector, the coding DNA sequences (CDSs) of *GmJAZ8* were amplified from Willimas 82 (Wm82) and ligated into the pBinGFP4 vector. The overexpression vector was subsequently introduced into *Agrobacterium tumefaciens* strain EHA105 and then transformed into *Arabidopsis*. All primers used to construct the vectors are listed in Supplementary Table S2.

Overexpression of *GmJAZ8* in *Arabidopsis*

To obtain the stable expression of *GmJAZ8* in *Arabidopsis*, we transformed the recombinant vector into *Agrobacterium tumefaciens* (EHA105 strain) and then transformed *Arabidopsis* via the foral dip method[24]. The transformants of the primary plants were placed on a Murashige and Skoog (MS) medium using kanamycin (0.05 mg/mL) for testing resistance. After 10 d, the resistant seedlings were transplanted into the soil. We collected the resulting seeds and tested them for resistance to kanamycin, selecting from three different generations to produce transformed homozygous plants. All primers used to construct the vectors are listed in Supplementary Table S2.

Transcriptome sequencing and analysis

For the RNA-seq analysis, total RNA was extracted from dry seeds for six soybean accessions, *GmJAZ8*-overexpressing *Arabidopsis* lines, and wild-type *Arabidopsis*, and seeds germinated at 0 and 24 HAI of *GmJAZ8*-overexpressing and wild-type *Arabidopsis* using Vazyme's FastPure® Universal Plant Total RNA Isolation Kit (RC411-01), following the manufacturer's guidelines. Both the *GmJAZ8*-overexpressing lines and the wild-type were cultivated under identical conditions. Three biological replicates were performed for each sample. The RNA libraries were sequenced on the Illumina NovaseqTM 6000 platform. and a bioinformatic analysis was conducted by LC Bio Technology Co., Ltd. (Hangzhou, China).

The sequence quality was verified using FastQC (including the Q20, Q30, and GC content of the clean data), and mapped to the soybean reference genome (Wm82.a4.v1) using the HISAT2 package. The mapped reads of each sample were assembled using StringTie with the default parameters. All transcriptomes from all samples were merged to reconstruct a comprehensive transcriptome using gffcompare software. After the final transcriptome was generated, StringTie and ballgown were used to estimate the expression levels of all transcripts and the expression abundance for mRNAs by calculating the FPKM (fragment per kilobase of transcript per million mapped reads) value.

DESeq2 was used to calculate differentially expressed genes (DEGs), using the threshold of a false discovery rate of < 0.05 and an absolute fold change of ≥ 2. Principal component analysis (PCA) was performed with the princomp function of R. Gene Ontology (GO) enrichment analyses of the DEGs were performed using the cloud platform provided by Shanghai OEbiotech with the default parameters.

Data analysis

The experimental data were analyzed using GraphPad Prism software (version 10.1.2). The significant differences among samples were compared using Student's *t*-test.

Results

GWAS for the rate of seed germination in soybean

To investigate the key genes affecting variation in the rate of seed germination in soybean, we analyzed the rate of seed germination at 36 and 48 HAI in 277 soybean accessions included in a previous resequencing population[23] (Supplementary Table S1). Subsequently, association analysis of the germination rate at 36 and 48 HAI was performed using the GLM and MLM. Finally, 59 significant SNPs were identified on chromosome 8 in 36HAI_GLM, whereas 36HAI_MLM identified 14 significant SNPs on chromosome 8. For 48HAI_GLM, 68 significant SNPs were identified on four

