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ORIGINAL ARTICLE

GmAOC4 modulates seed germination by regulating JA biosynthesis in soybean

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Abstract

Key message **An allene oxide cyclase 4, *GmAOC4*, was determined by GWAS and RT-PCR to be significantly associated with seed germination in soybean, and regulates seed germination by promoting more JA accumulation.**

Abstract The seed germination phase is a critical component of the plant lifecycle, and a better understanding of the mechanism behind seed germination in soybeans is needed. We used a genome-wide association study (GWAS) to detect a GWAS signal on chromosome 18. In this GWAS signal, SNP S18_56189166 was located within the 3'untranslated region of *Glyma.18G280900*, which encodes allene oxide cyclase 4 (named *GmAOC4*). Analysis of real-time PCR demonstrated that expression levels of *GmAOC4* in the low-germination variety (KF, carrying SNP S18_56189166-T) were higher than in the high-germination variety (NN, carrying SNP S18_56189166-C). In these two varieties, KF showed a higher JA concentration than NN at 0 and 24 h after imbibition. Moreover, the overexpression of *GmAOC4* led to an increase in the concentration of jasmonic acid (JA) in soybean hairy roots and *Arabidopsis thaliana*. Furthermore, it was found that *GmAOC4*-OE lines showed less seed germination than the wild type (WT) under normal conditions in *Arabidopsis*. After 7 days of ABA treatment, transgenic lines exhibited lower seed germination and higher expression levels of *AtABI5* compared with WT, indicating that the overexpression of *GmAOC4* resulted in hypersensitivity to ABA. Our findings demonstrate that *GmAOC4*, which promotes more JA accumulation, helps to regulate seed germination in soybeans.

Introduction

Seed vigor is measured by assessing seed traits that regulate the activity levels and performance of seed types with high germination rates under various environmental conditions (Perry 1978). Studies have demonstrated that seed vigor affects field emergence, seedling growth, and yield (R and Nikam 2008; Börner et al. 2018; Caverzan et al. 2018). Germination is a major component of seed vigor, and high germination rates can reduce the risk of lower crop yields. The propagation of crops is heavily influenced by whether or not seeds are successfully germinated and established, making seed germination important both economically and ecologically. Germination has been used as a major index indicating the seed vigor of different crops (Han et al. 2014; Hang et al. 2015; Hatzig et al. 2015; Zhang et al. 2019a), while genetic dormancy traits also influence seed germination (Rajjou et al. 2012; Jin et al. 2018; Song et al. 2020; Wang et al. 2020). A better understanding of how the genetic components contribute to successful germination is needed to breed vigorous crop varieties with uniform rapid growth characteristics.

Seed germination is a complicated process. As such, the genetic factors responsible for seed germination are hard to identify using typical physiological or genetic analytical methods. A genome-wide association study (GWAS) is the optimal method for identifying genomic locations associated

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with the complicated quantitative traits over a wide germplasm range to identify chromosomal locations that could hold candidate genes. GWAS can also significantly increase the accuracy of mapping QTLs by assessing different germplasm with common crossovers since their most recent evolutionary divergence. GWAS is an effective method of connecting complicated phenotypic characteristics with their related genetic factors in many crops, including soybean (Hatzig et al. 2015; Lekklar et al. 2019; Zhang et al. 2019a, b; Liu et al. 2020; Pang et al. 2020).

Due to its abundance of both protein and oil, it is easier for soybean [*Glycine max* (L.) Merr.] seeds to deteriorate and lose seed vigor than it is for other crops such as rice, wheat, and maize. This affects field emergence, seedling growth, and further reduces the yield (Caverzan et al. 2018). Therefore, it is necessary to explore the molecular mechanism behind the vigor and germination of soybean seeds. Significant progress has been achieved in identifying the seed germination and seed vigor of *Arabidopsis thaliana* (Jin et al. 2018; Pan et al. 2020), rice (He et al. 2020; Wang et al. 2020), *Brassica napus* (Hatzig et al. 2015), and maize (Li et al. 2016); however, there is limited research on the mechanism behind seed germination in soybeans. Therefore, a better understanding of the mechanism behind seed germination and breeding resistant soybean varieties can mitigate the loss of seed vigor and vitality.

