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WRKY45 is a negative regulator of *Botrytis cinerea* resistance through the JA/ET signaling pathway in *Arabidopsis*

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Botrytis cinerea is a necrotrophic fungal pathogen that causes significant crop damage, yet the molecular mechanisms underlying plant defense remain incompletely understood. Here, we identify WRKY45 as a negative regulator of *Arabidopsis* resistance to *B. cinerea* through the suppression of JA/ET-mediated defense signaling. Our results show that *WRKY45* expression was induced by *B. cinerea* infection, peaking 48 hours post-inoculation. Loss of *WRKY45* function enhanced resistance, while *WRKY45* overexpression increased susceptibility and cellular damage, as indicated by elevated electrolyte leakage, higher malondialdehyde levels, and reduced chlorophyll content. RNA-seq analysis identified 1,850 differentially expressed genes in *wrky45* mutants, with strong enrichment of JA/ET-responsive pathways. Defense-related genes, including *ORA59*, *PDF1.2*, *ERF104*, and *ERF1*, were markedly upregulated in *wrky45* but suppressed in overexpression lines, as confirmed by qRT-PCR. Electrophoretic mobility shift assays and dual-luciferase assays demonstrated that WRKY45 directly binds to the *ORA59* promoter inhibiting its transcription, and represses the expression of *PDF1.2*, *ERF104*, and *ERF1*. Together, these results show that WRKY45 functions as a negative regulator by suppressing the expression of JA/ET-mediated defense genes, thereby modulating plant resistance to *B. cinerea*.

KEYWORDS

Arabidopsis, *Botrytis cinerea*, defense response, molecular mechanism, WRKY45

1 Introduction

Necrotrophic fungal pathogens are a major threat to global agriculture by inducing host cell death and colonizing necrotic tissues as nutrient sources. Their airborne dissemination further accelerates disease spread. Among them, *B. cinerea* infects over 200 plant species and causes severe pre- and post-harvest losses worldwide (Dean et al., 2012; van Kan, 2006; Bi et al., 2021). Although jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) signaling pathways are known to orchestrate plant immune responses (Glazebrook, 2005; AbuQamar et al., 2017; Song et al., 2022), the transcriptional regulatory frameworks that integrate these signals and orchestrate robust immunity against necrotrophs are still not fully understood.

In *Arabidopsis*, the JA/ET signaling module plays a pivotal role in resistance to *B. cinerea*. Core defense-associated genes, including *ORA59*, *PDF1.2*, *ERF104*, and *ERF1*, function as critical hubs within this pathway, integrating hormonal cues and activating antimicrobial responses. *ORA59* functions as a central integrator of the JA and ET signaling pathways (Pré et al., 2008; Ju et al., 2017), whereas *PDF1.2* encodes a plant defensin that serves as a hallmark of JA/ET-mediated defense (Penninckx et al., 1996; Cheng et al., 2018). *ERF104* is an ET-responsive factor, and related studies indicate that it plays a crucial role in resisting non-adapted pathogens (Chen et al., 2013). *ERF1* amplifies resistance by coordinating a broad spectrum of JA/ET-dependent defense programs (Lorenzo et al., 2003; Rasool et al., 2015). The activities of these JA/ET-responsive genes are strongly shaped by upstream transcription factors, highlighting the importance of transcriptional regulation in controlling immune outputs.

Among transcription factors, WRKY proteins have emerged as master regulators of plant immunity. They constitute one of the largest transcription factor families in plants and exert diverse functions by binding to W-box cis-elements in target promoters (Chen et al., 2012; Jiang et al., 2017; Zhu et al., 2022). WRKY transcription factors act as critical regulators of stress responses, functioning either as activators or repressors of defense genes to fine-tune plant immunity (Wang et al., 2025; Chen et al., 2025; Du et al., 2023). For example, WRKY33 is a well-characterized positive regulator of resistance to *B. cinerea*, enhancing JA/ET signaling, reactive oxygen species (ROS) homeostasis, and secondary metabolism (Birkenbihl et al., 2012; Liu et al., 2015). WRKY75 similarly promotes JA-mediated immunity (Chen et al., 2021). OsWRKY67 positively regulates plant disease resistance through the modulation of defense-related genes (Liu et al., 2018). In contrast, several WRKYs—including SlWRKY3, PtrWRKY89, and VqWRKY52—function as negative regulators to prevent excessive immune activation (Luo et al., 2024; Jiang et al., 2016; Wang et al., 2017). These functional divergences highlight the context-dependent nature of WRKY function in plant immunity.