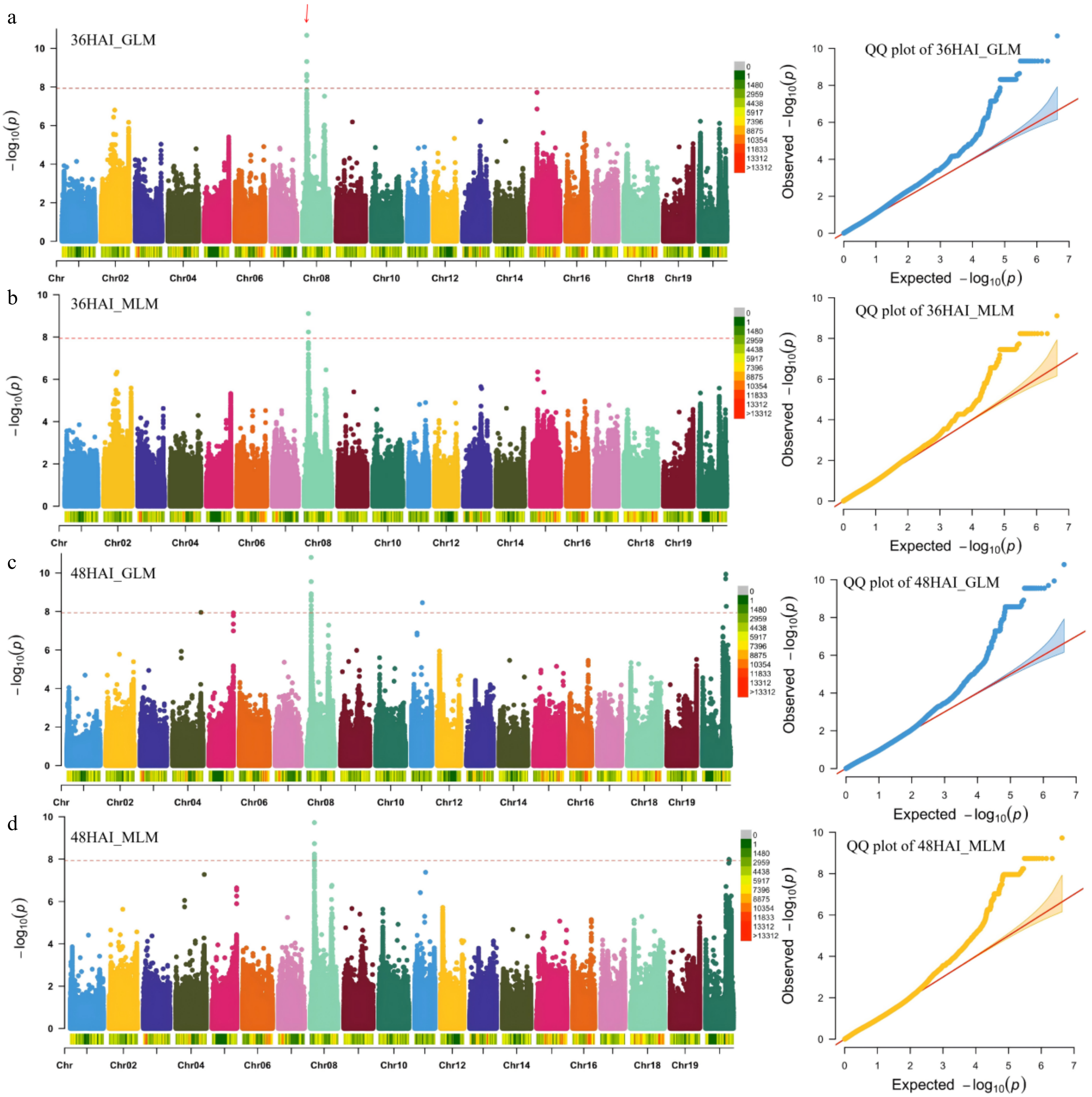


Fig. 1 GWAS of the rate of seed germination in soybean. (a)–(d) Manhattan plots and quantile–quantile (Q–Q) plots for the whole population of soybean accessions. The red arrows indicate the quantitative trait loci (QTLs) identified. GLM, general linear model; MLM, mixed linear model; HAI, hours after imbibition.

chromosomes (1 SNP on chromosome 4, 62 SNPs on chromosome 8, 1 SNP on chromosome 11, and 4 SNPs on chromosome 20). Meanwhile, 60 significant SNPs were located on chromosome 8 (59 SNPs) and chromosome 20 (1 SNP) for 48HAI_MLM (Supplementary Table S3). Notably, across all four GWAS results, 14 significant SNPs on chromosome 8 were consistently identified (Fig. 1a–h and Table 1). These SNPs are physically close on the chromosome, forming a continuous cluster within a 113.5-kb region (7,394,400–7,507,912 bp). We focused further on the genes within this 113.5-kb interval block.

Identification of potential candidate genes for seed germination

The potential candidate genes within the 113.5-kb interval block were analyzed, and a total of 15 genes were annotated according to the reference genome Wm82 (Wm82.a2.v1) (Table 2). We obtained the expression levels of 15 genes in flowers and at different seed developmental stages from SoyOmics (https://yanglab.hzau.edu.cn/SoyMD/#/tools/blast), and found that *Glyma.08G096500* was highly expressed in 10-week-old seeds. Further analysis of the expression patterns of these 15 genes during seed germination revealed that *Glyma.08G096500*, *Glyma.08G096800*, and *Glyma.08G097700* were

Table 1. Information of 14 significant SNPs within the 113.5-kb interval block (7,394,400–7,507,912 bp).

SNP	Alleles	36HAI_GLM *p*-value	36HAI_MLM *p*-value	48HAI_GLM *p*-value	48HAI_MLM *p*-value
Chr08_7394400	C/A	2.12E-11	7.74E-10	2.72E-09	1.90E-10
Chr08_7400080	C/T	4.86E-10	5.82E-09	1.57E-11	1.86E-09
Chr08_7416347	C/T	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7427722	G/T	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7459314	T/C	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7464017	G/A	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7465749	G/A	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7470131	A/G	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7476997	C/A	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7490743	T/A	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7490778	T/C	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7500442	T/C	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7500556	T/G	4.86E-10	5.82E-09	2.82E-10	1.86E-09
Chr08_7507912	A/T	4.86E-10	5.82E-09	2.82E-10	1.86E-09

HAI, hours after imbibition; GLM, general linear model; MLM, mixed linear model.

expressed throughout all stages of seed germination and were highly expressed in dry seeds (Supplementary Fig. S1a, S1b).

To identify the key genes involved in the rate of seed germination, transcriptomic sequencing of dry seeds was conducted in three soybean accessions with low germination rates (GDC198 with 37.5%, GDL060 with 57.5%, and GDL062 with 57.5%) and three soybean accessions with high germination rates (GDC018 with 90%, GDL023 with 100%, and GDL075 with 100%) (Fig. 2a). Among these 15 genes, the transcriptomic data revealed that 13 genes were successfully detected but two genes (*Glyma_08G097100* and *Glyma_08G097200*) remained undetected. In these 13 genes，the expression level of four genes was zero (Supplementary Table S4). Eight genes showed no significant differences between soybean accessions with low or high germination rates (Fig. 2b and Supplementary Table S4). Specifically, only *Glyma.08G096500* demonstrated a statistically significant difference and exhibited significantly higher expression levels in high-germination accessions compared with low-germination accessions (Fig. 2b). Further validation using