In this study, a GWAS signal on chromosome 18 related to germination rate, an index of seed vigor, was identified by GWAS during the seed germination stage in soybean. *GmAOC4* is the main candidate gene for this QTL and its regulatory functions on seed vigor were primarily investigated. Results demonstrated that the relative expression of *GmAOC4* in high-germination varieties was significantly lower than in low-germination varieties. *GmAOC4* is involved in JA biosynthesis in soybean and the overexpression of *GmAOC4* repressed seed vigor in *Arabidopsis*. Additionally, the hypersensitivity to ABA was observed with a high expression level of *AtABI5 in GmAOC4*-OE *Arabidopsis*. The possible mechanisms of *GmAOC4* involving the regulation of seed vigor could relate to modulating *GmABIs* expression during JA and ABA interactions in soybean germinating seeds. This study provides important insights into the role of *GmAOC4* in soybean seed vigor.

Materials and methods

Plant materials and phenotyping

A natural population of 264 Chinese soybean accessions (with 212 improved varieties and 52 landraces) was selected to produce an association mapping panel. All of the soybeans were planted in 2018 and 2019 in Nanjing City, Jiangsu Province. Field planting was performed according to a randomized complete block design, which used a plot with a single row and three replicates.

Before germination, chlorine gas was used to sterilize the seeds and reduce the risk of microbial contamination. Forty seeds were then placed on two pieces of filter paper, which were in sterilized Petri dishes. Fifteen mL of water was then added. These seeds were incubated at 25 ± 1 °C for 5 days in a dark growth chamber, after which the number of seeds that germinated was counted to obtain the germination rate (GR). The soybean seeds were considered germinated once the length of the radicle and plumule of the seeds exceeded the overall length of the seed. Three replicates were performed in this study; the average values of the data obtained from E1 and E2 were used for the GWAS.

Phenotypic data analysis

An analysis of variance (ANOVA) was performed for the phenotypic data using the PROC GLM in the software SAS version 9.0 (SAS Institute, Inc., Cary, NC, USA). The descriptive statistics and broad-sense heritability (h^2) were performed using the R software package (http://www.R-project.org/). Broad-sense heritability (h^2) was computed using the formula: $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{ge}/n + \sigma^2/nr)$ where σ^2_g is the genotypic variance, σ^2_{ge} is the genotype by environment interaction variance, σ^2 is the error variance, n is the number of environments, and r is the number of replications. The bar graphs and line graphs were generated with Origin 8.0 software.

GWAS for seed germination

To dissect the genetic mechanism of soybean seed germination, a genome-wide association study (GWAS) was conducted using the germination phenotype and a marker set of 2,597,425 SNPs as described in our previous study (Zhang et al. 2021). The association between the phenotypes and markers was evaluated using a mixed linear model that was implemented with an R package called Genome Association and Prediction Integrated Tool (GAPIT). GAPIT is a powerful tool and a series of methods for genome-wide association (GWAS), including the mixed linear model (MLM), compressed MLM (CMLM), and multiple locus mixed linear model (MLMM) (Lipka et al. 2012), while the population structure (Q) and kinship matrix (K) were calculated using the GAPIT program for GWAS with MLM. The threshold for significant association was $p < 3.85 \times 10^{-7}$ or $-\log_{10}(p) > 6.4$ as described in our previous study (Zhang et al. 2021).

Real-time PCR

Total RNA was isolated using an RNAsimple Total RNA Kit (TIANGEN Beijing, China), and the first-strand cDNA was reverse-transcribed with a TaKaRa Primer Script RT reagent kit and gDNA Eraser according to the manufacturer's instructions. Real-time PCR (RT-PCR) assessing gene expression was conducted with an ABI 7500 system (Applied Biosystems, Foster City, CA, USA) and an SYBR Green Real-time Master Mix (Toyobo). Tubulin transcription levels (GenBank accession number: AY907703) were used for quantitative controls. Table S1 lists the primers used in this study.

Agrobacterium rhizogenes-mediated transformation of soybean hairy roots

The *GmAOC4* coding sequence was cloned from KF and was subsequently subcloned to the vector pMDC83, which contained only the double CaMV 35S promoter. This generated the pMDC83-*GmAOC4* overexpression vector. Table S1 lists the primers used to construct these vectors.

The pMDC83-*GmAOC4* and its empty vector were transformed into the *Agrobacterium rhizogenes* strain K599 independently to generate soybean hairy roots, per the methods used in a previous study (Kereszt et al. 2007). One-week-old seedlings were injected with the resulting K599 and transferred to a germination chamber with a 12-h light/dark cycle, day/night temperatures of 28°C/25°C, and high humidity rates. After 2 or 3 weeks, the hairy roots were approximately 2–10 cm closer to the infection site than where they formed, at which point the primary root was removed. The seedling hairy roots were then placed in ½ Hoagland nutrient solution for 2 weeks.