WRKY45 is a member of the WRKY transcription factor family and has been reported to participate in various physiological processes in *Arabidopsis*, such as responses to phosphorus starvation, salt stress, cadmium toxicity, and leaf senescence (Wang et al., 2014; Zhou et al., 2025; Li et al., 2023; Chen et al.,

2017; Vátov et al., 2021; Barros et al., 2022). However, its role in plant immunity remains poorly understood, particularly in defense against necrotrophic pathogens. Considering the dual regulatory roles of WRKY transcription factors and the central importance of JA/ET-regulated defense genes such as *ORA59*, *PDF1.2*, *Thi2.1*, and *ERF1*, elucidating the function of WRKY45 in *Arabidopsis* immunity against *B. cinerea* is critical.

In this study, we investigate the role of WRKY45 in modulating *Arabidopsis* resistance to *B. cinerea*. We specifically assess whether WRKY45 regulates key JA/ET-dependent defense genes and dissect the transcriptional mechanisms underlying its activity. Our findings demonstrate that WRKY45 functions as a negative regulator of plant immunity, thereby providing new insights into the transcriptional networks shaping host responses to necrotrophic pathogens and offering potential targets for enhance disease resistance in crops.

2 Materials and methods

2.1 Plant materials and growth conditions

The experimental materials included *Arabidopsis* (Columbia ecotype, *Col-0* wild type), as well as *wrky45* mutant lines (*WRKY45-RNAi-3* and *wrky45*) (Wang et al., 2014; Chen et al., 2017), and *WRKY45* overexpression lines (35S:WRKY45-2 and 35S:WRKY45-6). Seeds of *Arabidopsis* were surface-sterilized with 20% bleach solution for 15 minutes, and then sown on half-strength Murashige and Skoog (MS) solid medium (pH 5.8). After stratification at 4°C for 3 days, the plates were transferred to a greenhouse under a 16-hour light/8-hour dark photoperiod at 22°C. After 7 days of growth, seedlings were transplanted into moist soil and covered with plastic film, which was removed after 2–3 days to allow further growth. Seeds of *Nicotiana benthamiana* were grown in a green house at 25°C under a 16 h light/8 h dark photoperiod. In all experiments, *Arabidopsis* seedlings used for *B. cinerea* inoculation were consistently two weeks old. The *WRKY45* overexpression lines were constructed and maintained at the Key Laboratory of Sustainable Utilization of Tropical Plant Resources, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Verification of the *WRKY45* overexpression lines (35S:WRKY45-2 and 35S:WRKY45-6) is provided in Supplementary Figure 1.

2.2 Pathogen inoculation with *B. cinerea*

B. cinerea strain B05.10 was cultured on potato dextrose agar (PDA) at 22°C under a 12-hour light/12-hour dark photoperiod (Xiang et al., 2020). For whole-plant inoculation, *B. cinerea* conidial suspension was evenly sprayed onto the leaves of 2-week-old *Arabidopsis* seedlings, which were then maintained under short-day, high-humidity, and dark conditions to promote infection (Scholz et al., 2023). Samples were collected at 0 and 5 days post-inoculation (dpi) for physiological measurements, and disease symptoms were monitored daily. For detached leaf inoculation,

B.cinerea conidia were suspended in SMB (40 g/L maltose, 10 g/L peptone) with 0.1% Tween 80, and the suspension was filtered through gauze to obtain a conidial solution. A 3 μ L droplet of this suspension (5×10^5 ; spores/mL) was applied to the abaxial surface of 14-day-old soil-grown *Arabidopsis* leaves, with 3 μ L of SMB solution as a control. The leaves were placed on moist filter paper in Petri dishes and incubated under low light and high humidity at 22 °C for 3 days. Disease symptoms were assessed, and qualitative evaluation was performed using GUS staining.

2.3 RNA extraction and quantitative real-time PCR analysis

Seeds of *Arabidopsis* (different genotypes) were germinated on half-strength Murashige and Skoog solid medium, stratified at 4°C for 3 days and grown for 7 days before transferring to moist soil for 2 weeks. Seedlings were then inoculated with *B. cinerea*, and samples were harvested at various time points post-inoculation. Total RNA was extracted from *Arabidopsis* seedlings using the water-saturated phenol method. First-strand cDNA was synthesized by reverse transcription, followed by quantitative real-time PCR (qRT-PCR). Reverse transcription and qRT-PCR kits were obtained from Takara (Dalian, China), and PCR was performed on a QuantStudio 5 real-time PCR machine using SYBR Premix Ex Taq™ II. *ACTIN2* (AT3G18780) was used as internal control in qRT-PCR. Primer sequences used in qRT-PCR are listed in [Supplementary Table S1](#).