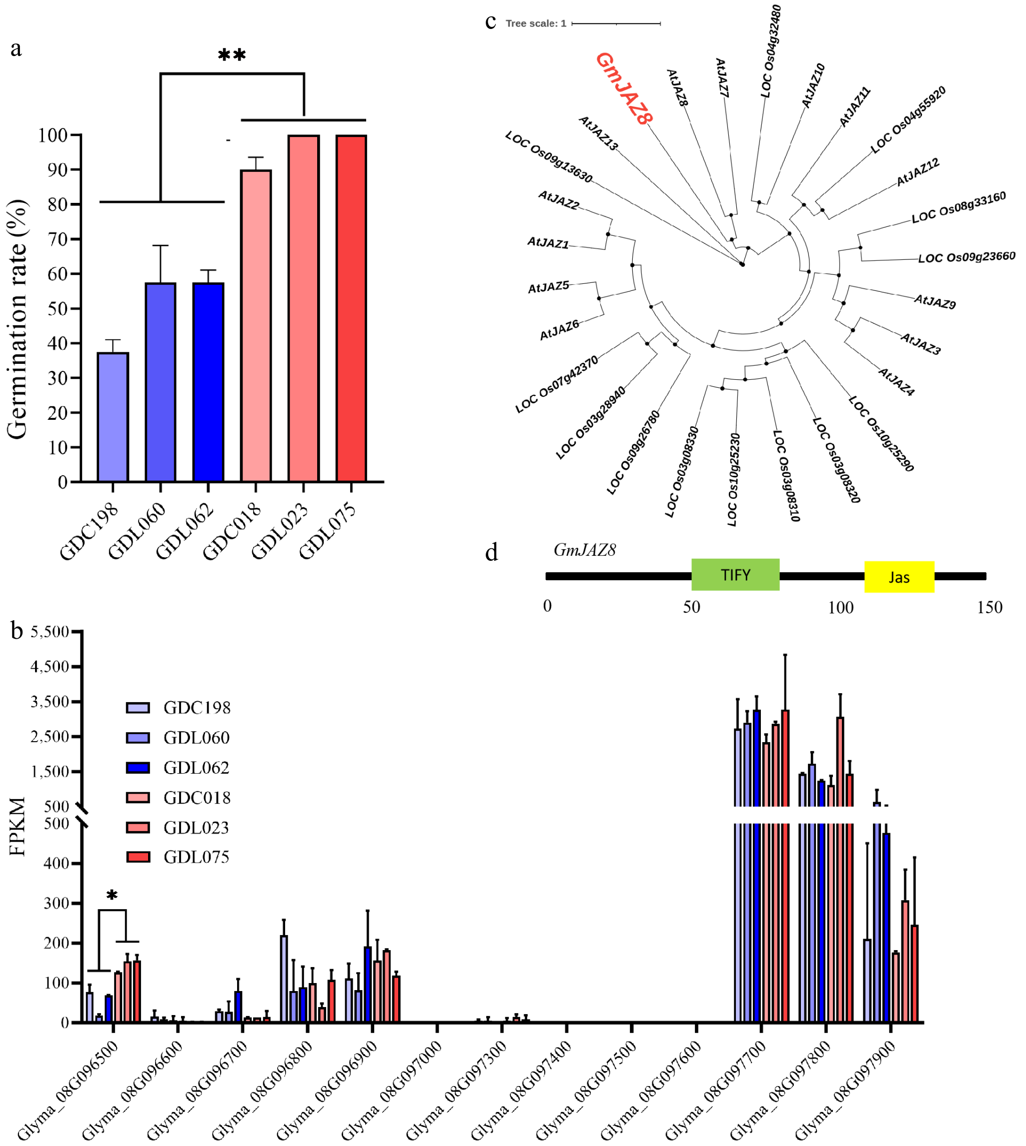


Fig. 2 Identification of *GmJAZ8* for soybean the rate of seed germination. (a) Germination rate of six soybean accessions. **, $p < 0.01$. (b) FPKM of nine genes. *, $p < 0.05$. ns, no significance. Only genes with FPKM values greater than 0 are displayed. (c) Phylogenetic analysis of the GmJAZ8 and JAZ family from *Arabidopsis* and rice. (d) Domain organization of GmJAZ8. The numbers under the black line indicate the position of the amino acids.

Table 2. Fifteen potential candidate genes within the 113.5-kb interval block (7,394,400–7,507,912 bp).

Gene ID	Position (bp)	Functional annotation	*Homologous Arabidopsis* genes
Glyma.08G096500	7,396,887–7,399,006	Jasmonate–ZIM domain protein 8	*AT1G30135*
Glyma.08G096600	7,404,774–7,409,459	(S)-2-hydroxy-acid oxidase	*AT4G18360*
Glyma.08G096700	7,412,769–7,417,800	(S)-2-hydroxy-acid oxidase	*AT4G18360*
Glyma.08G096800	7,418,970–7,424,007	(S)-2-hydroxy-acid oxidase	*AT3G14420*
Glyma.08G096900	7,424,892–7,426,785	Tetratricopeptide repeat (TPR)-like superfamily protein	*AT4G21065*
Glyma.08G097000	7,429,593–7,429,941	AA_trans domain-containing protein	*AT5G19875*
Glyma.08G097100	7,432,417–7,434,453	(S)-2-hydroxy-acid oxidase	*AT3G14420*
Glyma.08G097200	7,435,704–7,438,009	(S)-2-hydroxy-acid oxidase	*AT4G18360*
Glyma.08G097300	7,438,009–7,444,035	(S)-2-hydroxy-acid oxidase	*AT4G18360*
Glyma.08G097400	7,447,072–7,448,398	Leucine-rich repeat receptor-like protein kinase	*AT2G42800*
Glyma.08G097500	7,457,157–7,457,877	Cotton fiber expressed protein	*AT2G34610*
Glyma.08G097600	7,460,358–7,463,786	BED zinc finger	*AT3G42170*
Glyma.08G097700	7,468,455–7,476,125	RNA-binding KH domain-containing protein	*AT5G46190*
Glyma.08G097800	7,478,544–7,486,117	Ornithine aminotransferase	*AT5G46180*
Glyma.08G097900	7,489,719–7,494,791	TCP2 family transcription factor	*AT4G18390*

