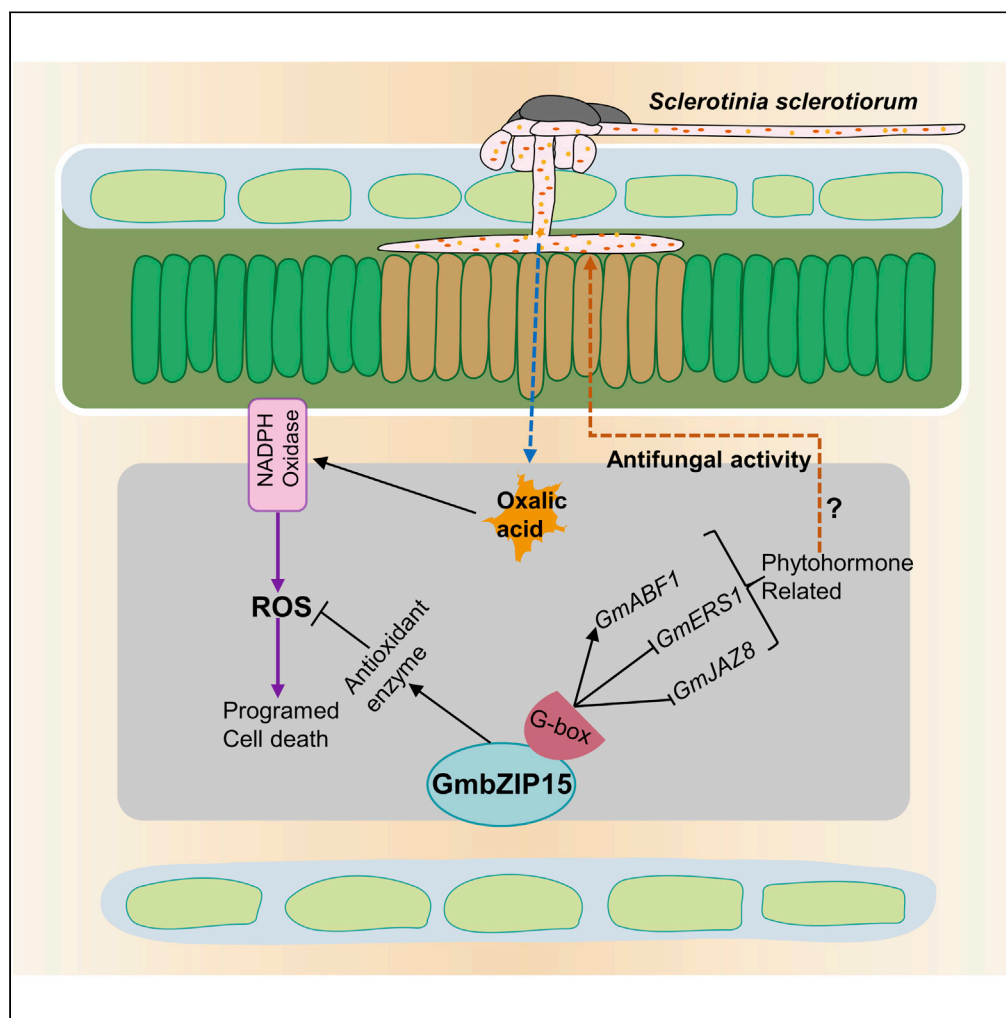**Article**

The bZIP transcription factor GmbZIP15 facilitates resistance against *Sclerotinia sclerotiorum* and *Phytophthora sojae* infection in soybean

Man Zhang,
Yanhui Liu, Zixian
Li, ..., Bingrui
Wang, Hanyang
Cai, Yuan Qin

brwang@mail.hzau.edu.cn
(B.W.)
caihanyang123@163.com
(H.C.)
yuanqin@fafu.edu.cn (Y.Q.)

Highlights

GmbZIP15 improves
resistance against
pathogen

GmbZIP15 modulates the
antioxidant defense
system

GmbZIP15 regulates
phytohormone signaling

GmbZIP15 can direct bind
to G-box

iScience

Article

The bZIP transcription factor GmbZIP15 facilitates resistance against *Sclerotinia sclerotiorum* and *Phytophthora sojae* infection in soybean

Man Zhang,[1] Yanhui Liu,[1] Zixian Li,[1] Zeyuan She,[2] Mengnan Chai,[1] Mohammad Aslam,[2] Qing He,[1] Youmei Huang,[1] Fangqian Chen,[1] Huihuang Chen,[1] Shikui Song,[3] Bingrui Wang,[4,*] Hanyang Cai,[1,*] and Yuan Qin[1,2,5,*]

SUMMARY

Soybean, one of the most valuable oilseed crops, is under constant pressure from pathogens. bZIP transcription factors (TFs) composing one of the largest TF families in plants have diverse functions. Biochemical and physiological analyses were performed to characterize the regulatory roles of soybean bZIP TF GmbZIP15 in response to pathogens. We found that transgenic soybean plants overexpressing *GmbZIP15* has increased resistance against *Sclerotinia sclerotiorum* and *Phytophthora sojae*. Besides, *GmbZIP15* regulates pathogen response by modulating the antioxidant defense system and phytohormone signaling. In addition, we performed chromatin immunoprecipitation sequencing to identify the downstream genes of *GmbZIP15* in response to *S. sclerotiorum* and found that *GmbZIP15* can activate or repress the expression of defense-related genes through direct promoter binding. Taken together, these results indicate that *GmbZIP15* plays a positive role in pathogen resistance in soybean, and this activity may be dependent on phytohormone signaling.

INTRODUCTION

Environmental signals elicit cellular responses in living organisms, and these responses are crucial for survival. Soybean (*Glycine max*), a major oilseed crop and source of plant proteins, hosts a wide variety of pathogens that cause significant yield loss (Ranjan et al., 2019). The agronomical importance of soybean has led to research focused on its interactions with pathogens, including *Phytophthora sojae*, *Phakopsora pachyrhizi*, and *Sclerotinia sclerotiorum* (Hoffman et al., 1998; Whitham et al., 2016). The oomycete pathogen *P. sojae* belongs to the kingdom Stramenopiles, and the stem and root rot caused by this destructive pathogen costs the soybean industry millions of dollars each year (Tyler, 2007; Tyler et al., 2006). The necrotrophic ascomycete *S. sclerotiorum* is the causative agent of Sclerotinia stem rot or white mold, which causes significant yield loss and economic damage to soybean production (Westrick et al., 2019; Zhang et al., 2014).

Plant-pathogen interactions are a two-way communication process: plants recognize pathogens and induce different defense mechanisms, while pathogens threaten plant functional physiology and counterattack plant defense strategies (Cui et al., 2018; Silva et al., 2018). Invasion by most pathogens is recognized through transmembrane plant proteins called pattern recognition receptors that detect microbe-derived molecules known as pathogen-associated molecular pattern molecules (PAMPs). PAMP-triggered immunity (PTI) is activated by endogenous plant signals released during pathogen attack (Boutrot and Zipfel, 2017; Jones and Dangl, 2006). Another plant strategy for recognizing virulence effectors or their actions is effector-triggered immunity (ETI) (Cui et al., 2015; Mine et al., 2018). Activation of ETI leads to transcriptional upregulation of defense-related genes and is often associated with rapid localized cell death at the infection site, known as the hypersensitive response (HR) (Betsuyaku et al., 2018; Dodds and Rathjen, 2010).

Phytohormones, including abscisic acid (ABA), jasmonic acid (JA), ethylene (ET), and salicylic acid (SA), play vital roles in the regulation of plant-pathogen recognition and signal transduction pathways (Adie et al., 2007; Betsuyaku et al., 2018; Cui et al., 2018; Guo and Stotz, 2007; Liu et al., 2015; Ranjan et al., 2019). In

plants under pathogen attack, PTI and ETI activate various hormone signaling pathways (Pieterse et al., 2012), resulting in a complex network of both complementary and antagonistic activities that ultimately fine-tune the defense response to pathogens (Adie et al., 2007; Betsuyaku et al., 2018; Di et al., 2017). A previous study has revealed that ABA can affect JA biosynthesis in the activation of defenses against *Pythium irregulare* in *Arabidopsis* (Adie et al., 2007). AcERF2, a halophyte *Atriplex canescens* ethylene-responsive factor (ERF), induced transcript accumulation of plant defense-related genes and increased *Arabidopsis* resistance to tomato *Botrytis cinerea* (Sun et al., 2018). *BnWRKY33*, as an *S. sclerotiorum*-responsive gene, positively regulates resistance to this pathogen by enhancing the expression of SA and JA-regulated genes in oilseed rape (Liu et al., 2018). Besides, overexpression of *GmKR3* in soybean was found to enhance viral resistance in part through ABA signaling (Xun et al., 2019).

Effective signal transduction leads to the activation of transcription factors (TFs) that regulate the bio-processes responsible for plant defense (Schluttenhofer and Yuan, 2015; Tsuda and Somssich, 2015). The bZIP genes encode plant-specific TFs composing one of the largest TF families in plants (Baloglu et al., 2014; Zhang et al., 2018) and plant bZIP TFs can be classified into three groups based on their DNA-binding specificity to G-box (CACGTG) or C-box (GACGTC) elements. It has reported that groupI proteins exhibit a stronger binding affinity for G-box elements and group II proteins bind G-box and C-box elements with comparable binding affinity, whereas group III proteins display a stronger binding affinity for C-box motif (Izawa et al., 1993). The bZIP TFs of all three groups play diverse and critical roles in abiotic stress responses, flower development, pathogen defense, and seed maturation (Chang et al., 2019; Dong et al., 2019; Eleblu et al., 2019; Gaguancela et al., 2016). For example, *StbZIP61* and *StNPR3L* cooperatively regulate the temporal activation of SA biosynthesis, which contributes to SA-mediated immunity against *Phytophthora infestans* infection in potato (Zhou et al., 2018). Overexpression of *MebZIP3* and *MebZIP5* enhanced callose deposition and improved disease resistance against cassava bacterial blight (Li et al., 2017). *OsbZIP79* overexpression in rice resulted in suppression of the elicitor-inducible diterpenoid phytoalexin biosynthesis genes, leading to reduced phytoalexin accumulation in rice cells (Miyamoto et al., 2015). However, the roles of bZIP genes in soybean are still poorly understood, especially in response to pathogens.

Based on the gene structure and phylogenetic analysis, we previously divided the bZIP family in soybean into 12 groups and group K includes only one member named *GmbZIP15* (Zhang et al., 2018). In this study, we isolated soybean *GmbZIP15* (Glyma.02G161100) gene and found that the encoded protein contains a typical bZIP domain and activates transcription in yeast cells. Furthermore, biochemical and physiological analyses were performed to reveal the regulatory roles of GmbZIP15 in pathogen response.

RESULTS

Sequence and domain analyses of GmbZIP15

Sequence analysis of GmbZIP15 was performed by aligning the amino acid sequence of 12 GmbZIP proteins with one member per subgroup. As shown in Figure S1, they shared the bZIP domain including a conserved 40–80 amino acid with two structural features, a basic DNA-binding region and a leucine zipper dimerization motif. The basic region contains an invariant N-×7-R/K-×9 motif of about 18 amino acid residues and the leucine zipper domain contains an L-×6-L-×6-L motif (Figures S1A and S1B). Surprisingly, only the member of the subgroup K GmbZIP15 contains a K1 domain, which is a transmembrane domain and has a conserved sequence QESAVL (Figures S1B and S1C). To further confirm whether K1 domain was conserved from different species or not, sequence alignment was performed with the six top-scoring matches of GmbZIP15 from basic local alignment search tool. Seven sequences from various plant species, including soybean, grape, poplar, rice, *Arabidopsis*, *Thellungiella salsuginea*, and *Brachypodium distachyon* display high similarity, including a bZIP domain and a K1 domain (Figure S1C), indicating that subgroup K own a specific K1 domain indeed. People have found this domain exists in *Arabidopsis*, rice, and black cottonwood (Corrêa et al., 2008), and we also found that this domain exists in grape, poplar, *T. salsuginea*, and *B. distachyon*.