2.4 RNA sequencing

Wild-type and *wrky45* mutant *Arabidopsis* lines, grown for two weeks, were inoculated with a suspension of *B. cinerea* spores and maintained under treatment conditions for five days. Samples were collected from both WT and *wrky45* mutant plants before and after inoculation. Total RNA was extracted from the seedlings using the water-saturated phenol method and subsequently sent to Kunming Hengyun Biotechnology Co., Ltd. for transcriptome sequencing. Three independent biological replicates were analyzed for each condition.

2.5 GUS staining

Histochemical GUS assays were performed as described previously ([Chen et al., 2021](#)). The GUS staining kit was purchased from Zhongke Ruitai Biotechnology Co., Ltd. (Beijing, China). Fourteen-day-old *WRKY45p::GUS* transgenic seedlings were detached and inoculated with *B. cinerea* for three days before GUS staining. Tissues were decolorized in 70% ethanol, with the solution replaced two to three times until the control tissues became completely white, and then photographed. Three independent biological replicates were processed under identical staining and imaging conditions to ensure reproducibility.

2.6 Electrolyte leakage assay

Electrolyte leakage was measured to assess membrane integrity in wild-type, *wrky45* mutant, and *WRKY45* overexpression seedlings at 0 and 5 dpi. Leaves were immersed in 10 mL of deionized water and gently shaken before measuring the initial conductivity (R_0). Samples were then incubated at room temperature for 2 hours to allow passive ion leakage, and conductivity was measured again (R_1). Subsequently, samples were boiled at 100°C for 20 minutes, cooled to room temperature, and final conductivity (R_2) was measured. Electrolyte leakage was calculated as $(R_1 - R_0)/(R_2 - R_0) \times 100$, following Bajji ([Bajji et al., 2002](#)). Three independent biological replicates were analyzed for each genotype.

2.7 Chlorophyll quantification

Fully expanded *Arabidopsis* leaves were collected, immediately frozen in liquid nitrogen, and ground to a fine powder. Photosynthetic pigments were extracted using ice-cold 80% (v/v) acetone, followed by centrifugation to remove debris. The Absorbance of the supernatant was measured at 663 nm and 645 nm using a UV-visible spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were calculated using the Lichtenthaler equations for 80% acetone ([Lichtenthaler, 1987](#)). Three independent biological replicates were analyzed for each genotype.

2.8 Determination of malondialdehyde content

Malondialdehyde (MDA) content was determined using a thiobarbituric acid (TBA) method. Samples were collected from wild-type, mutant, and *WRKY45*-overexpressing *Arabidopsis* seedlings before and after *B. cinerea* infection. Approximately 1 g of fresh tissue was weighed, cut into small pieces and homogenized in 10 mL of 10% (w/v) trichloroacetic acid (TCA) using a mortar and pestle. The homogenate was centrifuged at 4,000 rpm for 10 min, and 6 mL of the supernatant was mixed with an equal volume of 0.6% (w/v) thiobarbituric acid solution. The mixture was incubated in a boiling water bath at 100 °C for 15 min, rapidly cooled to room temperature, and centrifuged for 1 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm using a spectrophotometer, with 2 mL of distilled water as the blank control. MDA content was calculated according to the method described by Huang ([Huang et al., 2016](#)). Three independent biological replicates were analyzed for each genotype.

2.9 Electrophoretic mobility shift assays

To examine the potential interaction between *WRKY45* and the W-box element, electrophoretic mobility shift assays (EMSA) were

performed using a chemiluminescent EMSA kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions. DNA fragments corresponding to the *ORA59* promoter region, containing either the wild-type W-box sequence or a mutated W-box motif, were synthesized and end-labeled with biotin. Purified recombinant GST-WRKY45 fusion protein was incubated with the labeled probes in binding buffer at room temperature for 20 minutes. For competition assays, a 50- to 100-fold molar excess of unlabeled probe was added in the reaction. The DNA-protein complexes were separated on 6% non-denaturing polyacrylamide gels and subsequently transferred to nylon membranes (Brunello et al., 2024). Biotin-labeled DNA was detected according to the kit protocol. The primers used for the EMSA assay are listed in Supplementary Table 1.