Plasmid construction and overexpression of GmAOC4 in *Arabidopsis*

The coding sequence of *GmAOC4* was cloned from KF using primers harboring XbaI and BamHI sites (Supplemental Table S1) and was then subcloned into the vector pMDC83 with the CaMV 35S promoter as pMDC83-*GmAOC4*-35S::GFP. To obtain the stable expression of *GmAOC4* in *Arabidopsis*, we transformed the recombinant vector into *Agrobacterium tumefaciens* (EHA105 strain) and then transformed *Arabidopsis* via the floral dip method (Clough and Bent 1998). The transformants of the primary plants were placed on an MS medium using kanamycin (0.05 mg/ml) resistance, and after 10 days 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. Levels of *GmAOC4* overexpression were assessed via RT-PCR. TUBULIN (TUB) gene was used as an internal RT-PCR control, while Supplemental Table S1 displays the TUB primers.

Hormone quantification

For JA quantification, 1 g soybean hairy root, soybean seed, and *Arabidopsis* seed were prepared, respectively. Endogenous JA was extracted according to the manufacturer's instructions of the plant JA enzyme-linked immunosorbent assay (ELISA) kit (Dogesce Beijing, China). The procedure was performed according to the following: (1) Prepare all reagents before starting the assay procedure. All standards and samples be added in duplication on the microtiter plate. (2) Add 50 µl of standard or sample to the appropriate wells. The blank well doesn't add anything. (3) Add 100 µl of enzyme conjugate to standard wells and sample wells except for the black well, cover with an adhesive strip, and incubate for minutes at 37 °C. (4) Wash the microtiter plate four times. (5) Add substrate A 50 µl and substrate B 50 µl to each well. Gently mix and incubate for 15 min at 37 °C. (6) Add 50 µl Stop Solution to each well. (7) Read the optical density (OD) at 450 nm using a microtiter plate reader within 15 min.

Results

Phenotypic variation

The descriptive statistics and broad-sense heritability (h^2) of the germination rate (GR) were analyzed for 264 soybean varieties (Table 1). The average GR values in 2018 and 2019 were 94.2% and 94.8%, respectively, and the minimum GR values were 40% and 45%, respectively. The coefficients

Table 1 Descriptive statistics, ANOVA and h^2 of germination rate of the soybean diversity panel in 2 years

Trait	En	Mean ± SD	Min	Max	CV	Geno	Env	Geno × Env	h^2
GR	2018	94.2 ± 8.6	40	100	9.20	***	***	***	70.13%
	2019	94.8 ± 7.0	45	100	7.40				

GR, Germination rate; En., environment; Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient variations; h^2:heritability; Geno, genotype; Env, environment

***Significant at $P < 0.001$

of variation of GR were 9.2% and 7.4% in 2018 and 2019, respectively, and the heritability (h^2) was 70.13%. In addition, the population showed significant GR variation among genotypes well as among genotype × environment interactions estimated by ANOVA ($p < 0.001$) (Table 1), indicating that seed germination in soybeans is influenced by genetic factors and environmental factors (Table 1).

A GWAS signal associated with the germination rate

To analyze the genetic mechanism of seed germination in soybeans, a GWAS with high-density SNPs was performed to identify significant SNPs associated with germination rate (GR). A total of 28 SNPs ($-\log_{10}(p) > 6.4$) were significantly related to GR across two environments and were located on chromosomes 3, 4, and 18 (Fig. 1a and Table 2). Individual SNPs explained between 10.92 and 22.12% of phenotypic variation (Table 2). Among these SNPs, one SNP located on chromosome 3 was detected in 2018 and two SNPs located on chromosome 4 were detected in 2019, while 25 other SNPs were located on chromosome 18 (Fig. 1a; Table 2). In addition, 18 of these 25 SNPs were detected in both years of the experiments performed in the present study (2018 and 2019), explaining over 10% of the total phenotypic variation (Table 2), forming a cluster flanked by the SNP S18_56189166 and S18_56196037 with a physical position of 56,189,166–56,196,037 bp (Fig. 1b). These results indicated that this GWAS signal is a major QTL for soybean seed germination and suggests that it can identify a candidate gene for soybean seed germination.