qRT-PCR to assess the expression levels of the 15 genes in the high-germination and low-germination materials revealed that also only *Glyma.08G096500* showed a significant difference in its expression levels, which was consistent with the transcriptomic data (Supplementary Fig. S1c, S1d). According to the functional annotation of the soybean genome, *Glyma.08G096500* encodes JAZ protein 8 (Table 2) and is considered to be a candidate gene named *GmJAZ8*. In a comparative analysis of the amino acid sequence similarity between GmJAZ8 and JAZ proteins from *Arabidopsis thaliana* and rice, GmJAZ8 contains a ZIM and a Jas domain and shares higher homology with AtJAZ7 and AtJAZ8 in *Arabidopsis* (Fig. 2c, d). In *Arabidopsis*, AtJAZ7 interacts with AtARF10 and AtARF16, integrating hormone signaling to suppress the rate of seed germination[18]. AtJAZ1/5/8 interact with AtABI3/5, integrating JA and ABA signaling during seed germination[25].

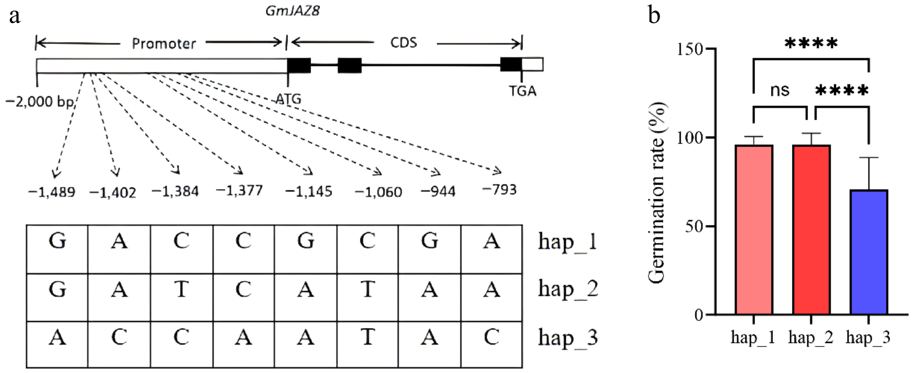
Allelic variations within the promoter and intragenic regions of *GmJAZ8* were detected in a resequenced population. Eight SNPs were identified within the promoter region (Supplementary Table S5). On the basis of these SNPs, the population was divided into three haplotype groups (hap_1 to hap_3) (Fig. 3a). The population primarily consisted of hap_1 (n = 215), hap_2 (n = 36), and hap_3 (n = 18). The rate of seed germination showed no significant differences between haplotype groups hap_1 and hap_2. However, the hap_3 haplotype group exhibited a significantly lower rate of seed germination than all other haplotype groups (Fig. 3b). According to these results, we propose that *GmJAZ8* (*Glyma.08G096500*) is likely to be a key gene regulating seed germination in soybean.

GmJAZ8 promotes seed germination

The candidate gene *Glyma.08G096500* encodes the JAZ protein named JASMONATE ZIMDOMAIN 8 (*GmJAZ8*). To validate the function of *GmJAZ8* in seed germination, *GmJAZ8* (*Glyma.08G096500*) overexpression vectors were constructed and then transformed into *Arabidopsis*, and two highly expressed lines (OE_6 and OE_28) were chosen by qRT-PCR analyses for subsequent experiments (Fig. 4a). Seeds of sterilized wild-type (Co10), OE_6, and OE_28 were first stratified at 4 °C for 3 d to break seed dormancy and were then sown in culture dishes with filter papers. The rate of seed germination was recorded at 12, 24, and 48 HAI. The results showed that the germination rate of all *GmJAZ8* overexpression lines was ssignificantly higher than that of the wild-type line at 24 HAI (Fig. 4b). The seeds from all lines eventually germinated at 48 HAI. These indicated that the overexpression of *GmJAZ8* promoted the speed of seed germination in OE_6 and OE_28 compared with the wild-type in *Arabidopsis* (Fig. 4b, c, and Supplementary Table S5).

GmJAZ8 altered expression of hormone signaling genes

To investigate the molecular mechanisms underlying the regulation of *GmJAZ8* in seed germination, RNA sequencing (RNA-seq) was performed on seeds of the wild-type (Col0) and *GmJAZ8* overexpressing lines (OE_6 and OE_28) at the 0 and 24 HAI timepoints. The PCA results showed that biological replicates clustered well (Fig. 5a). Notably, Col0 and the overexpression lines (OE_6 and OE_28) were



G	A	C	C	G	C	G	A	hap_1
G	A	T	C	A	T	A	A	hap_2
A	C	C	A	A	T	A	C	hap_3

Fig. 3 Allelic variations in the *GmJAZ8* gene (promoter and intragenic regions). (a) Haplotypes of *GmJAZ8*. (b) Box plots of the germination rate of the accessions of different haplotypes. Different colors indicate the different haplotypes. ****, $p < 0.0001$; ns, no significance.