2.10 Transcriptional activity assays

The native promoters (about 2.5 kb) of *ORA59*, *PDF1.2*, *ERF1*, and *ERF104* were cloned into pGWB35 to generate the reporter constructs *ORA59p:LUC*, *PDF1.2p:LUC*, *ERF1p:LUC*, and *ERF104p:LUC* using Gateway technology (Invitrogen, USA). The reporter plasmids and effector constructs carrying 35S:WRKY45 were introduced into *Agrobacterium tumefaciens* strain EHA105 (pSoup). *Agrobacterium* cultures harboring the respective plasmids were grown, collected, and resuspended in infiltration buffer (10 mM MgCl₂, 10 mM MES, and 200 μ M acetosyringone) to an OD₆₀₀ of 0.6 prior to infiltration (Kang et al., 2023). After infiltration, plants were maintained in a growth chamber under high humidity and low-light conditions for 72 hours. Before luminescence imaging, the infiltrated leaves were sprayed with fluorescein potassium salt solution (10 mL sterilized water + 10

μ L 10% Triton X-100 + 0.008 g fluorescein potassium salt), incubated in the dark for 5 minutes, and imaged using a cooled CCD imaging system (Olympus, Japan) to capture LUC luminescence (Zhang et al., 2022). Each experiment was performed with at least three independent biological replicates. The primers used for the transcriptional activity assays are listed in Supplementary Table 1.

2.11 Statistical analysis

All experiments were performed with at least three independent biological replicates, and data are presented as the mean \pm standard deviation (SD). One-way ANOVA was used for Figure 1A, a two-tailed Student's t-test for Figure 2E, and two-way ANOVA followed by Tukey's test for Figures 3, 5. Statistical significance was set at $P < 0.05$. Different lowercase letters indicate significant differences among multiple groups, while asterisks (* or **) denote significant differences in pairwise comparisons (* $P < 0.05$, ** $P < 0.01$).

3 Results

3.1 WRKY45 is transcriptionally activated by *B. cinerea* infection

To investigate the role of WRKY45 in resistance to *B. cinerea*, wild-type seedlings were inoculated with a suspension of *B. cinerea* spores, and *WRKY45* transcript levels were assessed at various time points. qRT-PCR analysis revealed that *WRKY45* expression was rapidly and significantly upregulated within 6 hours post-inoculation, with transcript levels progressively increasing and

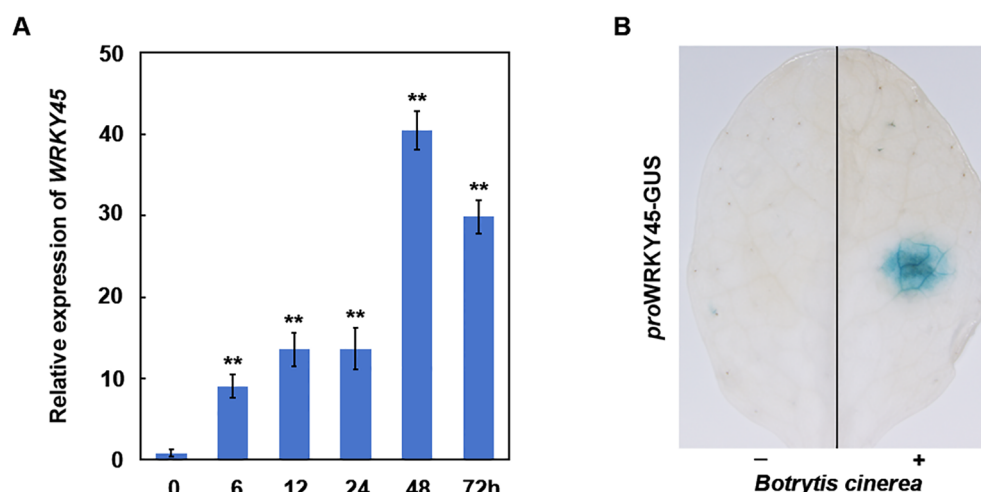


FIGURE 1

WRKY45 expression is induced by *B. cinerea*. (A) Time-course analysis of *WRKY45* transcript accumulation in *Arabidopsis* following inoculation with (B) *cinerea*. Relative expression levels were quantified by qRT-PCR at 0, 6, 12, 24, 48, and 72 hours post-inoculation (hpi), using *ACTIN2* as the internal reference gene. Data are presented as the mean \pm SD ($n = 3$ independent biological replicates). Statistical significance was determined using one-way ANOVA, and asterisks indicate significant differences compared to 0 h (** $P < 0.01$). (B) Histochemical GUS staining of *WRKY45p:GUS* transgenic plants after *B. cinerea* inoculation. Strong GUS activity was observed at the inoculation sites (+), compared to untreated control sites (–), confirming local induction of *WRKY45* expression by pathogen infection.