Identification of candidate gene for soybean seed germination

According to gene annotation on Phytozome 13 (https://phytozome.jgi.doe.gov/pz/portal.html), only one gene, *Glyma.18G280900*, was located within the above-described cluster. Moreover, SNP S18_56189166 was localized within the 3'-UTR of *Glyma.18G280900* and other SNPs were located downstream of *Glyma.18G280900* (Table 3) (Fig. 1b). Previous studies have demonstrated that 3'untranslated regions (3'UTRs) of messenger RNAs (mRNAs) are involved in regulating gene expression at post-transcript levels (Mayr 2017), and a real-time quantitative PCR (qRT-PCR) demonstrated that the expression levels of *Glyma.18G280900* in NN (carrying S18_56189166-C) were significantly lower those in KF (carrying the S18_56189166-T) after imbibition for 0 and 24 h. The germination rate and

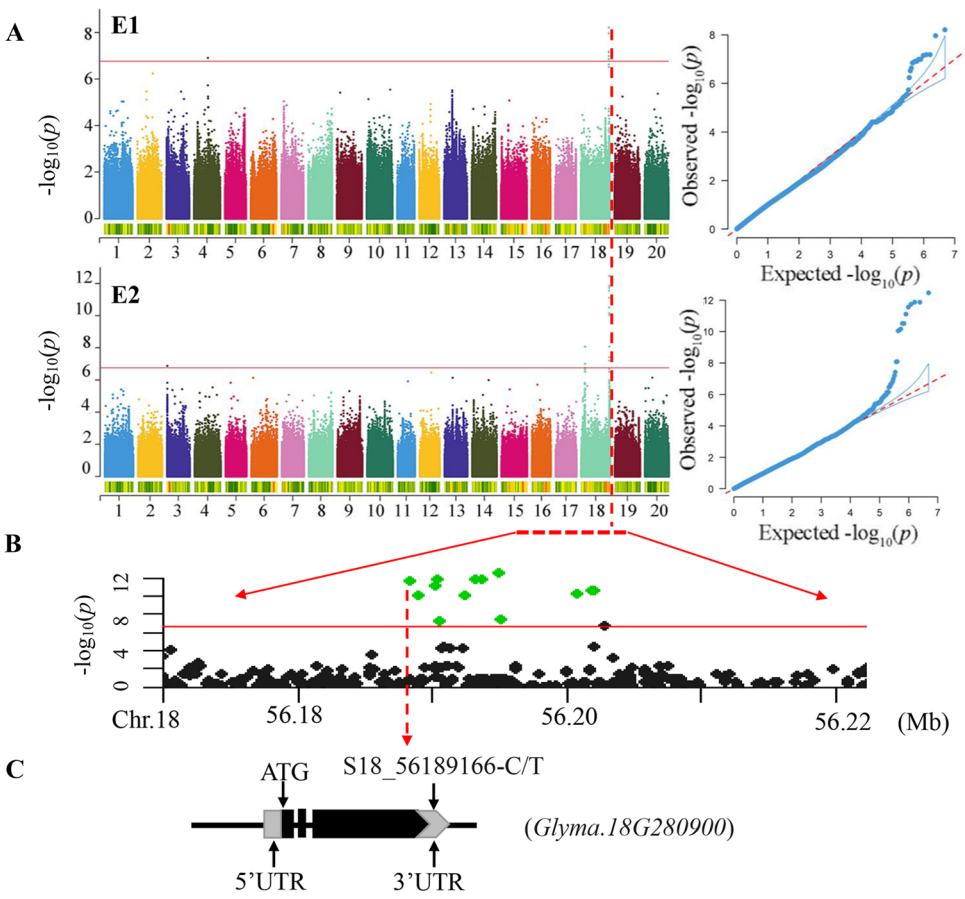

Fig. 1 Identification of *Glyma.18G280900* for regulating seed germination in soybean. **a** GWAS Manhattan and quantile–quantile plots for seed germination in E1 and E2. Red lines denote significance threshold ($-\log_{10}(p) = 6.4$). **b** Significant GWAS signal indicating seed weight on chromosome 18. Blue points represent SNP S18_56189166 (located within the 3'UTR of *Glyma.18G280900*)