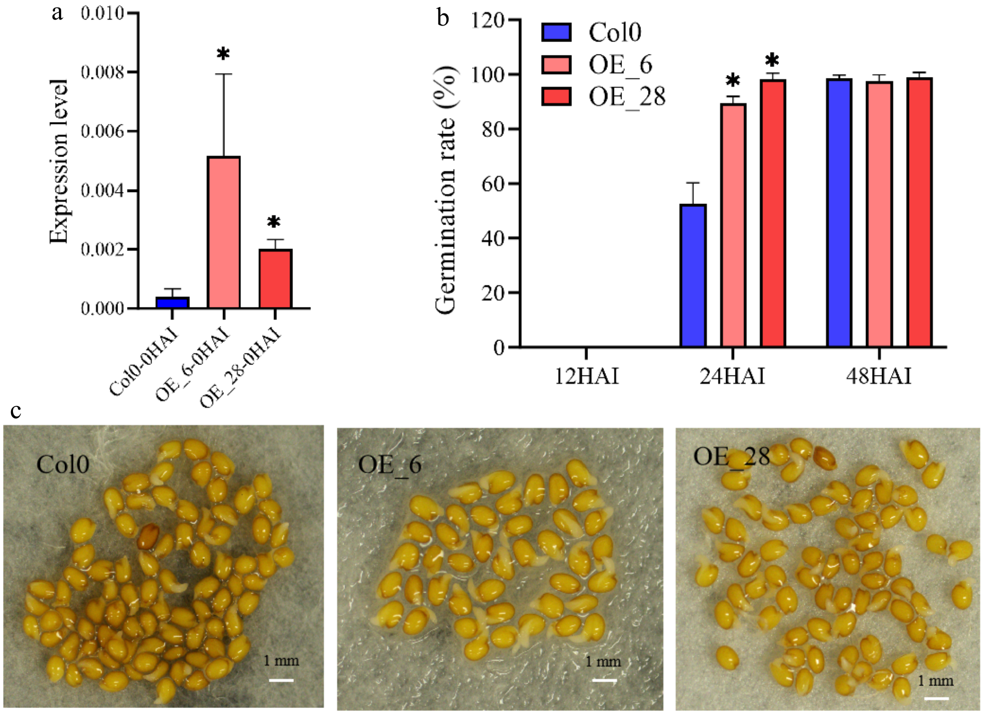


Fig. 4 *GmJAZ8* promotes seed germination. (a) Relative expression of *GmJAZ8* in Col0 and transgenic lines (OE_6, and OE_28) at 0 HAI. (b) The rate of germination of Col0 and the transgenic lines (OE_6, and OE_28). (c) Seedlings of the wild-type, OE_6, and OE_28 lines at 24 HAI. *, $p < 0.05$.

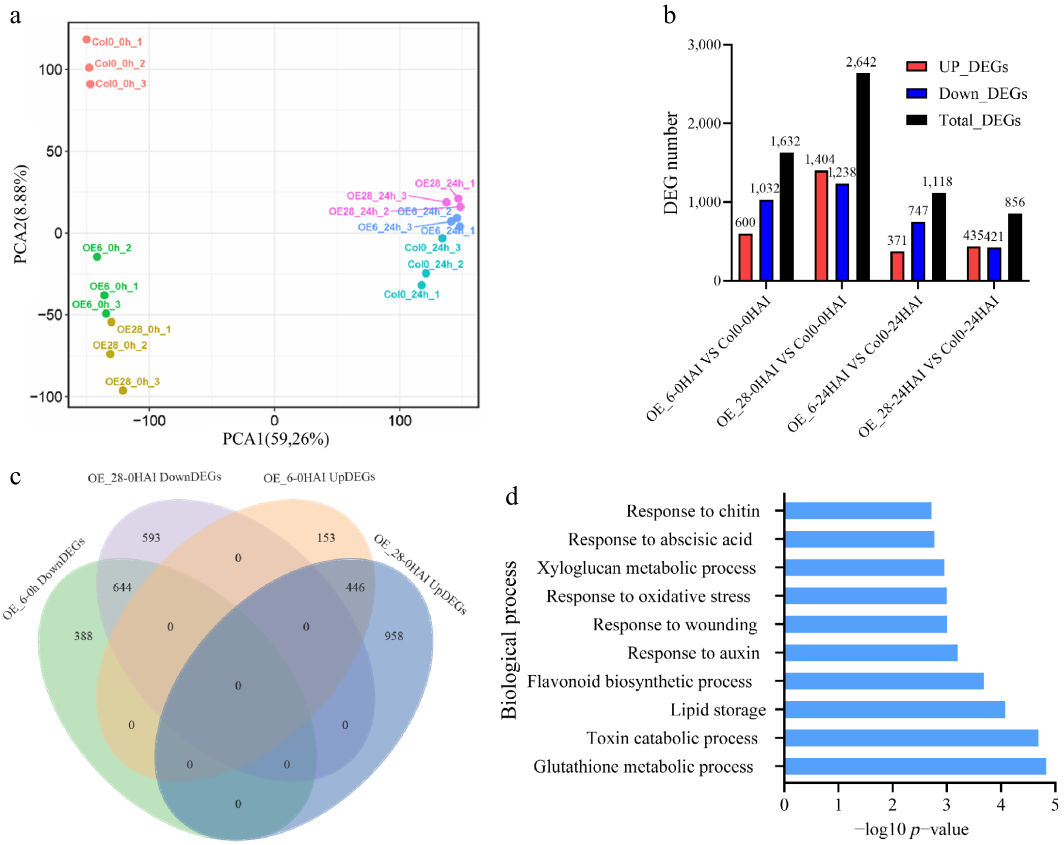


Fig. 5 Differentially expressed genes (DEGs) with $p < 0.05$ between the wild-type (Col0) and *GmJAZ8* overexpressing lines. (a) PCA of all samples. (b) The number of DEGs. (c) Overlapping DEGs between OE_6 and OE_28 at 0 HAI. (d) GO assay of downregulated overlapping DEGs at 0 HAI.

separated into two distinct clusters at 0 HAI, whereas Col0 and the overexpression lines (OE_6 and OE_28) grouped together within one cluster at 24 HAI (Fig. 5a), indicating that the differences in germination between Col0 and the overexpression lines primarily originated from the values at 0 HAI.