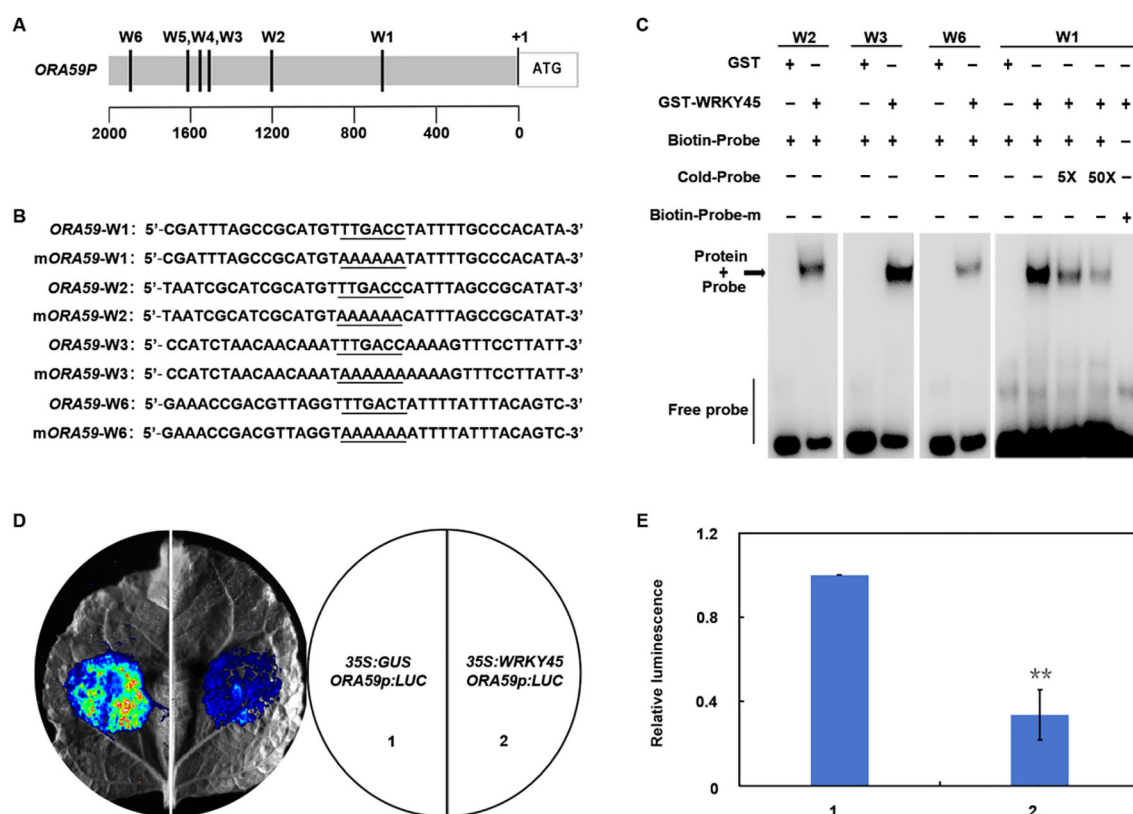


FIGURE 2

WRKY45 directly represses *ORA59* and downstream JA/ET-responsive defense genes. (A) Schematic representation of the *ORA59* promoter showing six putative W-box elements (W1–W6). (B) Nucleotide sequences of the W-box elements used in DNA-binding assays, with core motifs and mutated nucleotides underlined. (C) Electrophoretic mobility shift assay (EMSA) showing the interaction between GST–WRKY45 and W-box elements of the *ORA59* promoter. GST protein alone was used as negative control. “5x” and “50x” denote addition of fivefold and fiftyfold excess of unlabeled competitor probe, respectively; “+” and “–” denote presence or absence of competitor probes. (D) Transient dual-luciferase assays in *Nicotiana benthamiana* leaves. Representative luminescence images of co-infiltration with (1) 35S::GUS + *ORA59p::LUC* (control) and (2) 35S::WRKY45 + *ORA59p::LUC* are shown. (E) Quantitative analysis of luciferase activity. Data are presented as the mean \pm SD ($n = 3$ independent biological replicates). Statistical significance was determined using two-tailed Student's *t*-test, and asterisks indicate significant differences (** $P < 0.01$).

peaking at approximately 48 h (Figure 1A), indicating that WRKY45 expression is induced by *B. cinerea*. Subsequently, leaves of WRKY45p::GUS transgenic plants were inoculated with *B. cinerea*, and GUS staining was examined three days post-inoculation. A significantly stronger GUS signal was observed at the inoculation sites compared to the uninoculated regions (Figure 1B), further confirming the induction of WRKY45 expression by *B. cinerea*. Collectively, these results demonstrate that WRKY45 is a pathogen-inducible transcription factor, potentially modulating defense responses to *B. cinerea*.