Table 2 Significant SNPs associated with seed germination rate

Environment	SNP	Chromosome	Position	*P*-value	R^2
2018	S03_582611	3	582,611	0.000000132	0.11061
	S18_7603191	18	7,603,191	8.49E-09	0.133157
	S18_7605964	18	7,605,964	9.79E-08	0.113038
	S18_56189166	18	56,189,166	2.77E-12	0.202385
	S18_56189491	18	56,189,491	9.07E-11	0.171709
	S18_56190145	18	56,190,145	7.72E-12	0.193275
	S18_56190226	18	56,190,226	1.36E-12	0.20874
	S18_56190286	18	56,190,286	6.25E-08	0.116699
	S18_56191267	18	56,191,267	8.03E-11	0.172764
	S18_56191630	18	56,191,630	1.83E-12	0.206084
	S18_56191838	18	56,191,838	1.36E-12	0.20874
	S18_56192503	18	56,192,503	3.42E-13	0.221214
	S18_56192545	18	56,192,545	3.78E-08	0.12082
	S18_56195399	18	56,195,399	6.82E-11	0.174175
	S18_56195996	18	56,195,996	3.07E-11	0.18114
	S18_56196037	18	56,196,037	3.07E-11	0.18114
	S18_56230848	18	56,230,848	8.22E-09	0.133428
2019	S04_27789819	4	27,789,819	1.23E-07	0.110329
	S04_27789800	4	27,789,800	1.23E-07	0.110329
	S18_56192503	18	56,192,503	6.14E-09	0.134829
	S18_56189166	18	56,189,166	1.09E-08	0.13008
	S18_56190226	18	56,190,226	6.55E-08	0.115431
	S18_56191838	18	56,191,838	6.55E-08	0.115431
	S18_56195399	18	56,195,399	7.13E-08	0.114745
	S18_56195996	18	56,195,996	1.02E-07	0.111846
	S18_56196037	18	56,196,037	1.02E-07	0.111846
	S18_56190145	18	56,190,145	1.36E-07	0.109539
	S18_56191630	18	56,191,630	1.43E-07	0.109154

Table 3 Candidate genes identified based on the position between significant SNPs and genomic region

SNP ID	Chr	Position	Location site	Gene ID	Functional annotation
S18_56189166	18	**56,189,166**	3'UTR	*Glyma.18G280900*	Allene oxide cyclase 4
S18_56189491	18	56,189,491	Intrergenic	/	/
S18_56190145	18	56,190,145	Intrergenic	/	/
S18_56190226	18	56,190,226	Intrergenic	/	/
S18_56190286	18	56,190,286	Intrergenic	/	/
S18_56191267	18	56,191,267	Intrergenic	/	/
S18_56191630	18	56,191,630	Intrergenic	/	/
S18_56191838	18	56,191,838	Intrergenic	/	/
S18_56192503	18	56,192,503	Intrergenic	/	/
S18_56192545	18	56,192,545	Intrergenic	/	/
S18_56195399	18	56,195,399	Intrergenic	/	/
S18_56195996	18	56,195,996	Intrergenic	/	/
S18_56196037	18	56,196,037	Intrergenic	/	/
S18_56230848	18	56,230,848	Intrergenic	/	/

germination speed of NN exceeded those of KF (Fig. 2a). qRT-PCR analysis demonstrated that *Glyma.18G280900* in KF showed mRNA levels that were approximately 3.9 times and 2.2 times greater than those of NN at 0 and 24 h after imbibition, respectively (Fig. 2b). According to the gene annotation of 'Williams 82' (Bernard and Cremeens 1998)

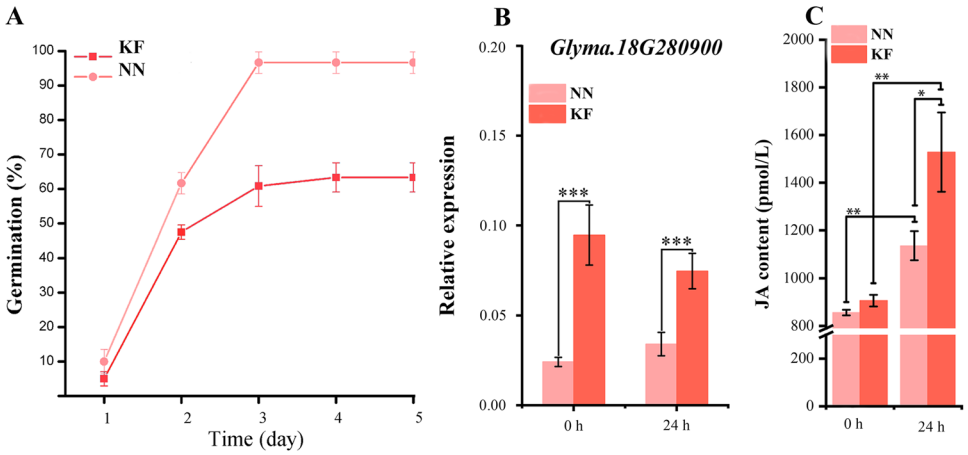

Fig. 2 **a** Seed germination of KF and NN under normal conditions for 5 days. **b** Relative expression of *Glyma.18G280900* in KF and NN seed after imbibition for 0 and 24 h. **c** JA content in KF and NN seeds after imbibition for 0 and 24 h. Three biological replicates were used to produce average SD and significance. Student's *t* test was performed. Asterisks indicate significant differences from the empty vector control. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