Compared with Col0, 1,632 significant DEGs were detected in OE_6, including 600 upregulated and 1,032 downregulated genes at 0 HAI, whereas 2,642 DEGs were identified in OE_28, comprising 1,404 upregulated and 1,238 downregulated genes (Fig. 5b and Supplementary Table S6). OE_6 exhibited 1,118 DEGs (371 upregulated and 747 downregulated), whereas OE_28 showed 856 DEGs (435 upregulated, 421 downregulated) at 24 HAI (Fig. 5b and Supplementary Table S6).

Further accurate analysis of DEGs in the *GmJAZ8* overexpressing lines was performed. We examined overlapping DEGs between OE_6 and OE_28 at 0 and 24 HAI. A cross-comparison of *GmJAZ8* overexpressing lines revealed 1,090 overlapping DEGs at 0 HAI (664 downregulated and 446 upregulated), decreasing to 497 overlapping DEGs at 24 HAI (300 downregulated and 197 upregulated) (Fig. 5c, Supplementary Fig. S2a, and Supplementary Table S6).

GO analyses revealed that upregulated DEGs at 0 HAI were enriched in biological processes including RNA modification, recognition of pollen, the innate immune response, glycolytic processes, microtubule-based movement, the response to zinc ions, pentose-phosphate shunt, oxidative branch, the response to light stimulus, intercellular transport, and cellular carbohydrate metabolic process, whereas the upregulated DEGs at 24 HAI were enriched in the regulation of lignin biosynthetic processes, the response to phenylpropanoid, phloem transport, seed coat development, xylem development, regulation of secondary cell wall biogenesis, cytokinin-activated signaling, defense response, L-phenylalanine biosynthetic processes, and lipid transport (Fig. 5d, Supplementary Figs S2c, S2d, and Supplementary Table S6). Downregulated DEGs at 0 HAI were primarily enriched in biological processes including glutathione metabolic processes, toxin catabolic process, lipid storage, flavonoid biosynthetic processes, response to auxin, response to wounding, response to oxidative stress, xyloglucan metabolic process, response to ABA, and response to chitin. However, downregulated DEGs at 24 HAI were mainly enriched in the chitin catabolic process, the polysaccharide catabolic process, leaf senescence, response to gibberellin, programmed cell death involved in cell development, the toxin catabolic process, transmembrane transport, the response to phenylpropanoid, cell wall macromolecule catabolic process, and aging. Notably, downregulated DEGs at 0 and 24 HAI showed enrichment in hormone signaling (Fig. 5d, Supplementary Fig. S2b, and Supplementary Table S6).

ABA and GA play important roles in controlling seed germination. Notably the ABA biosynthetic gene *NCED4* was significantly downregulated in OE_6 and OE_28, whereas the catabolic gene *CYP707A2* was upregulated (Fig. 6). In contrast, overexpression of *GmJAZ8* increased the expression of the GA biosynthetic gene *GA20OX3* and repressed the expression of the GA catabolic gene *GA2OX6* (Fig. 6). These suggested that a lower ABA/GA ratio was induced by the overexpression of*GmJAZ8*. Additionally, the overexpression of *GmJAZ8* also inhibited the expression of *AtDOG1*, which is another key component controlling seed germination (Fig. 6). Thus *GmJAZ8* controls seed germination by repression of the ABA/GA ratio and *DOG1* transcription.

ABI5, as a master regulator in ABA signaling, negatively regulates seed germination[19,26]. JAZ proteins interact with ABI5 and suppress