Transcriptional activation activity and subcellular localization of GmbZIP15

The bZIP domain is a highly conserved structural motif involved in transcriptional regulation (Hobo et al., 1999; Kim et al., 2004; Zhou et al., 2018). To test the transcription activation function of GmbZIP15, we performed a transient expression assay in yeast cells using a GAL4-responsive reporter system. As shown in Figure 1A, transformed yeast cells harboring DBD-P53+T-antigen (pGBKT7-53 + pGADT7-T, positive
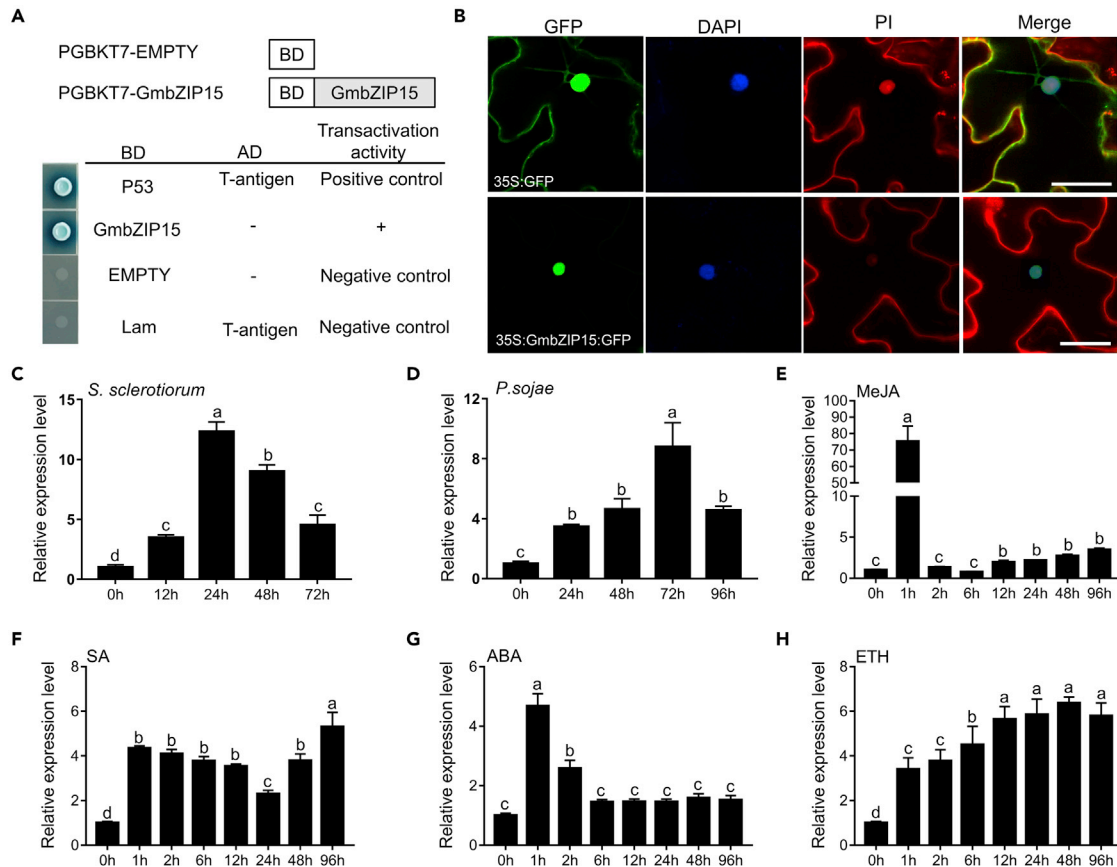
Figure 1. Transcriptional activation and expression pattern analysis of *GmbZIP15* in soybean

(A) Transcriptional activation analysis of GmbZIP15.

(B) Subcellular localization of *GmbZIP15* in tobacco.

(C and D) Response of *GmbZIP15* to pathogens infection in WT and *GmbZIP15* transcript levels were detected by qPCR with *S. sclerotiorum* (C) and *P. sojae* (D) infection at different time point in soybean.

(E–H) *GmbZIP15* transcript levels in soybean in response to hormone treatment. The relative expression level of *GmbZIP15* in WT soybean was determined by qRT-PCR at 0, 1, 2, 6, 12, 24, 48, and 96 h after different phytohormone treatments. (E) 100 μM MeJA (methyl jasmonate). (F) 1 mM SA (salicylic acid). (G) 100 μM ABA (abscisic acid). (H) 100 μM ETH (ethephon). Errors bars indicate $\pm$SD of three biological replicates. Significant differences between samples were determined by one-way ANOVA, $P < 0.05$.

control) and DBD-GmbZIP15 (pGBKT7-GmbZIP15) grew well in synthetic dropout medium without tryptophan, histidine, and adenine [SD (-Trp/-His/-Ade)] and had α-galactosidase (α-gal) activity. Yeast cells containing empty pGBKT7 (negative control) exhibited no α-gal activity. This result indicated that GmbZIP15 protein has a transcriptional activation activity.

To determine the subcellular location of GmbZIP15, we generated a 35S:GmbZIP15-GFP fusion construct and expressed the construct in *Nicotiana benthamiana* leaves. As shown in Figure 1B, GmbZIP15-GFP was exclusively localized in the nucleus, whereas the 35S-promoter-driven GFP control was observed in multiple subcellular compartments including the cytoplasm and nucleus. These results indicated that GmbZIP15 is a typical transcription factor.

Expression pattern of *GmbZIP15* in response to various treatments

To evaluate whether *GmbZIP15* is involved in response to pathogens infection, we performed quantitative polymerase chain reaction (qPCR) and examined the transcript levels of *GmbZIP15* after *S. sclerotiorum* or *P. sojae* infection in wild-type soybean C03-3. The results showed that the expression of *GmbZIP15* was significantly enhanced within 72 h postinoculation (hpi) with the *S. sclerotiorum* (Figure 1C). The transcript

levels of *GmbZIP15* were also elevated within 96 hpi with the *P. sojae* (Figure 1D). These results indicate that *GmbZIP15* is differentially expressed upon *S. sclerotiorum* and *P. sojae* infection in soybean.

Because soybean defense against pathogens is known to be mediated by plant hormones, such as SA, JA, ET, and ABA (Xun et al., 2019; Yang et al., 2019a, 2019b; Zhang et al., 2019), we quantified *GmbZIP15* transcript levels in response to hormone treatment by qPCR. *GmbZIP15* was induced rapidly by jasmonic acid methyl ester (MeJA), SA, ABA, and ethephon (ETH) (Figures 1E–1H). The *GmbZIP15* expression was rapidly induced by SA and ETH and kept a high expression level after 1-h treatment. *GmbZIP15* expression in response to MeJA and ABA peaked at 1 h then decreased rapidly. These results suggest that the expression of *GmbZIP15* is differentially regulated by different hormone treatments.

Functional analysis of *GmbZIP15* in response to *S. sclerotiorum*

To determine the function of *GmbZIP15* in response to *S. sclerotiorum*, we transformed soybean plants with a construct harboring *GmbZIP15-GFP* driven by the 35S promoter (*OX-GmbZIP15*) and a functional repressor form of *GmbZIP15* (*35S:bZIP15-SRDX*) via *Agrobacterium*-mediated transformation and two lines with higher expression levels were selected for further research, respectively (Zhang et al., 2020). Hypocotyl-wound inoculation and detached-leaf inoculation were used to assess the response to *S. sclerotiorum*. As shown in Figure 2A, hypocotyl-wound inoculation was performed in 15-day-old wild-type (WT), *OX-GmbZIP15* (OE), and *35S:GmbZIP15-SRDX* (SRDX) seedlings with 48 plants per sample, which led to complete wilting of about 80% WT and SRDX plants at about 5 days postinoculation (dpi), while about 70%–80% of two OE lines developed less-severe disease symptoms and showed less tissue damage than WT plants. Besides, the detached-leaf inoculation assay was performed with the first pair ternate palmate leaves, which showed that two OE line leaves display increased resistance to *S. sclerotiorum* at 24 hpi, with smaller lesions and relative biomass of *S. sclerotiorum* on the leaves; the degree of resistance was similar between the SRDX lines and WT (Figures 2B–2D).

To further confirm the function of *GmbZIP15*, virus-induced gene silencing (VIGS) was used to knock down the expression of *GmbZIP15*. The silencing efficiency was determined by qPCR and compared with empty vector control (TRV). To evaluate the efficacy of our VIGS system in soybean, we silenced the soybean phytoene desaturase (*GmPDS1*), a gene involved in carotenoid biosynthesis (Zhang et al., 2010), and obtained consistent photobleaching of the host plants (Figure 3A), which was used as an additional control to determine the success of gene silencing. TRV-PDS as an additional control in the VIGS system has been reported in many species, such as pepper (Cai et al., 2015) and tomato (Naing et al., 2019). After about 18–24 dpi, the TRV-GmPDS1-infected leaves began to become albino (Figure 3A) and the expression of the target genes (*GmbZIP15* and *GmPDS1*) were reduced to 20%–30% compared with the expression of these genes in empty vector control (Figure 3B). Compared with TRV-infected controls, plants infiltrated with TRV-GmPDS1 and TRV-GmbZIP15 displayed similar resistance to *S. sclerotiorum* (Figure 3C), with detached-leaf assay showed all plant leaves have similar lesion areas and relative biomass of *S. sclerotiorum* at 48 hpi (Figure 3D), which implies that the TRV construction did not affect plant resistance to *S. sclerotiorum* and knock down of *GmbZIP15* did not change the resistance of soybean against *S. sclerotiorum*. Taken together, these results indicate that overexpression of *GmbZIP15* improved resistance to *S. sclerotiorum* in soybean.

Functional analysis of *GmbZIP15* in response to *P. sojae*

To examine whether *GmbZIP15* also functions against *P. sojae*, we inoculated soybean plants with *P. sojae*. After hypocotyl-wound inoculation, about 80% WT and SRDX plants exhibited similar wilting symptoms and chlorosis at about 7 dpi, and most (~85%) OE plants were healthier than WT (Figure 4A). The detached-leaf inoculation result showed that the lesion areas and relative biomass of *P. sojae* were similar on the leaves of SRDX and WT plants (Figures 4B–4D). At 48 hpi, the OE lines had less lesion areas and relative biomass of *P. sojae* than WT, the difference was more obvious at 72 hpi (Figures 4C and 4D). In addition, TRV-GmPDS1 and TRV-GmbZIP15 displayed similar resistance to *P. sojae* compared with TRV-infected controls (Figures 3E and 3F). These results indicate that *GmbZIP15* also contributes resistance to *P. sojae* in soybean.

GmbZ1P15-mediated pathogen defense involves the antioxidant defense system

Reactive oxygen (ROS) are key signaling molecules produced under biotic and abiotic stress conditions and trigger a variety of plant defense responses (Huckelhoven and Kogel, 2003). To better understand
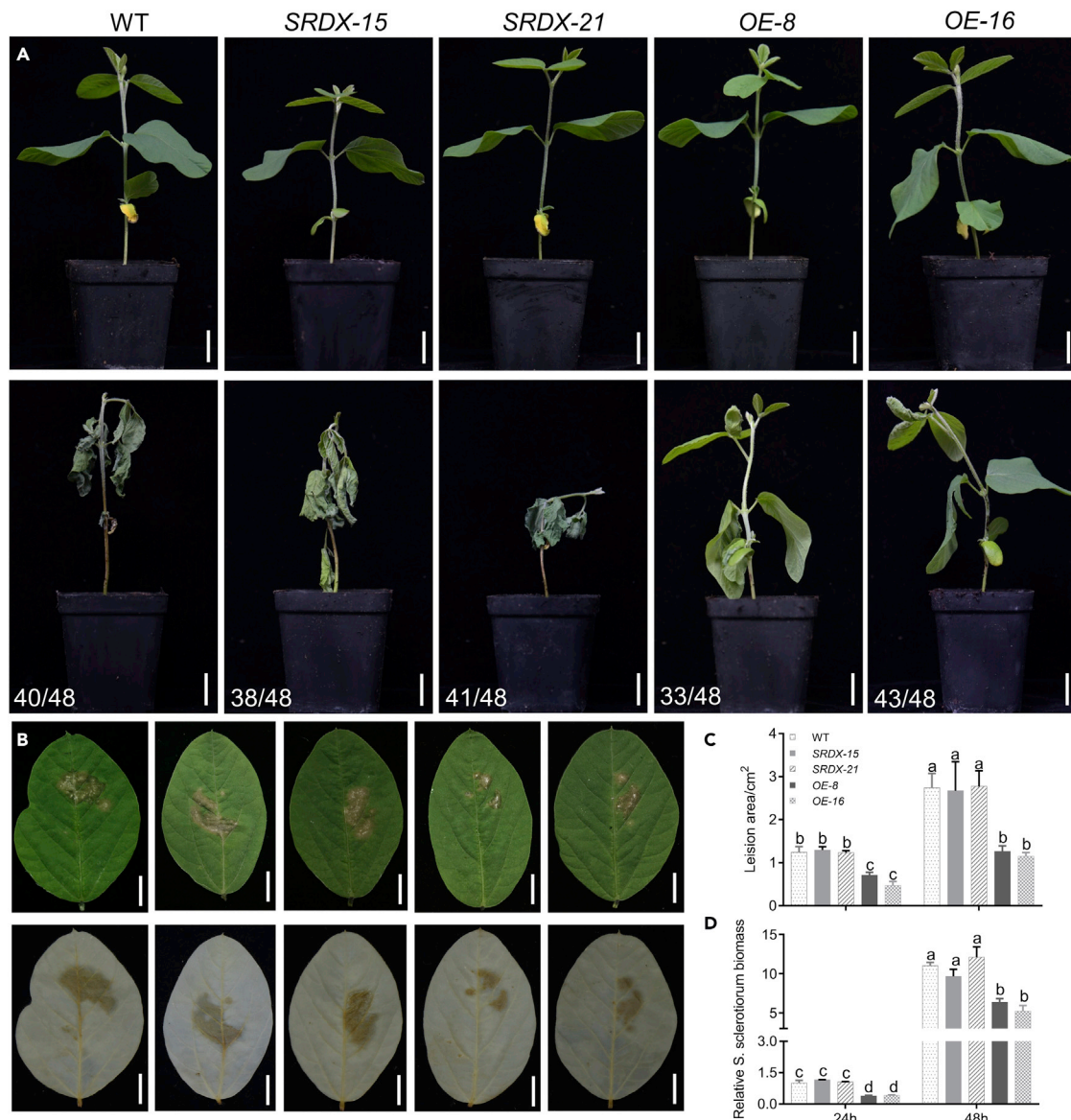
**Figure 2. Phenotype of *GmbZIP15* in response to *S. sclerotiorum***

(A)15-day-old WT and *GmbZIP15* transgenic soybean plants after about 5 days of *S. sclerotiorum* inoculation. Numbers in the panels denote the frequencies of the phenotypes shown. Bar = 1 cm.