reference genome on Phytozome 13 (https://phytozome-next.jgi.doe.gov/), *Glyma.18G280900* encodes an allene oxide cyclase 4 (named *GmAOC4*), of which the homologous gene was reported to regulate the biosynthesis of the JA precursor 12-oxo-phytodienoic acid (12-OPDA) in *Arabidopsis* (Dave et al. 2011). This indicates that *GmAOC4* controls the biosynthesis of JA in soybean, leading us to speculate that high transcriptional levels of *GmAOC4* in KF could induce more JA accumulation than NN. To assess this hypothesis, we analyzed the levels of endogenous JA in both the mature and germinating seeds of NN and KF with a plant JA ELISA kit. The results demonstrated that KF accumulated more JA in germinating seeds when compared with NN at 24 h after germination ($p>0.05$), but not in mature seeds (0 h) (Fig. 2c). Additionally, the contents of JA in KF and NN significantly increased after imbibition for 24 h ($p<0.01$) (Fig. 2c). It has been reported that Jasmonate (JA) negatively regulates seed germination in plants by enhancing abscisic acid (ABA) functioning, which decreases the rates of seed germination and subsequent growth (Wang et al. 2020). These results indicate that *GmAOC4* is likely the candidate gene that plays a role in regulating seed germination in soybeans.

GmAOC4 is involved in the synthesis of JA and repressing seed germination

To assess whether *GmAOC4* is involved in the synthesis of JA in soybean, we generated *GmAOC4*-overexpression soybean hairy roots, following the *Agrobacterium rhizogenes*-mediated hairy root transformation system (Kereszt et al. 2007). The average levels of *GmAOC4* expression in the hairy roots of *GmAOC4*-OE were 5.3 times higher than in the wild-type strain K599-generated (WT) control hairy roots (Fig. S1A), and the concentration of endogenous JA in *GmAOC4*-OE soybean hairy roots significantly exceeded that in the WT ($P<0.05$) (Fig. S1B). This suggests that JA is regulated by *GmAOC4* in soybeans.

To further explore how *GmAOC4* regulates seed germination, *GmAOC4* was overexpressed in *Arabidopsis* and three highly expressed T3 (OE-3, OE-4, and OE-7) lines were chosen by quantitative real-time PCR analyses for subsequent experiments. These experiments were named OE-3, OE-4, and OE-7, and detected additional JA concentrations when compared with the wild-type Col-0 (WT) (Fig. 3a). The seeds of WT, OE-3, OE-4, and OE-7 were grown in culture dishes with filter paper for 7 days, after which the phenotypes of germination were observed. The overexpression of *GmAOC4* inhibited the seed germination speed of OE-3, OE-4, and OE-7 and slightly repressed the seed germination rate compared with WT in *Arabidopsis* (Fig. 3b). To confirm these observations, several germination-related genes were analyzed in WT and *GmAOC4*-OE germinating seeds, including EM1, EM6, and LEA. Transcription levels of EM1, EM6, and LEA improved in seeds germinating *GmAOC4*-OE compared with the WT, which was consistent with the phenotype (Fig. 3c–e). These results indicate that *GmAOC4* is involved in the synthesis of JA and the repression of seed germination.

Overexpression of GmAOC4 exhibited hypersensitivity to ABA during the seed germination stage

It was reported that JA acts with ABA to regulate the germination of seeds in *Arabidopsis* (Dave et al. 2011; Wang et al. 2020). We then examined the germination phenotypes of WT seeds and *GmAOC4*-OE that were treated with and without ABA. The rates of seed germination of *GmAOC4*-OE plants were slightly lower than WT without ABA treatment (Fig. 4), while the germination and establishment of both WT and *GmAOC4*-OE seeds were repressed following 7 days of ABA treatment (Fig. 4a, b). However,