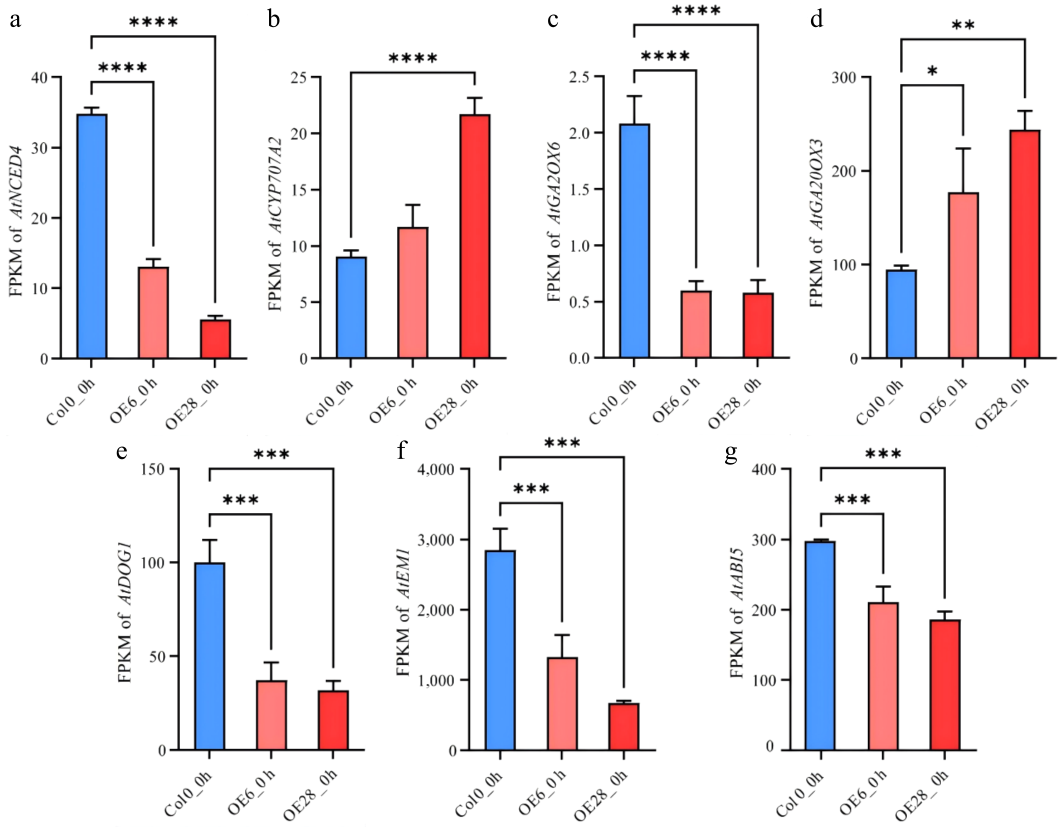


Fig. 6 *GmJAZ8* altered expression of hormonal signaling genes. the FPKM values of (a) *AtNCED4*, (b) *AtCYP707A2*, (c) *AtGA2OX6*, (d) *AtGA20OX3*, (e) *AtDOG1*, (f) *AtEM1* and (g) *AtABI5*. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

its transcriptional activity, thereby modulating EM1 expression levels to ultimately regulate seed germination[16,27,28]. In our results, the expression levels of *AtEM1* and *AtABI5* were also significantly suppressed in both the OE_6 and OE_28 lines (Fig. 6). As a result, overexpression of *GmJAZ8* altered the expression of genes involved in ABA and GA biosynthesis and signaling. Taken together, these findings demonstrate that *GmJAZ8*-mediated regulation of seed germination may involve coordinated ABA/GA pathway interactions (Fig. 6 and Supplementary Table S7). The expression levels of these genes were validated using qRT-PCR, and the results were consistent with the transcriptomic data (Supplementary Fig. S3).

Discussion

Seed germination is the process by which a mature, dry seed begins with imbibition and progresses to the gradual elongation of the hypocotyl until it breaks through the seed coat. As the first and most critical stage in a plant's growth cycle, seed germination plays a crucial role in determining crop yield[1]. Studies have shown a significantly positive correlation between the rate of seed germination and crop yield in soybean[4]. Soybean seeds are short-lived seeds that are prone to deterioration, leading to a significant decline in seed vigor and, consequently, a reduced germination rate. In this study, we combined GWAS and transcriptomic analyses to identify a major QTL on chromosome 8 associated with the rate of seed germination. Within this locus, *GmJAZ8* was pinpointed as a key candidate gene. Functional validation in *Arabidopsis* demonstrated that the overexpression of *GmJAZ8* accelerates seed germination. Further transcriptomic profiling revealed that *GmJAZ8* likely exerts its function by modulating the expression of genes involved in ABA and GA biosynthesis and signaling, thereby lowering the ABA/GA ratio.

To increase soybean production, it is essential to dissect and understand the mechanisms involved in the regulation of seed germination. GWAS has been widely used in plant research to identify loci that are significantly associated with seed germination[29−34]. At present, several genetic loci for seed germination in soybean have been detected via linkage mapping and GWAS[3,34,35]. Sharmin *et al.*[35] found a major SNP, Gm_08_46239716, for the germination rate and identified a candidate gene *Glyma.08G348500* encoding uridine diphosphate glycosyltransferase. Zhang *et al.*[3] detected a GWAS signal on chromosome 18 which was significantly associated with seed germination in soybean. In this study, GWAS revealed that 14 significant SNPs were co-localized in close proximity across multiple environments on chromosome 8, distributed within a 113.5-kb interval block (7,394,400–7,507,912 bp) (Fig. 1a–h and Supplementary Table S3).

A 113.5-kb interval block (7,394,400–7,507,912 bp) on chromosome 8 for soybean germination was identified, which contained 14 SNPs. Moreover, the key candidate gene *Glyma.08G096500*, which encodes a JAZ protein, was pinpointed by integrating transcriptomic analysis, homology analysis, and haplotype analysis (Figs 2, 3, and Table 2), which might be responsible for seed germination in soybean.

JAZ proteins constitute a plant-specific subfamily of the TIF[F/Y]XG domain (TIFY) superfamily characterized by TIFY and jasmonate domains. Studies have demonstrated that JAZ domains exhibit relatively conserved functional mechanisms in *Arabidopsis*[36,37]**.** The number of JAZ proteins exhibits substantial variation across plant species, with *Arabidopsis thaliana* possessing 12[38], rice having 15[39], *Zea mays* (maize) having 16[40], and soybean having 28 members (Wm82.a4.v1). Research indicates that different JAZ proteins play diverse and versatile roles in regulating distinct phenotypic traits, with JAZ1 inducing flowering[41], whereas JAZ11 regulates seed width and weight[42]. Although JAZ protein families in *Arabidopsis* and rice have been extensively characterized, considerably less attention has been devoted to their soybean orthologs. ASMONATE ZIM-DOMAIN 5 (OsJAZ5) involving an OsABI3-mediated ABA pathway was verified, which positively regulates seed germination in rice[18]. JAZ proteins interact with ABI3/ABI5 and suppress their transcriptional activity, thereby affecting seed germination in wheat and *Arabidopsis*[15,17]. Similar to previous studies[15,17,18], our *GmJAZ8* overexpression lines of *Arabidopsis* exhibited significantly higher germination rates than the wild-type plants (Fig. 4), providing evidence that *GmJAZ8* functions to promote seed germination.