(B) *S. sclerotiorum* lesion progression on soybean leaves.

(C and D) Lesion areas and relative *S. sclerotiorum* biomass measurement at 24 and 48 hpi. Errors bars indicate $\pm$ SD of three biological replicates. Significant differences between samples were determined by one-way ANOVA, $P < 0.05$.

the resistance mechanism of *GmbZIP15* transgenic soybean plants, we collected *S. sclerotiorum*- or *P. sojae*-inoculated leaves of WT and transgenic plants after 48 hpi or 72 hpi. We then stained them with 3,3-diaminobenzidine (DAB) to visualize H_2O_2 accumulation. After pathogen infection, more staining was observed in leaves of SRDX and WT plants, while less in leaves of OE plants exhibiting enhanced resistance to the pathogen (Figures 2B and 4B).

Malondialdehyde (MDA) is a typical product of lipid peroxidation, and its content indirectly reflects the degree of damage and antioxidant capacity (Yu et al., 2016). After infection with *S. sclerotiorum* and *P. sojae*, the MDA level was significantly elevated but that was distinctly lower in OE plants than that of WT plants, and the MDA concentration did not significantly differ between WT and SRDX plants
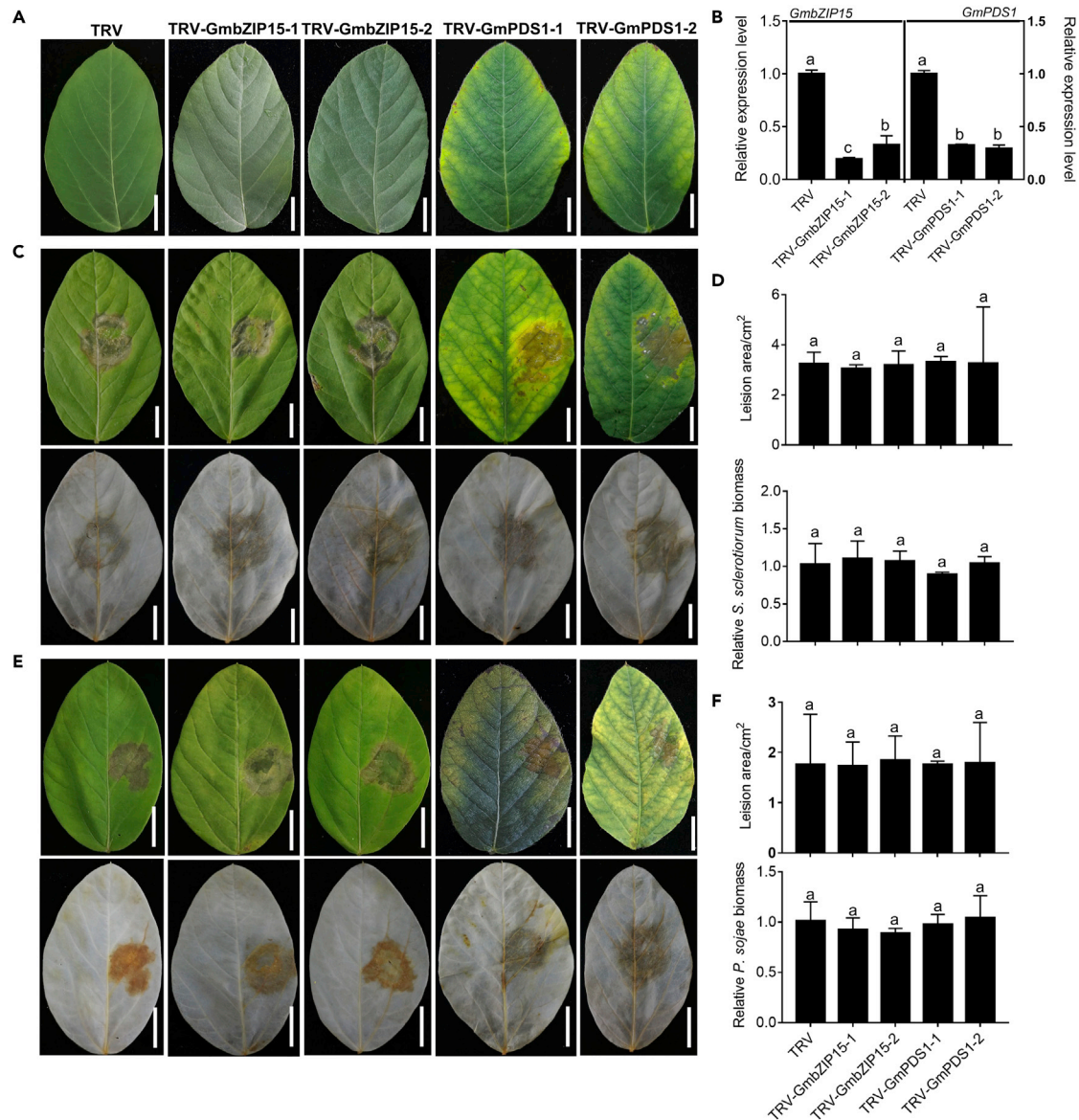
Figure 3. Virus-induced gene silencing (VIGS) of GmbZIP15 showed similar resistance to wild type

(A) Soybean plants infected with TRV, TRV-GmbZIP15 or TRV-GmPDS1.

(B) Transcript level detection of GmbZIP15 and GmPDS1 in the first pair ternate palmate leaves of soybean.

(C) Phenotype of soybean plants with *S. sclerotiorum* inoculation for 48 h.

(D) Lesion areas and relative *S. sclerotiorum* biomass measurement at 48 h.

(E) Phenotype of soybean plants with *P. sojae* inoculation for 72 h.

(F) Lesion areas and relative *P. sojae* biomass measurement at 72 h. Errors bars indicate ±SD of three biological replicates. Significant differences between samples were determined by one-way ANOVA, P < 0.05.

(Figures S2A and S2B). To study whether the increased lipid peroxidation and ROS were caused by altered antioxidant activities, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were detected. These enzymes are important to plants for eliminating ROS (Alscher et al., 2002; Manju and Nair, 2006). Under mock and pathogen infection conditions, SOD, POD, and CAT activities were much higher in OE plants than in WT plants (Figures S2A and S2B). These results suggest that *GmbZIP15* may reduce ROS accumulation in soybean by increasing antioxidant enzymatic activities.
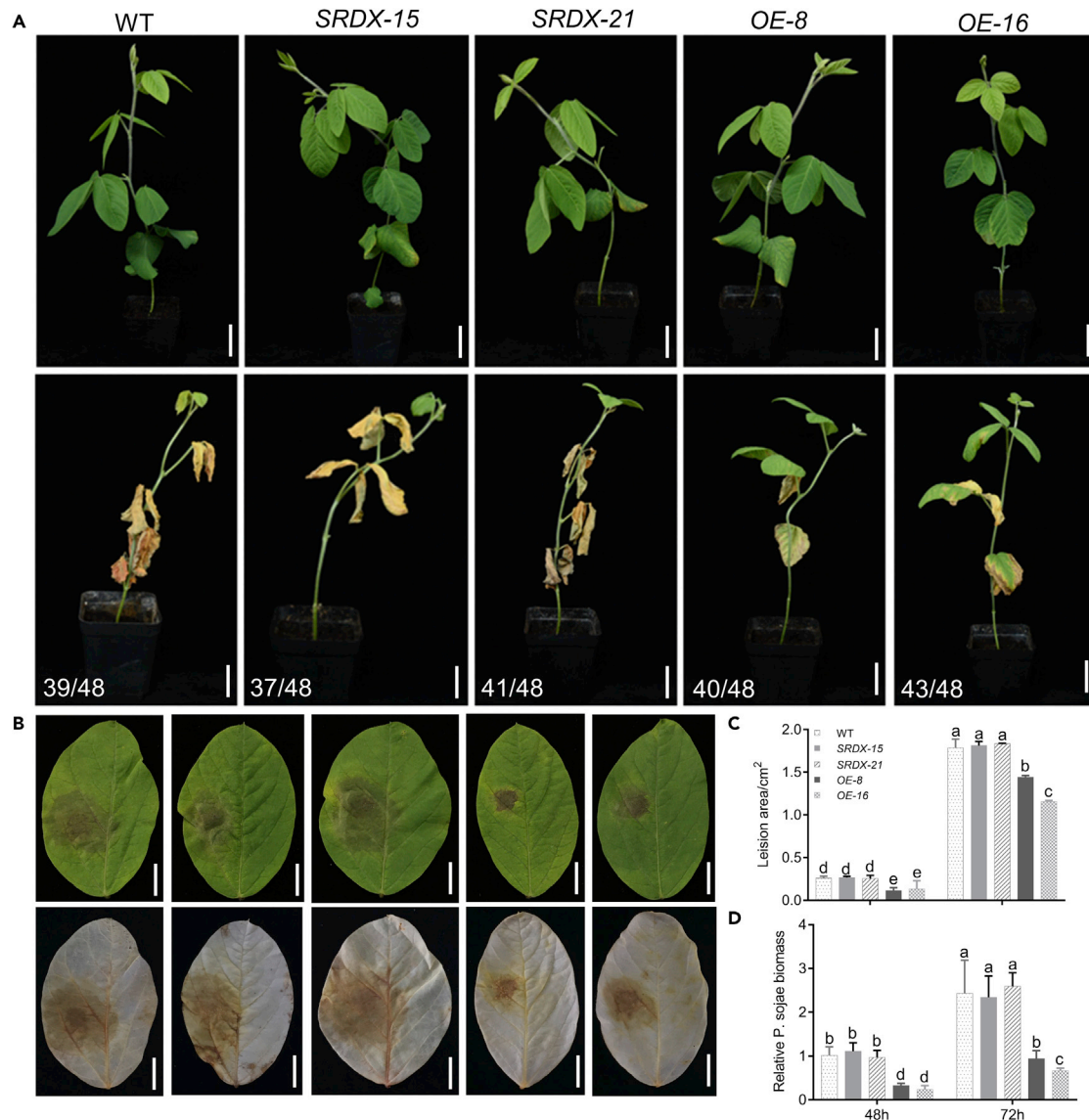
Figure 4. Phenotype of *GmbZIP15* in response to *P. sojae*

(A) Phenotypes of 15-day-old WT and *GmbZIP15* transgenic soybean plants after about 7 days of *P. sojae* inoculation. Numbers in the panels denote the frequencies of the phenotypes shown.

(B) *P. sojae* lesion progression on soybean leaves. Bar = 1 cm.

(C and D) Lesion areas and relative *P. sojae* biomass measurement at 48 and 72 hpi. Errors bars indicate ±SD of three biological replicates. Significant differences between samples were determined by one-way ANOVA, $P < 0.05$.