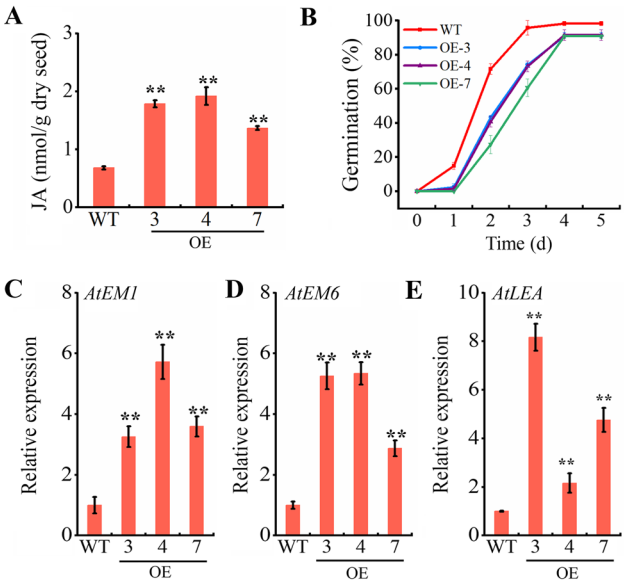

Fig. 3 Effect of overexpression of *GmAOC4* on seed germination. **a** JA content of *GmAOC4*-OE lines (OE-3, OE-4, and OE-7) in *Arabidopsis* dry seed. **b** Germination rates of WT and transgenic-line (OE-3, OE-4, and OE-7) seeds on culture dish with filter paper for 5 days under normal conditions. **c–e** Relative expression of several germination-related genes (AtEM1, *AtEM6*, and *AtLEA*) in WT and transgenic-line (OE-3, OE-4, and OE-7) seeds. Three biological replicates were used for germination assays with 120 seeds. Three biological replicates were used to produce average SD and significance. Student's *t* test was performed. Asterisks indicate significant differences from the empty vector control. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

GmAOC4-OE seeds exhibited much lower rates of germination when treated with ABA compared to WT. Additionally, the levels of *AtABI5* expression in *GmAOC4*-overexpressed *Arabidopsis* were significantly upregulated compared with WT under normal conditions, and the transcript levels of *AtABI5* were enhanced when treated with ABA in transgenic lines (Fig. 4c). It was also found that the mRNA abundance of two ABI5-like genes (*Glyma.10G071700* and *Glyma.19G194500*) was much higher in the hairy roots of *GmAOC4*-OE soybeans compared to the hairy roots of WT soybeans (Fig. S1C). This indicates that *GmAOC4* overexpression could improve *ABI5* transcript levels and exhibit hypersensitivity to ABA during seed germination.

Discussion

To improve crop production, seedlings should must germinate quickly and uniformly after sown. It is difficult to rectify differences at this stage, which can significantly affect crop yield. Recent studies have indicated there is significant potential for improving the germination of seeds through genetic methods (Ju et al. 2019; He et al. 2020; Wang et al. 2020). While many quantitative trait loci (QTLs) have been found in different plants that are related to the germination rate (Al-Chaarani et al. 2005; Xie et al. 2014; Hatzig et al. 2015), there is limited research on the mechanism of soybean seed germination (Zhang et al. 2019a). Recently, Zhang et al. (2019a) have mapped