3.2 WRKY45 is a negative regulator of *Arabidopsis* responses to *B. cinerea*

To investigate the role of WRKY45 in *Arabidopsis* resistance to *B. cinerea*, we assessed the disease responses of wild-type, *wrky45* knockout mutants, *WRKY45-RNAi* lines, and *WRKY45* overexpression plants following *B. cinerea* inoculation. All lines exhibited comparable growth under normal conditions, with no discernible phenotypic differences. At 5 days post-inoculation,

wrky45 and RNAi plants exhibited significantly enhanced resistance compared with wild-type, whereas 35S::WRKY45 plants displayed pronounced susceptibility (Figure 3A). To quantify pathogen biomass, we examined the accumulation of *B. cinerea* β -tubulin transcripts at 0 and 5 dpi. At 0 dpi, β -tubulin levels were nearly undetectable across all genotypes. However, at 5 dpi, β -tubulin transcript abundance was markedly reduced in the *wrky45* and RNAi lines but substantially increased in the *WRKY45* overexpression plants (Figure 3B). These results consistently suggest that WRKY45 functions as a negative regulator of *Arabidopsis* resistance to *B. cinerea*.

To further evaluate the physiological consequences of WRKY45 activity, we measured membrane integrity, oxidative stress, and chlorophyll retention. The *wrky45* and RNAi plants exhibited significantly lower electrolyte leakage and malondialdehyde levels compared with WT, indicating reduced cellular damage, whereas 35S::WRKY45 lines showed exacerbated membrane injury and oxidative stress (Figures 3C, D). Moreover, chlorophyll content was better preserved in *wrky45* and RNAi plants but was strongly depleted in the *WRKY45* overexpression lines, correlating with their increased disease severity (Figure 3E). Collectively, these findings