GmbZ1P5-mediated pathogen defense involves phytohormone signaling

To further investigate how *GmbZIP15* promotes resistance to *S. sclerotiorum* and *P. sojae* infection, we analyzed the expression of defense-related genes in WT and *GmbZIP15* transgenic soybean plants. First, two HR-related genes (*GmNPR1* and *GmRAR1*) were significantly elevated after pathogens infection (Figures S3 and S4). In addition, the expression of phytohormone-responsive genes, including ABA response genes (*GmAA O 3A* and *GmNCED1*), ET and JA response gene (*GmERF1*), and SA response gene (*GmPR2*), was also induced in OE lines compared with WT plants by *S. sclerotiorum* and *P. sojae* infection in a different degree, while the transcript level of these marker genes in SRDX plants was similar or slightly lower than that in WT plants (Figures S3 and S4). In addition, the expression of auxin-related gene *GmWUS* showed no significantly difference in all plants after pathogens infection. We also detected the biosynthetic

genes of SA and JA and found that the transcript level of JA biosynthetic gene *GmAOS* was significantly higher in OE plants and that was similar or lower in SRDX plants compared with WT plants after *S. sclerotiorum* infection and was higher in SRDX plants than in WT plants after *S. sclerotiorum* and *P. sojae* infection. The expression of SA biosynthetic gene *GmPAL* showed no obvious difference in all plants (Figures S3 and S4).

Conservation of the *GmbZIP15*-mediated response to *S. sclerotiorum* in soybean and *Arabidopsis*

To investigate the functional conservation of *GmbZIP15* in *S. sclerotiorum* plant interactions, we generated *OX-GmbZIP15* and *35S:GmbZIP15-SRDX* transgenic *Arabidopsis* plants (Zhang et al., 2020) and evaluated the effect of *GmbZIP15* on the response to *S. sclerotiorum* in *Arabidopsis*. As shown in Figure S5, the *OX-GmbZIP15* plants had improved resistance to *S. sclerotiorum* compared with WT plant, while there was no obvious difference between *35S:GmbZIP15-SRDX* plants and WT plants. The *S. sclerotiorum* lesion areas were about 0.6 cm^2 on the leaves of WT and two *35S:GmbZIP15-SRDX* lines and about 0.3 cm^2 on the leaves of two *OX-GmbZIP15* lines at 24 hpi, and the relative biomass of *S. sclerotiorum* was significantly lower in two *OX-GmbZIP15* lines than in WT plants (Figure S5A). These results suggest that *GmbZIP15* enhanced the resistance of *Arabidopsis* to *S. sclerotiorum*. We also analyzed the function of *AtbZIP60*, the *Arabidopsis* homolog of *GmbZIP15*. The *Atbzip60* mutant was more sensitive to *S. sclerotiorum* compared to WT and had larger lesion areas and relative biomass of *S. sclerotiorum* than WT, which was consistent with the DAB staining assay (Figure S5A). Besides, *OX-GmbZIP15* could partially restore *S. sclerotiorum* resistance in *Atbzip60* in lines (Figure S5A).

To further understand the causal factors behind the pathogen resistance of *GmbZIP15* transgenic *Arabidopsis* plants, the expression levels of some defense-related genes were tested in WT, *Atbzip60* mutant, and *GmbZIP15* transgenic *Arabidopsis* after *S. sclerotiorum* infection. The transcript levels of *AtABI2*, *AtABI5*, *AtERF1*, *AtACS6*, and *AtPDF1.2* were significantly elevated, whereas the transcript levels of *AtAOC3*, *AtICS1*, *AtLOX4*, and *AtPR1* had no noticeable difference in *OX-GmbZIP15* transgenic *Arabidopsis* plants compared with WT plants (Figure S5B). The expression level of most marker genes in *Atbzip60* mutant was lower than or similar to that of WT. Besides, the expression level of most marker genes in *35S:GmbZIP15-SRDX* transgenic *Arabidopsis* plants was lower than or equal to that of WT, except for *AtICS1* and *AtPDF1.2* at 12 h and *AtABI2*, *AtABI5*, *AtACS6*, *AtERF1*, *AtLOX4*, and *ATPR1* at 24 h (Figure S5B). On one hand, ectopic overexpression *35S:GmbZIP15-SRDX* construction might affect other endogenous genes expression in *Arabidopsis,* and somehow, these genes might influence the marker genes at only some time point. On the other hand, this result might cause by little differences between different individuals or by a little difference in the process of pathogen inoculating. Taken together, these results suggest that *GmbZIP15* confers *S. sclerotiorum* resistance in *Arabidopsis* as it does in soybean.

Identification of GmbZIP15-binding sites by chromatin immunoprecipitation sequencing

To identify the binding sites of GmbZIP15 throughout the genome, chromatin immunoprecipitation sequencing (ChIP-seq) was performed using the leaf tissues of *OX-GmbZIP15-16* (*35S:GmbZIP15:GFP*) transgenic soybean plants under normal growth condition and a GFP antibody to pull down the putative GmbZIP15-bound DNA. After sequencing, we obtained 10,458,022 uniquely mapped reads, which were mainly located within 500 bp upstream of transcription start sites (TSS; Figure 5A). Using model-based ChIP-seq analysis software, we identified 1,865 peaks in OE plants (Table S2). As per the distribution of the GmbZIP15-binding sites in the soybean genome, 44% were within the 3,000-bp promoter regions upstream of the TSS and 41% were in the 5′ intergenic areas (Figure 5B). To analyze the binding motifs of GmbZIP15, flanking sequences (±100 bp) around the peaks were submitted to MEME-ChIP (http://meme-suite.org/tools/meme-chip) to identify consensus sequences. The most frequent sequence (about 45% of all identified peaks) in the analysis was ACGTG(G/T) (C/A) (Figure 5C), which is similar to the previously identified G-box (T/G/C/)ACGT(G/T)GC, with both containing an ACGT core (Hobo et al., 1999; Nijhawan et al., 2008; Zhang et al., 2017). The variation of the bases surrounding the ACGT core may determine the binding specificity of individual bZIP TFs. Among the peaks, we identified 1,647 candidate genes which may be directly bound by GmbZIP15. Gene ontology (GO)-based analysis of these genes revealed that the significantly enriched GO terms include the genes involved in transcriptional regulation, defense functions, including plant-pathogen interaction, plant hormone signal transduction, and some antioxidant metabolism processes (e.g., oxidative phosphorylation, phenylalanine metabolism, glutathione metabolism, SOD, and POD activity) (Figure 5D). These findings suggest that GmbZIP15 regulates
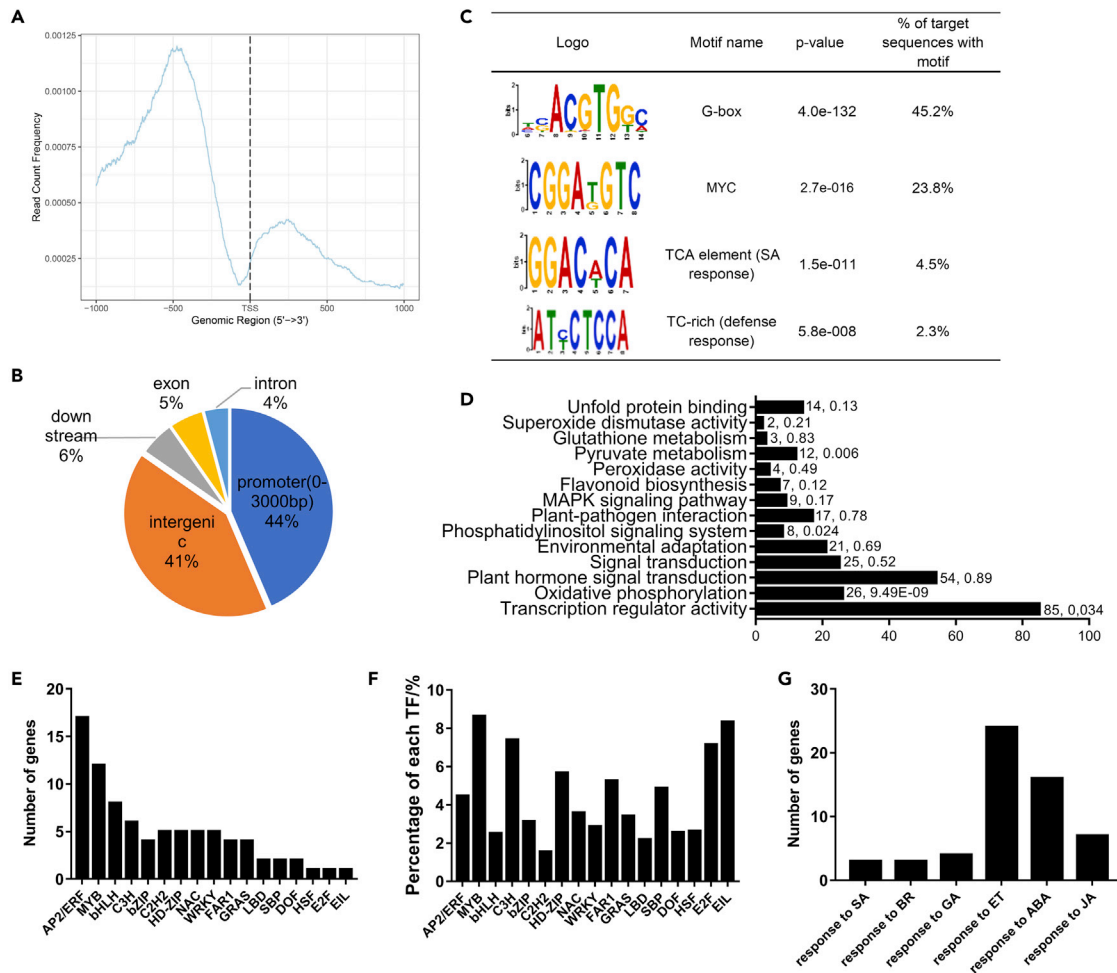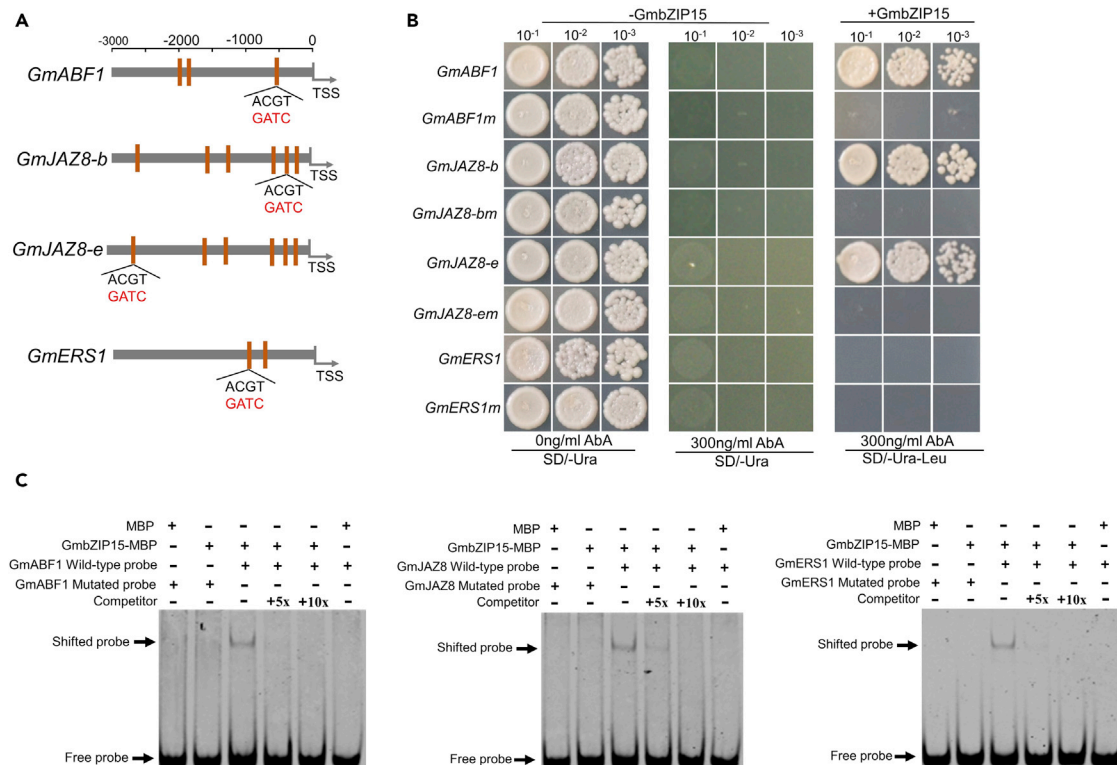
Figure 5. Identification of *GmbZIP15*-binding sites based on ChIP-seq data

(A) Enrichment of GmbZIP15 binding peaks in the promoter region. TSS, transcription start site.

(B) GmbZIP15 binding peak distribution in the soybean genome.

(C) Consensus sequence identified by MEME-ChIP among the GmbZIP15 binding peaks.

(D) Gene ontology analysis of genes targeted by GmbZIP15.

(E) Number of transcription factors targeted by GmbZIP15.