ABA and GA levels in seeds are significantly correlated with seed germination[43,44]. ABA inhibits seed germination, whereas ABI5 is a key component of ABA signaling[18]. In wheat, the JAZ1 protein can directly interact with ABI5, inhibiting its transcriptional activation activity, thereby negatively regulating the ABA response and promoting seed germination[16]. Simultaneously, *Arabidopsis* JAZ proteins can also interfere with the activation of AtABI5 by the Indole-3-acetic acid (IAA)-dependent transcription factors AtARF10 and AtARF16, thus affecting seed germination[18]. In rice, OsJAZ6/7 can interact with OsABI3/5, influencing the transcriptional activity of OsABI3/5, and OsABI3/5 can participate in the process of regulating seed germination mediated by the UDP glucosyltransferase OsUGT75A[45]. Overall, these findings reveal that JAZ proteins can participate in ABA hormone signaling in coordination to regulate seed germination.

ABA is biosynthesized by *NCED* and *ABA genes*[8,46,47], and catalyzed by *CYP707As*[48]. GA is mainly biosynthesized by *GA3OXs*[49,50] and *GA20OXs*[51,52], and catalyzed by *GA2OXs*[53,54]. JAZ proteins regulate seed germination through a dual mechanism. By suppressing the activity of the transcription factor MYC2, they modulate the stability of Asp-Glu-Leu-Leu-Ala (DELLA) proteins, thereby indirectly but effectively influencing the expression of GA biosynthesis/metabolism-related genes[36]. This results in the suppression of GA biosynthetic genes (e.g., *GA20OX*) and the activation of GA catabolic genes (e.g., *GA2OX*). Simultaneously, JAZ proteins regulate ABA biosynthesis/metabolism genes by inhibiting transcription factors such as MYC2, which can bind to the promoters of ABA biosynthesis genes (e.g., *NCED*). This leads to downregulation of *NCED* transcription and reduced ABA levels. Seed germination is ultimately controlled through by coordinated regulation[36]. When the expression levels of ABA synthesis and GA metabolic genes decrease, or the expression levels of GA synthesis and ABA metabolic genes increase, the ABA/GA content ratio decreases, which promotes seed germination[55,56].

In our results, *GmJAZ8*-overexpressing lines exhibited upregulated expression of the ABA catabolic gene *AtCYP707A2* but downregulated expression of the ABA biosynthesis gene *AtNCED4* (Fig. 6). GA–ABA antagonism fundamentally operates through bidirectional suppression of hormone biosynthesis, with GA inhibiting ABA production and ABA repressing GA synthesis, thus collectively gatekeeping the initiation of germination[5,57,58]. By upregulating a GA biosynthetic gene (*GA20OX*) while suppressing a GA catabolic gene (*GA2OX*), seed germination can be effectively promoted[59]. Consistent with this established mechanism, our findings demonstrate that in *GmJAZ8*-overexpressing lines, *AtGA20OX3* expression was significantly upregulated, whereas *AtGA2OX6* transcript levels were markedly downregulated (Fig. 6). Transcription factors such as ABI3 and ABI5 function as master regulators in ABA signaling and negatively regulate seed germination[25]. Studies have shown that JAZ proteins interact with ABI3/ABI5, suppress their transcriptional activity, and modulate ABA signaling, thereby affecting seed germination in species such as wheat, rice, and *Arabidopsis*[15,18]. JAZ proteins interact with ABI5 and suppress its transcriptional activity, thereby modulating EM1 expression levels to ultimately regulate seed germination[15,26,27]. In the *GmJAZ8*-overexpressing lines (OE_6 and OE_28), transcript levels of ABI5 and EM1 were significantly downregulated, whereas the germination rate markedly increased (Fig. 6). We therefore propose that the accelerated germination in *GmJAZ8*-overexpressing lines may result from the altered expression of hormone signaling components.

Collectively, this GWAS pinpointed *GmJAZ8* as a pivotal regulator of the rate of seed germination. Functional validation demonstrated that *GmJAZ8* overexpression enhances the germination rate. Mechanistically, *GmJAZ8* likely modulates the germination rate by integrating ABA and GA signaling cascades.

Conclusions

In this study, we performed a GWAS and identified a QTL for the rate of seed germination in soybean on chromosome 8. By using transcriptomic analysis, cross-species homology alignment, and haplotype-based selection analysis, *GmJAZ8* was identified as a key candidate gene which regulates soybean's germination rate. *GmJAZ8* was then confirmed to accelerate the rate of seed germination by regulating the transcriptional levels of genes involved in ABA and GA signaling. Thus *GmJAZ8* might be useful for improving the rate of seed germination in soybean breeding programs.

Author contributions

The authors confirm their contributions to the paper as follows: funding support, study conception and design: Yuan F, Chen X, Zhao J; material treatment, data analysis, and manuscript writing: Xu W, Yang Y, Liu B, Chen Y; material preparation: Deng P, Yu H. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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