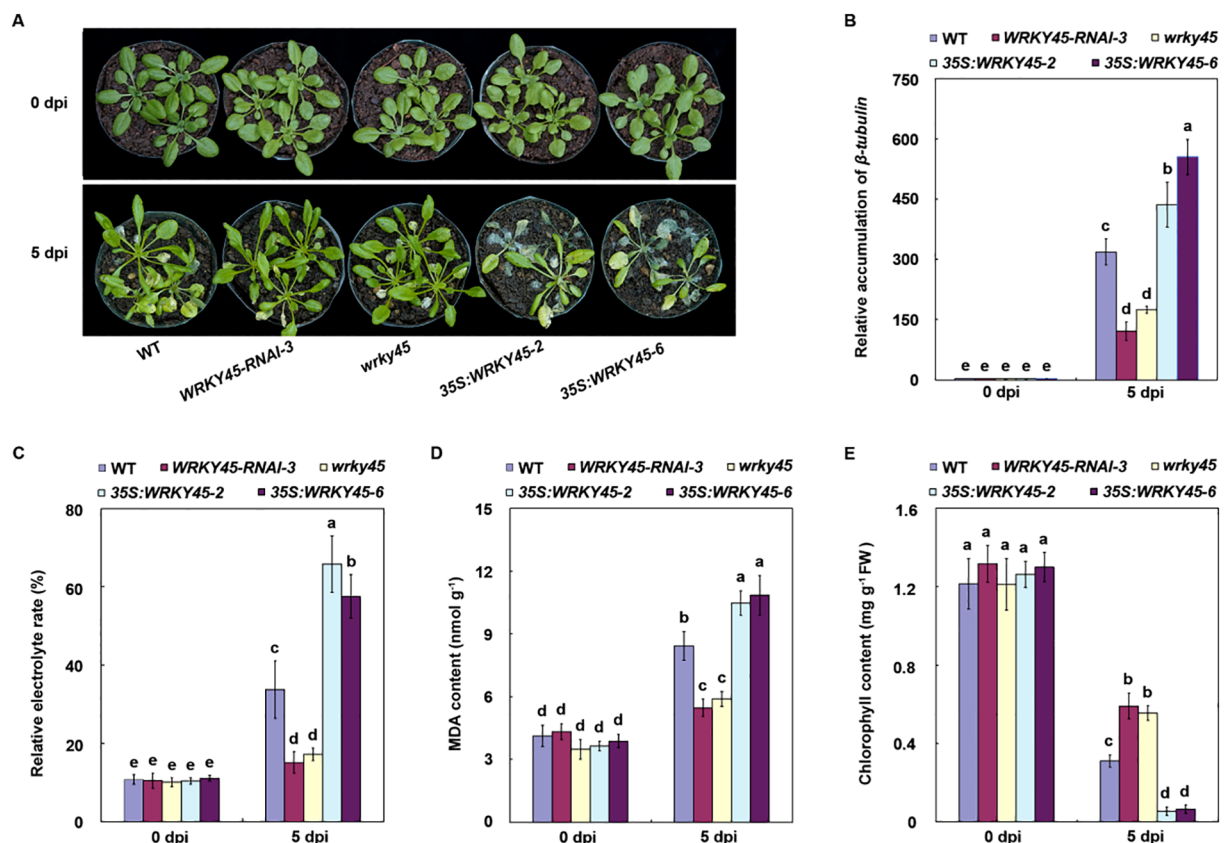


FIGURE 3

WRKY45 negatively regulates *Arabidopsis* resistance to *B. cinerea*. (A) Disease phenotypes of wild-type plants, *wrky45* mutants, *WRKY45*–RNAi lines, and *WRKY45* overexpression plants at 0 and 5 days post-inoculation (dpi) with *B. cinerea*. (B) Fungal biomass in infected leaves quantified by β -tubulin transcripts using qRT-PCR, with *ACTIN2* as the internal reference gene for normalization. (C–E) Electrolyte leakage, malondialdehyde content, and chlorophyll content at 0 and 5 dpi. Data are presented as the mean \pm SD ($n = 3$ independent biological replicates). Statistical significance was determined using two-way ANOVA followed by Tukey's test. Different letters above the bars indicate significant differences ($P < 0.05$).

demonstrate that WRKY45 functions as a negative regulator of *Arabidopsis* resistance to *B. cinerea*.

3.3 RNA-seq analysis to identify the potential WRKY45-involved pathway to control defense response

To elucidate the transcriptional basis of WRKY45-mediated defense, we performed RNA-seq analysis on wild-type and *wrky45* plants, with samples collected before and after inoculation with *B. cinerea*. A Venn diagram analysis revealed 15,408 differentially expressed genes (DEGs) in WT versus WT-*B. cinerea*, while 1,850 DEGs were identified in *wrky45* versus WT under infection conditions, with 1,692 genes shared between the two comparisons (Figure 4A). Among the 1,850 DEGs detected in the *wrky45* mutant, 857 were up-regulated and 993 were down-regulated (Figure 4B). Hierarchical clustering of representative defense-related genes showed that loss of *WRKY45* significantly enhanced the expression of several JA/ET marker genes, including *PDF1.2*, *PDF1.2b*, *PDF1.2c*, and *ORA59*, as well as stress-associated

transcription factors such as *WRKY33*, *MYB77*, and multiple *ERF* genes (e.g., *ERF1*, *ERF4*, *ERF5*, *ERF6*) (Figure 4C). Notably, these genes are well-established regulators of immunity against necrotrophic pathogens, indicating that WRKY45 negatively modulates JA/ET-mediated defense transcriptional networks. Gene Ontology (GO) enrichment analysis further revealed that DEGs between WT and *wrky45* plants upon infection were predominantly enriched in biological processes associated with immunity, such as “response to stimulus,” “immune system process,” “detoxification,” and “regulation of biological process” (Figure 4D). Collectively, these results demonstrate that WRKY45 deficiency reprograms transcriptional responses toward enhanced activation of defense-related pathways, thereby strengthening resistance to *B. cinerea*.

3.4 WRKY45 represses the expression of JA/ET-dependent defense genes

To validate the transcriptional regulation of defense genes revealed by the RNA-seq analysis, we examined the expression