(F) The percentage of TFs in each TF family.

(G) Number of phytohormone-response genes targeted by GmbZIP15.

the transcription of genes associated with a variety of plant defense processes (Figure 5D). As transcription factors (TFs) and phytohormones play a pivotal role in plant defense response, we picked TFs and phytohormone response genes up in detail (Table S3). We found that in total, there are 6 TF familie, including MYB, C3H, HD-ZIP, FAR1, E2F and EIL, containing more than 5% of family members were targeted by GmbZIP15 (Figures 5E and 5F and Table S3), indicating these genes are potentially regulated by GmbZIP15. Besides, there are four bZIP genes (*GmbZIP60*, *GmABF*, *GmbZIP137*, and *GmbZIP159*), accounting for about 3% of bZIP genes were targeted by GmbZIP15 (Figure 5E and Table S3). There are also many GmbZIP15 target genes response to ABA, ET and JA signaling regulation processes (Figure 5G and Table S4), such as *GmABF1*, *GmERS1*, *GmJAZ8*, *GmLOX2* and *GmLOX3*.

GmbZIP15 directly binds to the promoters of *GmABF1*, *GmJAZ8* and *GmERS1*

As phytohormones critically regulate the plant defense process (Lorenzo et al., 2003; Mine et al., 2018), three genes associated with ABA, JA and ET signaling (*GmABF1*, *GmJAZ8* and *GmERS1*) were chosen randomly to examine their *in vivo* binding with GmbZIP15 and transcription profiles by ChIP-qPCR. As

Figure 6. Validation of GmbZIP15 binding site and function analysis of three binding genes

(A) Diagram of the G-box distribution of *GmABF1*, *GmJAZ8*, and *GmERS1* promoter fragments and wild-type G-box element (ACGT) was mutated to mG-box element (GATC).

(B) Yeast one-hybrid assay using vectors contains GmbZIP15 binding site or fragments with introduced mutations. Yeast cells carting or lacking pGAD-GmbZIP15 were grown on SD/-Ura or SD/-Ura, SD/-Ura/-Leu containing 300 ng/mL AbA.

(C) EMSA of the binding sites of GmbZIP15 protein to the promoter of *GmABF1*, *GmJAZ8*, and *GmERS1*. 5x or 10x competitor probe DNA (unlabeled) were added as control in the assay.

shown in Figure S5, GmbZIP15 physically interacted with at least one site containing the G-box motif localized within the promoter region of each of the three candidate genes. A distinctive peak was detected approximately 1 kb upstream of the TSS of *GmABF1*(*GmbZIP157*) (Figure S6A), and ChIP-qPCR showed that GmbZIP15 specifically binds to the 600 bp region upstream of the *GmABF1* TSS (Figure S6B). GmbZIP15 was also found to bind sites immediately upstream of the TSS of *GmJAZ8* (sites b and e) and *GmERS1* (sites b), as shown by ChIP-qPCR (Figure S5B). Besides, the binding specifity can also be found after *S. sclerotiorum* infection (Figure S6C).

To further confirm this result, yeast one-hybrid assay (Y1H) was performed. We mutated the core elements of binding site ACGT to GATC (Figure 6A), and a WT fragment and a mutated fragment of three genes were used as bait and cloned into the pAbAi vector, and GmbZIP15 was used as prey. Aureobasidin A (AbA) is an antifungal antibiotic produced by *Aureobasidium pullulans* R106, and AURI is a novel gene conferring aureobasidin resistance on *Saccharomy cescerevisia*, which can be used for screening in Y1H assay (Hashida-Okado et al., 1996; Takesako et al., 1993). As shown in Figure 6B, the yeast cells of all samples grew well on the screening medium (SD/-Ura). Moreover, cell growth of all mutated samples-GmbZIP15 prey transformats were completely inhibited by 300 ng/mL AbA, while most normal samples-GmbZIP15 prey transformats were survived except for JAZ8-e-GmbZIP15 transformat. The Y1H assay indicated that GmbZIP15 can only bind to the site b of *GmJAZ8* promoter. We subsequently validated the binding regions of GmbZIP15 by performing an electrophoretic mobility shift assay (EMSA). MBP-GmbZIP15 fusion proteins could bind to the DNA probes, and the DNA-binding intensity of GmbZIP15 fusion proteins decreased due to competition from non-labeled probes, while the mutated probe was completely abolished (Figure 6C), indicating a binding specificity of GmbZIP15. Y1H and EMSA experimental evidence together suggest that GmbZIP15 can directly bind to the specific loci of *GmABF1*, *GmJAZ8* and *GmERS1* promoters.
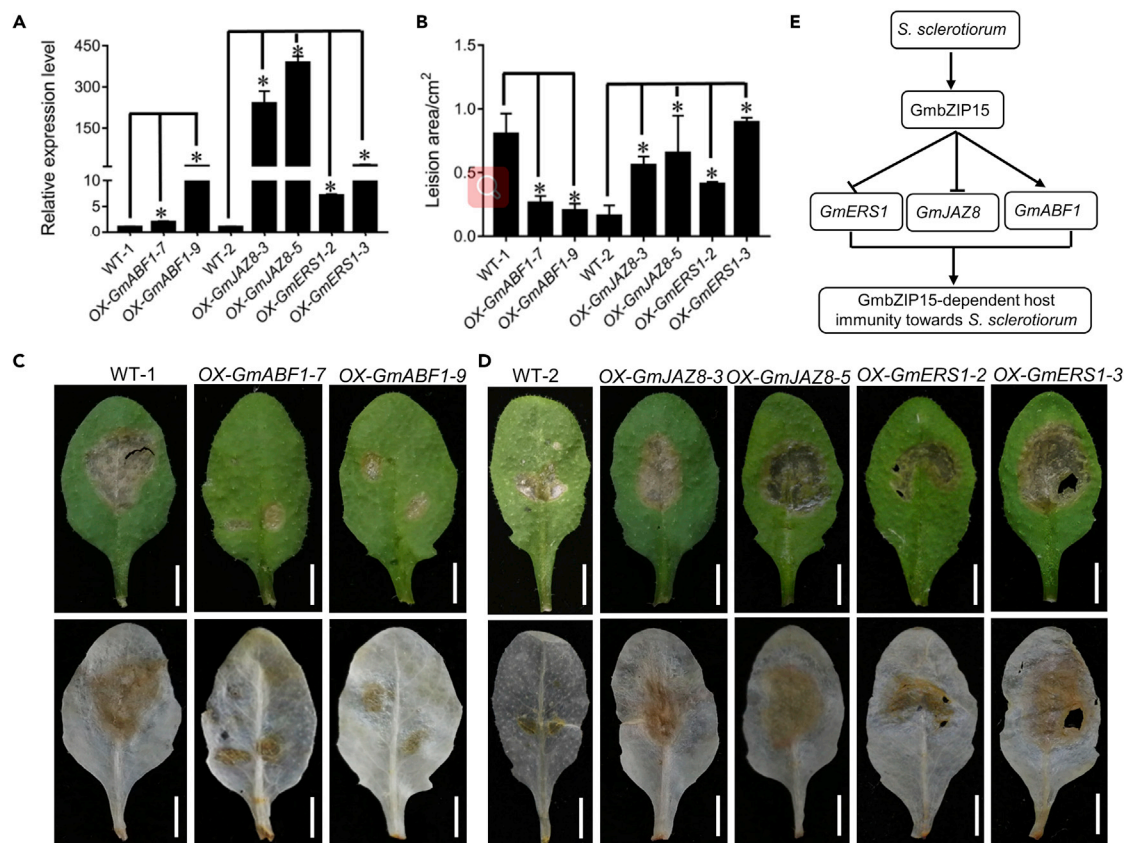
Figure 7. Function analysis of three binding genes

(A) Transcript level of three genes overexpression in *Arabidopsis*.

(B) Lesion areas measurement after *S. sclerotiorum* infection. Errors bars indicate ±SD of three biological replicates. Significant differences between samples labeled with asterisk were determined by one-way ANOVA, $P < 0.05$.

(C) Phenotype observation of *GmABF1* with *S. sclerotiorum* infection 24 h.

(D) Phenotype observation of *GmJAZ8* and *GmERS1* with *S. sclerotiorum* infection 12 h.

(E) Working model of GmbZIP15 responding to pathogen infection. GmbZIP15 play a positive role in the response of soybean to *S. sclerotiorum* infection. The expression of *GmbZIP15* is induced by pathogen infection, and GmbZIP15 activates the phytohormone signaling pathways by binding to G-box of the promoter of the phytohormone-related genes, such as GmbZIP15 directly represses ET response genes such as *GmERS1* and the JA signaling repressor GmJAZ8, but it directly enhances ABA signaling by activating *GmABF1* to improve pathogen resistance.

GmbZIP15 regulates the expression of *GmABF1*, *GmJAZ8* and *GmERS1* in response to *S. sclerotiorum*

To further investigate the function of these three genes, we detected the expression profile of these three genes by qPCR. Upon infection of *OX-GmbZIP15* soybean plants with *S. sclerotiorum*, the transcript level of *GmABF1*, genes associated with ABA, was increased, suggesting direct and positive regulation by *GmbZIP15* in response to pathogen infection (Figure S6D). As described above, GmbZIP15 also bound to *GmERS1* and *GmJAZ8*, genes involved in ET and JA catabolism. Still, their transcript levels decreased in OE plant after infection (Figure S6D), indicating negative regulation by GmbZIP15 in response to pathogen infection. Subcellular localization analysis of the proteins encoded by these three genes showed that they were mainly located at the nucleus, except for GmERS1, which was detected in the cytoplasm and nucleus (Figure S7).

To confirm these expression changes after pathogen infection, these three genes were overexpressed in transgenic *Arabidopsis*, and their transcript levels were detected by qPCR in the T$_2$ homozygous transgenic lines. As expected, the expression levels of the three genes in the corresponding transgenic lines were dramatically increased compared to WT (Figure 7A). *GmABF1* overexpression in transgenic *Arabidopsis* conferred enhanced resistance to *S. sclerotiorum*, based on the smaller lesion areas and reduced DAB

staining (Figures 7B and 7C), while the *GmERS1* and *GmJAZ8* overexpression lines showed hypersensitivity to this pathogen (Figure 7D). Therefore, in response to *S. sclerotiorum*, GmbZIP15 could directly activate *GmABF1* expression and repress *GmJAZ8* and *GmERS1* by binding to their promoters (Figure 7E).

DISCUSSION

Plant resistance to pathogens, such as *S. sclerotiorum*, remains unclear due in part to the complex network of responses to these pathogens. bZIP TFs are involved in the regulation of various biological processes, such as plant development, abiotic stress and pathogen defense responses (Kim et al., 2004; Lee et al., 2006; Li et al., 2017). In soybean, the functions of bZIP proteins in response to pathogen infection largely remain uncharacterized. In this study, we investigated the roles of *GmbZIP15* in response to pathogens infection. As a group K member, GmbZIP15 contains a K1 transmembrane domain, with a similar phenotype found in *Arabidopsis* and rice. Overexpression of *GmbZIP15* in soybean did not cause any unexpected visible changes in agronomic traits. Notably, the *OX-GmbZIP15* transgenic soybean plants have significantly increased resistance to *S. sclerotiorum* and *P. sojae*. However, the resistance of *35S:GmbZIP15-SRDX* transgenic and TRV-GmbZIP15 penetration soybean plants were comparable to that of WT plants against these two pathogens.

ROS function as signaling molecules and is involved in host-pathogen interactions, including interactions with fungal and oomycete pathogens. Once plant-pathogen interaction is established, the rapid generation and accumulation of ROS, such as H_2O_2 or O^{2-}, trigger programmed cell death (PCD), commonly known as the hypersensitive response (HR). HR may result in penetration failure of pathogens in their attempt to invade plant epidermal cells (Barna et al., 2003; Cheng et al., 2018). Upon pathogens challenge, the generation of H_2O_2 (Figures 2B and 4B) and the significantly elevated transcript levels of two HR-related genes *GmNPR1* and *GmRAR1* in *OX-GmbZIP15* plants compared to WT (Figure S3), indicate a positive reaction of GmbZIP15 on pathogen invasion. Biotrophs feed on living host tissue, whereas necrotrophs kill host tissue and feed on the remains. However, in the case of necrotrophs, it seems that programmed cell death in the host would merely make life easier for the pathogen (Glazebrook and Jane, 2005). So in the next stage, other defense-related processes might be activited, such as POD, SOD, and CAT enzyme activities, induces to counter ROS deleterious effect and to prevent cell death (Yu et al., 2016). In this study, SOD, POD and CAT activities were much higher in *OX-GmbZIP15* plants than in WT after pathogens infection (Figure S2A), which can eliminate the harmful substances produced by organisms during various metabolic processes (Choi et al., 2004). We also observed the enrichment of genes targeted by GmbZIP15 related to glutathione metabolism, SOD and POD activity (Figure 5D), as these processes are important in preventing oxidative damage imposed by pathogens (Morel et al., 2009; Ranjan et al., 2019). Thus, it is suggested that the scavenging of ROS in *OX-GmbZIP15* transgenic plants is an important defense mechanism against *S. sclerotiorum*, which might in part be owing to the activation of antioxidant enzymatic activities in soybean.