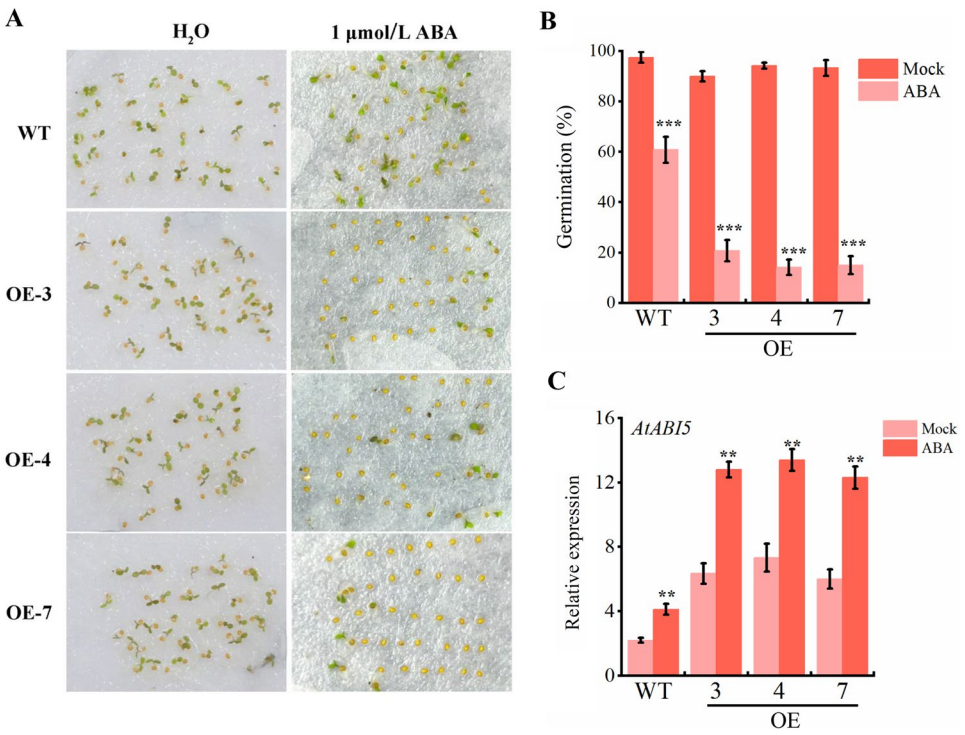

Fig. 4 Overexpression of *GmAOC4* and ABA synergistically inhibits seed germination. **a** Seedlings of WT and *GmAOC4*-OE lines treated with 1 μM ABA for 7 days. **b** Germination rates of WT and transgenic-line (OE-3, OE-4, and OE-7) seeds treated with 1 μM ABA for 7 days on culture dish with filter paper. **c** Relative expression of *AtABI5* in WT and *GmAOC4*-OE line seeds in *Arabidopsis* treated with 1 μM ABA for 24 h. Three biological replicates were used for germination assays with 120 seeds. Three biological replicates were used to produce average SD and significance. Student's *t* test was performed. Asterisks indicate significant differences from the empty vector control. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Cluster-18, containing three QTLs, for seed germination on chromosome 18. *Cluster-18* explained 7.30–16.03% of the phenotypic variation for seed germination in two recombinant inbred lines (RIL) populations with a physical range of approximately 5.4–7.4 Mb (Zhang et al. 2019a). In this study, we used GWAS to dissect the genetic architecture of seed germination using a high-resolution genetic map and detected a GWAS signal on chromosomes 18, which was significantly associated with seed germination in soybean. This GWAS signal was approximately 50 Mb far from the *Cluster-18* identified by Zhang et al. (2019a). It is believed that this is the first study assessing the significant association between this major GWAS signal on chromosome 18 and seed germination in soybean.

Jasmonic acid, a plant hormone, is involved in important processes related to the growth and development of plants, as well as stress response (Qi et al. 2015; Du et al. 2017). Previous studies have demonstrated that JA is related to seed germination in many plants, including rice, bread wheat, and *Arabidopsis* (Ju et al. 2019; Pan et al. 2020; Wang et al. 2020). Allene oxide cyclase (AOC) is a rate-limiting gene in JA biosynthesis (Wasternack and Hause 2013; Claus and Susheng 2017), and the mutation of AOC was unable to produce the JA precursor 12-oxo-phytodienoic acid (12-OPDA), decreasing levels of JA (Hazman et al. 2015). However, the particular function and the underlying molecular mechanism behind how JA regulates the germination of soybean seeds are not well understood. In the present study, SNP S18_56189166 is located within the 3'-UTR of *GmAOC4* and was significantly associated with soybean seed germination. Overexpression of *GmAOC4* resulted in a higher concentration of JA accumulation in *Arabidopsis* and soybean hairy root (Fig. 3a and Fig. S1B). Our results also demonstrated that *GmAOC4*-OE lines showed repressed seed germination and were more sensitive to ABA compared to WT in *Arabidopsis* (Fig. 3b and Fig. 4a, b). The improved ABA sensitivity indicates that AOC could serve an important role in ABA-JA interaction and ABA signaling when regulating soybean seed germination. ABA helps regulate the biosynthesis of JA, which inhibits the germination of rice seeds via the 'SAPK10-bZIP72-*AOC*' pathway in rice (Wang et al. 2020). Additionally, we determined that the levels of *AtABI5* expression in *GmAOC*-OE lines were upregulated compared to WT, regardless of whether or not they were treated with ABA. Additionally, the mRNA abundance of two ABI5-like genes was significantly enhanced in the hairy roots of *GmAOC4*-OE soybean compared with the hairy roots of WT soybeans (Fig. S2), which suggested that JA could inhibit seed germination growth by stimulating ABA responses during seed germination, which requires functional ABI5 (Pan et al. 2020). Altogether, these effects can be explained by the fact that *GmAOC4* modulates JA biosynthesis and thus negatively regulates seed germination in soybean.

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Author Contribution statement ZW, WX, HZ, and SL were all involved in the experimental design and the collection of phenotypic data; ZW and XC conducted the phenotypic analysis; WX, LS, XL, YZ, and CX helped revise the manuscript; ZW assessed the experimental results; ZW and HT wrote the paper. The manuscript was reviewed by all authors, who each contributed suggestions.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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