However, defense signaling regulation pathways involved in the interaction between plants and pathogen are complex. The present study reveals that phytohormones (such as SA, JA, ET, and ABA) and their responsive marker genes play important roles in plants response to biotic stresses (Adie et al., 2007; Jing et al., 2019; Luan et al., 2019; Mazarei et al., 2007). In this study, the expression of *GmbZIP15* after various hormone treatments (Figures 1E–1H) indicates its potential involvement in phytohormone pathways. Consistently, several marker genes responsive to ABA, JA, ET, and SA were significantly upregulated in the *OX-GmbZIP15* transgenic soybean lines upon pathogen exposure (Figures S3 and S4). Furthermore, GmbZIP15 could directly bind to the promoter regions of *GmABF1*, *GmERS1*, and *GmJAZ8*, which are associated with the ABA, ET, and JA pathways, and these three genes were found to impact *S. sclerotiorum* resistance (Figures 7C and 7D). Moreover, *GmbZIP15*-dependent signaling through these pathways was demonstrated using well-characterized marker genes, such as *AtAOC3*, *AtPDF1.2 AtERF1*, *AtACS6*, *AtABI2*, *AtABI5*, *AtICS1*, and *AtPR1* in *Arabidopsis* (Figure S5B). These results suggest that *GmbZIP15* activates phytohormone signaling in response to pathogen invasion and that this pathway is conserved in soybean and *Arabidopsis*. The SA signaling pathway has been shown to protect against biotrophic fungi, oomycetes, and bacteria, such as *Erysiphe orontii* and *Pseudomonas syringae*, whereas JA signaling activates defense responses against many necrotrophic fungi, such as *Alternaria brassicicola* and *B. cinerea* (Glazebrook, 2005; Thomma et al., 1998). However, our study clearly indicated that the SA, ET/JA, and ABA signaling pathways were all involved in the response to *S. sclerotiorum* in the *GmbZIP15*-overexpressing soybean plants.

Some TFs also function to activate phytohormone signals upon pathogens or bacterial infection. In our study, TFs, such as ERF, bZIP, WRKY, and MYB gene families (Table S3), were targeted by GmbZIP15 and their homolog in *Arabidopsis* has been found to function in pathogen resistance. For example, over-expression of ethylene-responsive *ERF1* in *Arabidopsis* confers resistance to some necrotrophic pathogens (Berrocal-Lobo et al., 2002), and *ERF1* expression can be activated synergistically by ET and JA (Lorenzo et al., 2003). Overexpression of *MYB102* in *Arabidopsis* increased susceptibility to green peach aphids (*Myzus persicae*) by promoting ET biosynthesis (Zhu et al., 2018). AtWRKY6 positively influenced pathogen-defense-associated PR1 promoter activity, most likely involving NPR1 function (Robatzek and Somssich, 2002). These results suggest that phytohormones play a critical role in pathogen response.

Taken together, these results suggest that altered expression of GmbZIP15 affects the pathogen-induced defense responses, including the trigger of HR response and scavenging of ROS and expression of defense genes associated with SA, ABA, JA, and ET signaling. Based on the results presented here, a working model of GmbZIP15 responding to pathogen infection can be proposed (Figure 7E). GmbZIP15 play a positive role in the response of soybean to *S. sclerotiorum* infection. The expression of *GmbZIP15* is induced by pathogen infection, and GmbZIP15 activates the phytohormone signaling pathways by binding to G-box of the promoter of the phytohormone-related genes, such as GmbZIP15 directly represses ET response genes such as *GmERS1* and the JA signaling repressor GmJAZ8, but it directly enhances ABA signaling by activating *GmABF1* to improve pathogen resistance (Figure 7E). Collectively, our findings provide key insights into the pathogen response mechanisms in soybean.

Limitations of the study

In this study, we revealed that *GmbZIP15* improved resistance against *S. sclerotiorum* and *P. sojae* by activating or repressing the expression of plant hormone-related genes (*GmABF1*, *GmJAZ8*, and *GmERS1*) through binding to the G-box of their promoters. *GmbZIP15* regulates pathogen response by modulating the antioxidant enzymes activities. Additional studies are required to elucidate whether other phytohormones or antioxidant-system-related genes are involved in the downstream of *GmABF1*, *GmJAZ8,* or *GmERS1* to regulate pathogen responses.

STAR★METHODS

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

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Soybean plant material
  - Pathogens infection
- METHOD DETAILS
  - Vector construction and transformation
  - Virus-induced gene silencing of GmbZIP15 in soybean plants
  - Soybean seedling phytohormone treatments
  - *Arabidopsis* plant material and pathogen infection
  - Diaminobenzidine staining
  - RNA extraction and quantitative real-time PCR
  - Transcriptional activation analysis in yeast cells
  - Determination of antioxidant enzyme activity
  - Subcellular localization
  - Chromatin immunoprecipitation assay
  - Yeast one-hybrid assays
  - Electrophoretic mobility shift assays
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102642.

AUTHOR CONTRIBUTIONS

MZ performed vector construction and phenotype analysis. YL performed RNA-seq and ChIP-seq analysis. ZL, MC, and QH calculated all the data and prepared the plastic sections. ZS, YH, and FC performed qPCR analysis. HC performed yeast hybridization, SS performed soybean transformation, and HC and YQ designed the research. MZ and YQ wrote the manuscript. MA, BW, and YQ revised the manuscript. All authors have read and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Adie, B.A., Perez-Perez, J., Perez-Perez, M.M., Godoy, M., Sanchez-Serrano, J.J., Schmelz, E.A., and Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. Plant Cell 19, 1665–1681. https://doi.org/10.1105/tpc.106.048041.

Alscher, R.G., Erturk, N., and Heath, L.S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 53, 1331–1341. https://doi.org/10.1093/jexbot/53.372.1331.

Baloglu, M.C., Eldem, V., Hajyzadeh, M., and Unver, T. (2014). Genome-wide analysis of the bZIP transcription factors in cucumber. PLoS One 9, e96014. https://doi.org/10.1371/journal.pone.0096014.

Barna, B., Fodor, J., Pogany, M., and Kiraly, Z. (2003). Role of reactive oxygen species and antioxidants in plant disease resistance. Pest Manag. Sci. 59, 459–464. https://doi.org/10.1002/ps.706.

Berrocal-Lobo, M., Molina, A., and Solano, R. (2002). Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant J. 29, 23–32.

Betsuyaku, S., Katou, S., Takebayashi, Y., Sakakibara, H., Nomura, N., and Fukuda, H. (2018). Salicylic acid and jasmonic acid pathways are activated in spatially different domains around the infection site during effector-triggered immunity in Arabidopsis thaliana. Plant Cell Physiol 59, 8–16. https://doi.org/10.1093/pcp/pcx181.

Boutrot, F., and Zipfel, C. (2017). Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu. Rev. Phytopathol. 55, 257–286. https://doi.org/10.1146/annurev-phyto-080614-120106.

Cai, H.Y., Yang, Y., Xiao, Z.L., Cheng, J.B., and Qiu, A.L. (2015). CaWRKY6 transcriptionally activates CaWRKY40, regulates Ralstonia solanacearum resistance, and confers high-temperature and high-humidity tolerance in pepper. J. Exp. Bot. 66, 3163–3174.

Cai, H., Zhao, L., Wang, L., Zhang, M., Su, Z., Cheng, Y., Zhao, H., and Qin, Y. (2017). ERECTA signaling controls Arabidopsis inflorescence architecture through chromatin-mediated activation of PRE1 expression. New Phytol. 214, 1579–1596. https://doi.org/10.1111/nph.14521.

Chang, H.C., Tsai, M.C., Wu, S.S., and Chang, I.F. (2019). Regulation of ABI5 expression by ABF3 during salt stress responses in Arabidopsis thaliana. Bot. Stud. 60, 16. https://doi.org/10.1186/s40529-019-0264-z.

Cheng, Q., Dong, L., Gao, T., Liu, T., Li, N., Wang, L., Chang, X., Wu, J., Xu, P., and Zhang, S. (2018). The bHLH transcription factor GmPIB1 facilitates resistance to Phytophthora sojae in Glycine max. J. Exp. Bot. 69, 2527–2541. https://doi.org/10.1093/jxb/ery103.

Choi, D.G., Yoo, N.H., Yu, C.Y., de Los Reyes, B., and Yun, S.J. (2004). The activities of antioxidant enzymes in response to oxidative stresses and hormones in paraquat-tolerant Rehmannia glutinosa plants. J. Biochem. Mol. Biol. 37, 618–624. https://doi.org/10.5483/bmbrep.2004.37.5.618.

Corrêa, L., Riaño-Pachón, D., Schrago, C.G., Santos, R., Mueller-Roeber, B., and Vincentz, M. (2008). The role of bZIP transcription factors in green plant evolution: adaptive features emerging from four founder genes. PLoS One 3, e2944.

Cui, H., Qiu, J., Zhou, Y., Bhandari, D.D., Zhao, C., Bautor, J., and Parker, J.E. (2018). Antagonism of transcription factor MYC2 by EDS1/PAD4 complexes bolsters salicylic acid defense in Arabidopsis effector-triggered immunity. Mol.

Plant 11, 1053–1066. https://doi.org/10.1016/j.molp.2018.05.007.

Cui, H., Tsuda, K., and Parker, J.E. (2015). Effector-triggered immunity: from pathogen perception to robust defense. Annu. Rev. Plant Biol. 66, 487–511. https://doi.org/10.1146/annurev-arplant-050213-040012.

Dai, X., Bai, Y., Zhao, L., Dou, X., Liu, Y., Wang, L., Li, Y., Li, W., Hui, Y., Huang, X., et al. (2018). H2A.Z represses gene expression by modulating promoter nucleosome structure and enhancer histone modifications in Arabidopsis. Mol. Plant 11, 635. https://doi.org/10.1016/j.molp.2018.03.011.

Di, X., Gomila, J., and Takken, F.L.W. (2017). Involvement of salicylic acid, ethylene and jasmonic acid signalling pathways in the susceptibility of tomato to Fusarium oxysporum. Mol. Plant Pathol. 18, 1024–1035. https://doi.org/10.1111/mpp.12559.

Dodds, P.N., and Rathjen, J.P. (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. Nat. Rev. Genet. 11, 539–548. https://doi.org/10.1038/nrg2812.

Dong, Q., Xu, Q., Kong, J., Peng, X., Zhou, W., Chen, L., Wu, J., Xiang, Y., Jiang, H., and Cheng, B. (2019). Overexpression of ZmbZIP22 gene alters endosperm starch content and composition in maize and rice. Plant Sci. 283, 407–415. https://doi.org/10.1016/j.plantsci.2019.03.001.

Eleblu, J.S.Y., Haraghi, A., Mania, B., Camps, C., Rashid, D., Morin, H., Dogimont, C., Boualem, A., and Bendahmane, A. (2019). The gynoecious CmWIP1 transcription factor interacts with CmbZIP48 to inhibit carpel development. Sci. Rep. 9, 15443. https://doi.org/10.1038/s41598-019-52004-z.

Gaguancela, O.A., Zuniga, L.P., Arias, A.V., Halterman, D., Flores, F.J., Johansen, I.E., Wang,

A., Yamaji, Y., and Verchot, J. (2016). The IRE1/bZIP60 pathway and bax inhibitor 1 suppress systemic accumulation of potyviruses and potexviruses in Arabidopsis and Nicotiana benthamiana plants. Mol. Plant Microbe Interact 29, 750–766. https://doi.org/10.1094/MPMI-07-16-0147-R.

Glazebrook, and Jane. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227. https://doi.org/10.1146/annurev.phyto.43.040204.135923.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227. https://doi.org/10.1146/annurev.phyto.43.040204.135923.

Guo, X., and Stotz, H.U. (2007). Defense against Sclerotinia sclerotiorum in Arabidopsis is dependent on jasmonic acid, salicylic acid, and ethylene signaling. Mol. Plant Microbe Interact 20, 1384–1395. https://doi.org/10.1094/MPMI-20-11-1384.

Hashida-Okado, T., Ogawa, A., Endo, M., Yasumoto, R., Takesako, K., and Kato, I. (1996). AUR1, a novel gene conferring aureobasidin resistance on Saccharomyces cerevisiae: a study of defective morphologies in Aur1p-depleted cells. Mol. Gen. Genet. 251, 236–244.

Hobo, T., Kowyama, Y., and Hattori, T. (1999). A bZIP factor, TRAB1, interacts with VP1 and mediates abscisic acid-induced transcription. Proc. Natl. Acad. Sci. U S A 96, 15348–15353. https://doi.org/10.1073/pnas.96.26.15348.

Hoffman, D.D., Hartman, G.L., Mueller, D.S., Leitz, R.A., Nickell, C.D., and Pedersen, W.L. (1998). Yield and seed quality of soybean cultivars infected with Sclerotinia sclerotiorum. Plant Dis. 82, 826–829. https://doi.org/10.1094/PDIS.1998.82.7.826.

Huckelhoven, R., and Kogel, K.H. (2003). Reactive oxygen intermediates in plant-microbe interactions: who is who in powdery mildew resistance? Planta 216, 891–902. https://doi.org/10.1007/s00425-003-0973-z.

Izawa, T., Foster, R., and Chua, N.H. (1993). Plant bZIP protein DNA binding specificity. J. Mol. Biol. 230, 1131–1144. https://doi.org/10.1006/jmbi.1993.1230.

Jing, Y., Liu, J., Liu, P., Ming, D., and Sun, J. (2019). Overexpression of TaJAZ1 increases powdery mildew resistance through promoting reactive oxygen species accumulation in bread wheat. Sci. Rep. 9, 5691. https://doi.org/10.1038/s41598-019-42177-y.

Jones, J.D., and Dangl, J.L. (2006). The plant immune system. Nature 444, 323–329. https://doi.org/10.1038/nature05286.

Kim, S., Kang, J.Y., Cho, D.I., Park, J.H., and Kim, S.Y. (2004). ABF2, an ABRE-binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance. Plant J. 40, 75–87. https://doi.org/10.1111/j.1365-313X.2004.02192.x.

Lee, S.C., Choi, H.W., Hwang, I.S., Choi, D.S., and Hwang, B.K. (2006). Functional roles of the pepper pathogen-induced bZIP transcription

factor, CAbZIP1, in enhanced resistance to pathogen infection and environmental stresses. Planta 224, 1209–1225. https://doi.org/10.1007/s00425-006-0302-4.

Li, X., Fan, S., Hu, W., Liu, G., Wei, Y., He, C., and Shi, H. (2017). Two cassava basic leucine zipper (bZIP) transcription factors (MebZIP3 and MebZIP5) confer disease resistance against cassava bacterial blight. Front. Plant Sci. 8, 2110. https://doi.org/10.3389/fpls.2017.02110.

Liu, F., Li, X., Wang, M., Wen, J., Yi, B., Shen, J., Ma, C., Fu, T., and Tu, J. (2018). Interactions of WRKY15 and WRKY33 transcription factors and their roles in the resistance of oilseed rape to Sclerotinia infection. Plant Biotechnol. J. 16, 911–925. https://doi.org/10.1111/pbi.12838.

Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R.P., and Somssich, I.E. (2015). Negative regulation of ABA signaling by WRKY33 is critical for Arabidopsis immunity towards Botrytis cinerea 2100. Elife 4, e07295. https://doi.org/10.7554/eLife.07295.

Lorenzo, O., Piqueras, R., Sanchez-Serrano, J.J., and Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15, 165–178. https://doi.org/10.1105/tpc.007468.

Luan, Q., Chen, C., Liu, M., Li, Q., Wang, L., and Ren, Z. (2019). CsWRKY50 mediates defense responses to Pseudoperonospora cubensis infection in Cucumis sativus. Plant Sci. 279, 59–69. https://doi.org/10.1016/j.plantsci.2018.11.002.

Manju, L., and Nair, R.R. (2006). Magnesium deficiency augments myocardial response to reactive oxygen species. Can. J. Physiol. Pharmacol. 84, 617–624. https://doi.org/10.1139/y06-017.

Mazarei, M., Elling, A.A., Maier, T.R., Puthoff, D.P., and Baum, T.J. (2007). GmEREBP1 is a transcription factor activating defense genes in soybean and Arabidopsis. Mol. Plant Microbe Interact 20, 107–119. https://doi.org/10.1094/MPMI-20-2-0107.

Mine, A., Seyfferth, C., Kracher, B., Berens, M.L., Becker, D., and Tsuda, K. (2018). The defense phytohormone signaling network enables rapid, high-amplitude transcriptional reprogramming during effector-triggered immunity. Plant Cell 30, 1199–1219. https://doi.org/10.1105/tpc.17.00970.

Miyamoto, K., Nishizawa, Y., Minami, E., Nojiri, H., Yamane, H., and Okada, K. (2015). Overexpression of the bZIP transcription factor OsbZIP79 suppresses the production of diterpenoid phytoalexin in rice cells. J. Plant Physiol. 173, 19–27. https://doi.org/10.1016/j.jplph.2014.09.001.

Morel, M., Ngadin, A.A., Droux, M., Jacquot, J.P., and Gelhaye, E. (2009). The fungal glutathione S-transferase system. Evidence of new classes in the wood-degrading basidiomycete Phanerochaete chrysosporium. Cell Mol. Life Sci. 66, 3711–3725. https://doi.org/10.1007/s00018-009-0104-5.

Naing, A.H., Kyu, S.Y., Pe, P.P.W., Park, K.I., Lee, J.M., Lim, K.B., and Kim, C.K. (2019). Silencing of the phytoene desaturase (PDS) gene affects the expression of fruit-ripening genes in tomatoes. Plant Methods 15, 110. https://doi.org/10.1186/s13007-019-0491-z.

Nijhawan, A., Jain, M., Tyagi, A.K., and Khurana, J.P. (2008). Genomic survey and gene expression analysis of the basic leucine zipper transcription factor family in rice. Plant Physiol. 146, 333–350. https://doi.org/10.1104/pp.107.112821.

Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055.

Ranjan, A., Westrick, N.M., Jain, S., Piotrowski, J.S., Ranjan, M., Kessens, R., Stiegman, L., Grau, C.R., Conley, S.P., Smith, D.L., and Kabbage, M. (2019). Resistance against Sclerotinia sclerotiorum in soybean involves a reprogramming of the phenylpropanoid pathway and up-regulation of antifungal activity targeting ergosterol biosynthesis. Plant Biotechnol. J. https://doi.org/10.1111/pbi.13082.

Robatzek, S., and Somssich, I.E. (2002). Targets of AtWRKY6 regulation during plant senescence and pathogen defense. Genes Dev. 16, 1139–1149. https://doi.org/10.1101/gad.222702.

Schluttenhofer, C., and Yuan, L. (2015). Regulation of specialized metabolism by WRKY transcription factors. Plant Physiol. 167, 295–306. https://doi.org/10.1104/pp.114.251769.

Silva, M.S., Arraes, F.B.M., Campos, M.A., Grossi-de-Sa, M., Fernandez, D., Candido, E.S., Cardoso, M.H., Franco, O.L., and Grossi-de-Sa, M.F. (2018). Review: potential biotechnological assets related to plant immunity modulation applicable in engineering disease-resistant crops. Plant Sci. 270, 72–84. https://doi.org/10.1016/j.plantsci.2018.02.013.

Sun, X., Yu, G., Li, J., Liu, J., Wang, X., Zhu, G., Zhang, X., and Pan, H. (2018). AcERF2, an ethylene-responsive factor of Atriplex canescens, positively modulates osmotic and disease resistance in Arabidopsis thaliana. Plant Sci. 274, 32–43. https://doi.org/10.1016/j.plantsci.2018.05.004.

Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y., Kato, I., Uchida, K., Hiratani, T., and Yamaguchi, H. (1993). Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. J. Antibiot. 46, 1414–1420.

Thomma, B.P., Eggermont, K., Penninckx, I.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P., and Broekaert, W.F. (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. U S A 95, 15107–15111. https://doi.org/10.1073/pnas.95.25.15107.

Tsuda, K., and Somssich, I.E. (2015). Transcriptional networks in plant immunity. New Phytol. 206, 932–947. https://doi.org/10.1111/nph.13286.

Tyler, B.M. (2007). Phytophthora sojae: root rot pathogen of soybean and model oomycete. Mol. Plant Pathol. 8, 1–8. https://doi.org/10.1111/j.1364-3703.2006.00373.x.

Tyler, B.M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R.H., Aerts, A., Arredondo, F.D., Baxter, L., Bensasson, D., Beynon, J.L., et al. (2006). Phytophthora genome sequences uncover evolutionary origins and mechanisms of

pathogenesis. Science *313*, 1261–1266. https://doi.org/10.1126/science.1128796.

Westrick, N.M., Ranjan, A., Jain, S., Grau, C.R., Smith, D.L., and Kabbage, M. (2019). Gene regulation of Sclerotinia sclerotiorum during infection of Glycine max: on the road to pathogenesis. BMC Genomics *20*, 157. https://doi.org/10.1186/s12864-019-5517-4.

Whitham, S.A., Qi, M., Innes, R.W., Ma, W., Lopes-Caitar, V., and Hewezi, T. (2016). Molecular soybean-pathogen interactions. Annu. Rev. Phytopathol. *54*, 443–468. https://doi.org/10.1146/annurev-phyto-080615-100156.

Xun, H., Yang, X., He, H., Wang, M., Guo, P., Wang, Y., Pang, J., Dong, Y., Feng, X., Wang, S., and Liu, B. (2019). Over-expression of GmKR3, a TIR-NBS-LRR type R gene, confers resistance to multiple viruses in soybean. Plant Mol. Biol. *99*, 95–111. https://doi.org/10.1007/s11103-018-0804-z.

Yang, B., Wang, Y., Guo, B., Jing, M., Zhou, H., Li, Y., Wang, H., Huang, J., Wang, Y., Ye, W., et al. (2019a). The Phytophthora sojae RXLR effector Avh238 destabilizes soybean Type2 GmACSs to suppress ethylene biosynthesis and promote infection. New Phytol. *222*, 425–437. https://doi.org/10.1111/nph.15581.

Yang, X., Yang, J., Wang, Y., He, H., Niu, L., Guo, D., Xing, G., Zhao, Q., Zhong, X., Sui, L., et al. (2019b). Enhanced resistance to sclerotinia stem rot in transgenic soybean that overexpresses a wheat oxalate oxidase. Transgenic Res. *28*, 103–114. https://doi.org/10.1007/s11248-018-0106-x.

Yu, H., Gao, Q., Dong, S., Zhou, J., Ye, Z., and Lan, Y. (2016). Effects of dietary n-3 highly unsaturated fatty acids (HUFAs) on growth, fatty acid profiles, antioxidant capacity and immunity of sea cucumber Apostichopus japonicus (Selenka). Fish Shellfish Immunol. *54*, 211–219. https://doi.org/10.1016/j.fsi.2016.04.013.

Zhang, C., Bradshaw, J.D., Whitham, S.A., and Hill, J.H. (2010). The development of an efficient multipurpose bean pod mottle virus viral vector set for foreign gene expression and RNA silencing. Plant Physiol. *153*, 52–65.

Zhang, C., Gao, H., Li, R., Han, D., Wang, L., Wu, J., Xu, P., and Zhang, S. (2019). GmBTB/POZ, a novel BTB/POZ domain-containing nuclear protein, positively regulates the response of soybean to Phytophthora sojae infection. Mol. Plant Pathol. *20*, 78–91. https://doi.org/10.1111/mpp.12741.

Zhang, C., Li, C., Liu, J., Lv, Y., Yu, C., Li, H., Zhao, T., and Liu, B. (2017). The OsABF1 transcription factor improves drought tolerance by activating the transcription of COR413-TM1 in rice. J. Exp. Bot. *68*, 4695–4707. https://doi.org/10.1093/jxb/erx260.

Zhang, H., Wu, Q., Cao, S., Zhao, T., Chen, L., Zhuang, P., Zhou, X., and Gao, Z. (2014). A novel protein elicitor (SsCut) from Sclerotinia sclerotiorum induces multiple defense responses in plants. Plant Mol. Biol. *86*, 495–511. https://doi.org/10.1007/s11103-014-0244-3.

Zhang, M., Liu, Y., Cai, H., Guo, M., Chai, M., She, Z., Ye, L., Cheng, Y., Wang, B., and Qin, Y. (2020). The bZIP transcription factor GmbZIP15 negatively regulates salt- and drought-stress responses in soybean. Int. J. Mol. Sci. *21*. https://doi.org/10.3390/ijms21207778.

Zhang, M., Liu, Y., Shi, H., Guo, M., Chai, M., He, Q., Yan, M., Cao, D., Zhao, L., Cai, H., and Qin, Y. (2018). Evolutionary and expression analyses of soybean basic Leucine zipper transcription factor family. BMC Genomics *19*, 159. https://doi.org/10.1186/s12864-018-4511-6.

Zhang, W., Wu, Y., Schnable, J.C., Zeng, Z., Freeling, M., Crawford, G.E., and Jiang, J. (2012). High-resolution mapping of open chromatin in the rice genome. Genome Res. *22*, 151–162. https://doi.org/10.1101/gr.131342.111.

Zhao, L.H., Cai, H.Y.,Z.X., Wang, L.L., Huang, X.Y., and Zhang. (2018). KLU suppresses megasporocyte cell fate through SWR1-mediated activation of WRKY28 expression in Arabidopsis. Proc. Natl. Acad. Sci. U S A. https://doi.org/10.1073/pnas.1716054115.

Zhou, X.T., Jia, L.J., Wang, H.Y., Zhao, P., Wang, W.Y., Liu, N., Song, S.W., Wu, Y., Su, L., Zhang, J., et al. (2018). The potato transcription factor StbZIP61 regulates dynamic biosynthesis of salicylic acid in defense against Phytophthora infestans infection. Plant J. *95*, 1055–1068. https://doi.org/10.1111/tpj.14010.

Zhu, L., Guo, J., Ma, Z., Wang, J., and Zhou, C. (2018). Arabidopsis transcription factor MYB102 increases plant susceptibility to aphids by substantial activation of ethylene biosynthesis. Biomolecules *8*. https://doi.org/10.3390/biom8020039.

Zong, W., Zhong, X., You, J., and Xiong, L. (2013). Genome-wide profiling of histone H3K4-tri-methylation and gene expression in rice under drought stress. Plant Mol. Biol. *81*, 175–188. https://doi.org/10.1007/s11103-012-9990-2.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
GV3101	This paper	N/A
Experimental models: Organisms		
Arabidopsis: OX-GmABF1	This paper	N/A
Arabidopsis: OX-GmERS1	This paper	N/A
Arabidopsis: OX-GmJAZ8	This paper	N/A
Arabidopsis: SALK-050203C	Tair	N/A
Soybean: *OX-GmbZIP15*	This paper	N/A
Soybean: *35S:GmbZIP15-SRDX*	This paper	N/A
Software		
MEME-ChIP	This paper	http://meme-suite.org/tools/meme-chip
Deposited data		
Raw ChIP-seq data	This paper	PRJEB44708
Recombinant DNA		
Plasmid: pGWB605	Cai et al., 2017	N/A
TRV-GmbZIP15	This paper	N/A
35S:GmbZIP15	This paper	N/A
35S:GmbZIP15-SRDX	This paper	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yuan Qin (yuanqin@fafu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The original ChIP-seq data is available at European Nucleotide Archive (ENA) under accession number PRJEB44708.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Soybean plant material

Soybean ecotype C03-3 was used in this study. Soybean seeds were sown in soil and grown at 25°C with a 16-h light/8-h dark photoperiod. *Agrobacterium*-mediated transformation was performed with an expression construct harboring the *OX-GmbZIP15* and *35S: GmbZIP15-SRDX* (Yang et al., 2019b). To screen the transgenic plants progenies and obtain homozygous lines, both glufosinate spraying and polymerase chain reaction (PCR) analysis were conducted.

Pathogens infection

Fifteen-day-old soybean plants were infected with *Sclerotinia sclerotiorum* and *Phytophthora sojae*, which were collected from Fujian Agriculture and Forestry University, Fuzhou, China. The fungus *S. sclerotiorum* was germinated to produce hyphal inoculum on potato dextrose agar and *P. sojae* was cultivated at 25 °C for 7 days on V8 juice agar. The detached-leaf inoculation assay was performed based on the description

reported by Yang et al. In a Petri dish, mycelial plugs were placed on the completely expanded leaves from 15-day-old soybean plants. Eight leaves were inoculated for each transgenic line and WT plants. Lesion areas on the detached leaves were monitored and photographed at 24 h or 48 h after inoculation. Hypocotyl-wound inoculation was performed based on previous research with 48 plants per samples. Briefly, a wound about 1 cm in length was cut into the hypocotyl epidermis 1 cm below the cotyledon of 15-day-old soybean plants, and then, it was inoculated with mycelial plugs. After inoculation, plants were placed in a moisturizing room at 23–25°C with 100% humidity for 48 h and then transferred to a greenhouse for further cultivation at 25°C.

METHOD DETAILS

Vector construction and transformation

To generate the overexpression construct, the coding DNA sequences (CDS) of *GmbZIP15* (Glyma.02G161100), *GmABF1* (*GmbZIP157*, Glyma.20G049200), *GmJAZ8* (Glyma.05G141200), and *GmERS1* (Glyma.03G216700) were amplified, and *35S: GmbZIP15-SRDX* was generated by amplifying *GmbZIP15* cDNA sequence and an SRDX motif was added to the end of the cDNA sequence (ctagatctggatctagaactccgtttgggtttcgcttaa). All the PCR fragment was cloned into the pENTR/D-TOPO vector (Invitrogen), and the pENTR clones were recombined into the destination vector pGWB605 (with 35S promoter) using LR Clonase II (Invitrogen). The resulting construct also contained the selectable marker BAR for glufosinate resistance (Cai et al., 2017; Zhao et al., 2018). All the primers used in this article are listed in Table S1.

Virus-induced gene silencing of GmbZIP15 in soybean plants

To make the GmbZIP15 silencing construct, primers (Forward: 5'-ctcacgcgtctcgaggcccCCGGGTATTA-CAAGCTCTTCGAG-3', Reverse: 5'-atgtcttcgggacatgcccCACATACAACTTCTTCCTCTCCC-3') were used to amplify a 212-bp fragment. The amplified fragment was ligated into the vector pTRV: RNA2 at BamHI and SmlI sites. Silencing was monitored using the construct pTRV: RNA2-GmPDS1, which targets the soybean phytoene desaturase (PDS), leading to photobleaching of plants. The *Agrobacterium* was then resuspended in agroinfiltration buffer (10 mM MgCl2, 10 mM MES pH 5.7, 100 μM acetosyringone, OD600=0.8). Each agrosuspension containing the TRV2 derivatives (TRV2-GmPDS1 and TRV2-GmbZIP15) was mixed with TRV1 and then infiltrated in ten-day-old soybean leaves. After about 18-24 dpi, the transcript levels of the first pair ternate palmate leaves were examined by qPCR.

Soybean seedling phytohormone treatments

For phytohormone treatments, SA (1 mM), MeJA (100 μM), ETH (100 μM), and ABA (100 μM) were dissolved in 0.01% Tween 20 and sprayed onto 15-day-old WT soybean young leaves, and soybean leaves were collected at 0, 1, 2, 6,12, 24, and 48 h. The control leaves were sprayed with an equal volume of 0.01% (v/v) Tween 20.

Arabidopsis plant material and pathogen infection

Arabidopsis ecotype Col-0 was used in this study. All the constructs were transformed into WT *Arabidopsis* (Col-0) plants by the floral dip method, and transgenic plants screening was performed as described previously. The T-DNA mutant *Atbzip60* (SALK_050203) was obtained from the Arabidopsis Biological Resource Center. *Arabidopsis* seeds were sterilized and placed on 1/2 MS medium. After stratification at 4°C for 3 days, the plates were kept at 22°C with a 16-h light/8-h dark photoperiod for plant growth, followed by infection with *S. sclerotiorum* in 4-week-old plants. The detached-leaf inoculation assay was performed.

Diaminobenzidine staining

Following the method reported by Zhang et al. for hydrogen peroxide (H_2O_2) detection (Zhang et al., 2014), diaminobenzidine (DAB) staining was performed in the leaves of pathogen-treated plants. Briefly, the harvested leaves were vacuum-infiltrated for 20 min with Tris-HCl (pH 7.4) containing 1 % (w/v) DAB. The leaves were placed in light for 10 h and then boiled for 20 min in 75 % ethanol.

RNA extraction and quantitative real-time PCR

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed using the PrimeScript RT-PCR kit (TaKaRa). Real-time PCR was performed with specific primers to analyze the relative transcript levels of selected genes (Table S1), the Bio-Rad Real-time PCR system (Foster City, CA, USA)

using SYBR Premix Ex Taq II (TaKaRa Perfect Real Time). The qPCR program was 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 34 s; and 95°C for 15 s. *GmActin* was used for normalization.

Transcriptional activation analysis in yeast cells

The *GmbZIP15* ORF was introduced into pGBKT7 to generate pGBKT7-GmbZIP15. The yeast strain AH109 was transformed with pGBKT7-53 + pGADT7-T, pGBKT7-GmbZIP15, and pGBKT7. The transformed cells were grown on SD (-Trp), SD (-Trp/–His/-Ade), and SD (-Trp/-His/-Ade/α-gal). The transactivation activity of proteins was detected by the growth status and α-gal activity.

Determination of antioxidant enzyme activity

Superoxide dismutase, peroxidase, catalase, and malondialdehyde enzymes were extracted from approximately 0.1 g of leaves using 1 mL extraction solution. The enzyme activities were measured as per the protocol from the Solarbio Biochemical Assay Division.

Subcellular localization

To examine GmbZIP15 localization *in planta*, the *Agrobacteria* containing the construct 35S:GmbZIP15-GFP was resuspended in infiltration media (10 mM ethanesulfonic acid [pH 5.8], 10 mM $MgCl_2$, and 200 μM acetosyringone) before infiltration into the leaves of 30-day-old *Nicotiana benthamiana* plants. Two days later, leaf discs were observed under a confocal microscope (Leica TCS SP8X DLS) for GFP.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) was performed using a GFP antibody as previously described (Dai et al., 2018) with minor modifications. For each ChIP experiment, 3.0 g of soybean leaves was used, and three biological replicates were performed because the mapping rate of two samples was too low to conduct further analysis, so only one sample data could be used for further analysis. First, cross-linked chromatin was fragmented using 4 units of micrococcal nuclease (Sigma) in 1 ml of MNase digestion buffer (10 % sucrose, 50 mM Tris–HCl [pH 7.5], 25 mM $MgCl_2$, 1 mM $CaCl_2$), and then, the digestion was stopped with 5 Mm EDTA. All ChIP experiments were performed in a buffer containing 50 mM HEPES (pH 7.5), 1 mM EDTA (pH8.0), 150 mM NaCl, 1% Triton X-100, and 13 protease inhibitor cocktail (Roche). The ChIP-seq libraries were prepared as previously described (Zhang et al., 2012) then sequenced on the Illumina HiSeq 2500 platform. The ChIP-seq sequence reads were mapped to the phytozome v12.1 reference genome (https://phytozome.jgi.doe.gov/pz/portal.html) (Table S2). ChIP-Seq data processing and analysis were performed as described previously (Zong et al., 2013).

Yeast one-hybrid assays

GmbZIP15 CDS without a stop codon was amplified and then integrated into the pGADT7-T vector by an In-Fusion cloning kit (Clontech, Takara) to form a pGADT7-GmbZIP15 bait report vector. Based on the predicted binding site in our ChIP-seq data, the normal or mutational fragments were synthesized by DNA synthesis technology (Sangon Biotech, Shanghai, China; Table S1) and cloned into a pABAi vector by In-Fusion technology to form pABAi-GmABF1/pABAi-GmABF1m, pABAi-GmERS1/pABAi-GmERS1m, pABAi-GmJAZ8/pABAi-GmJAZ8m, and pABAi-GmEIX1/pABAi-GmEIX1m prey report vectors.

Yeast one-hybrid was carried out as per instructions provided by Clontech (Takara). Prey was transformed into Y1H gold yeast strain and cultured on SD/-Ura or SD/-Ura/-Leu medium with or without 300 ng/mL aureobasidin A (AbA) for 3 days. Furthermore, the yeast cells cotransformed by prey and bait were cultured on SD/-Ura/-Leu medium containing 300 ng/mL AbA for 3 days.

Electrophoretic mobility shift assays

The CDS of *GmbZIP15* was amplified and inserted into the vector pMAL (NEB) to fuse with the maltose-binding protein (MBP) CDS, generating the recombinant MBP-GmbZIP15 protein. The resulting construct was expressed in *Escherichia coli* strain BL21(DE3) cells, the recombinant protein was expressed and purified using Amylose Resin (New England Biolabs, cat. no. E8021S). The probes were synthesized and labeled with Cy5, and different concentrations of nonlabeled probe were added to the reactions for competition. Finally, the DNA-protein complexes were electrophoresed on 6 % nondenaturing polyacrylamide gels in an ice water bath.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analysis of variance was used to calculate the least significant differences between mean values by the t-test at $P = 0.05$ using SPSS software (v. 17.0). The type of statistical test, the number of replicates and whether error bars denote standard deviation or the standard error of the mean, is specified in each figure legend.