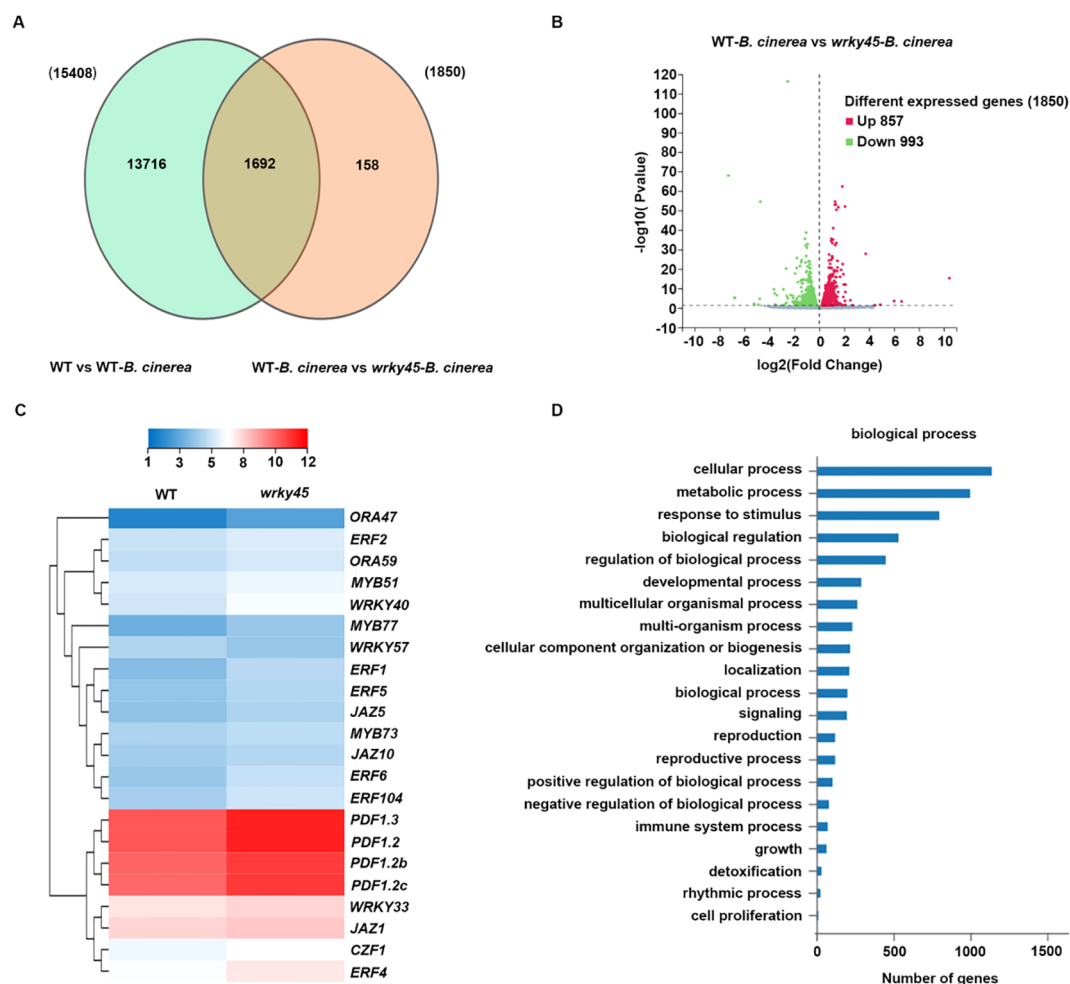


FIGURE 4

Transcriptomic analysis reveals *WRKY45*-dependent regulation of defense-associated genes in response to *B. cinerea*. **(A)** Venn diagram showing the overlap of genes co-regulated by *B. cinerea* and *WRKY45* deficiency. The overlapping region represents genes co-regulated in the comparisons WT vs. WT-*B. cinerea* and WT-*B. cinerea* vs. *wrky45*-*B. cinerea*. **(B)** Volcano plot displaying differentially expressed genes (DEGs) between WT-*B. cinerea* vs. *wrky45*-*B. cinerea*. DEGs were defined using thresholds $|\log_2$ fold change| ≥ 1 and adjusted P value (P_{adj}) < 0.05 . The X-axis represents the fold change of the difference after conversion to \log_2 , and the Y-axis represents the significance value after conversion to $-\log_{10}$. Red and green dots indicate up-regulated and down-regulated DEGs, respectively. **(C)** Hierarchical clustering of representative JA/ET-responsive, defense-related genes in the *wrky45* mutant following *B. cinerea* infection. **(D)** Gene Ontology (GO) enrichment analysis of 1,850 DEGs in the *wrky45* mutant after *B. cinerea* treatment, highlighting significant enrichment in biological processes related to plant immunity.

levels of multiple JA/ET-dependent genes in different plant lines following *B. cinerea* infection. qRT-PCR analysis showed that the expression levels of *ORA59*, *PDF1.2*, *ERF104*, and *ERF1* in the *wrky45* mutant and *WRKY45*-RNAi plants were not significantly different from those in the wild type at 0 dpi, but were markedly upregulated at 5 dpi relative to the wild type (Figures 5A–D). By contrast, their transcript levels were significantly repressed in *WRKY45* overexpression lines, consistent with their enhanced susceptibility (Figures 5A–D). These findings, together with the RNA-seq results, demonstrate that *WRKY45* functions as a negative regulator of JA/ET-mediated defense by repressing key downstream immune genes, thereby attenuating the host immune response against *B. cinerea*.

3.5 *WRKY45* directly binds to the *ORA59* promoter and represses its transcriptional activity

To investigate whether *WRKY45* directly regulates *ORA59*, we analyzed the *ORA59* promoter and identified several putative W-box elements (W1–W6) (Figures 2A, B). Electrophoretic mobility shift assays showed that recombinant GST-*WRKY45* protein strongly bound to probes containing the W2, W3, and W6 elements, whereas while no binding signal was detected with the mutated W1 probe (Figure 2C). Competitive binding assays further demonstrated that an excess of unlabeled probes effectively blocked binding, confirming the specificity of these interactions (Figure 2C).

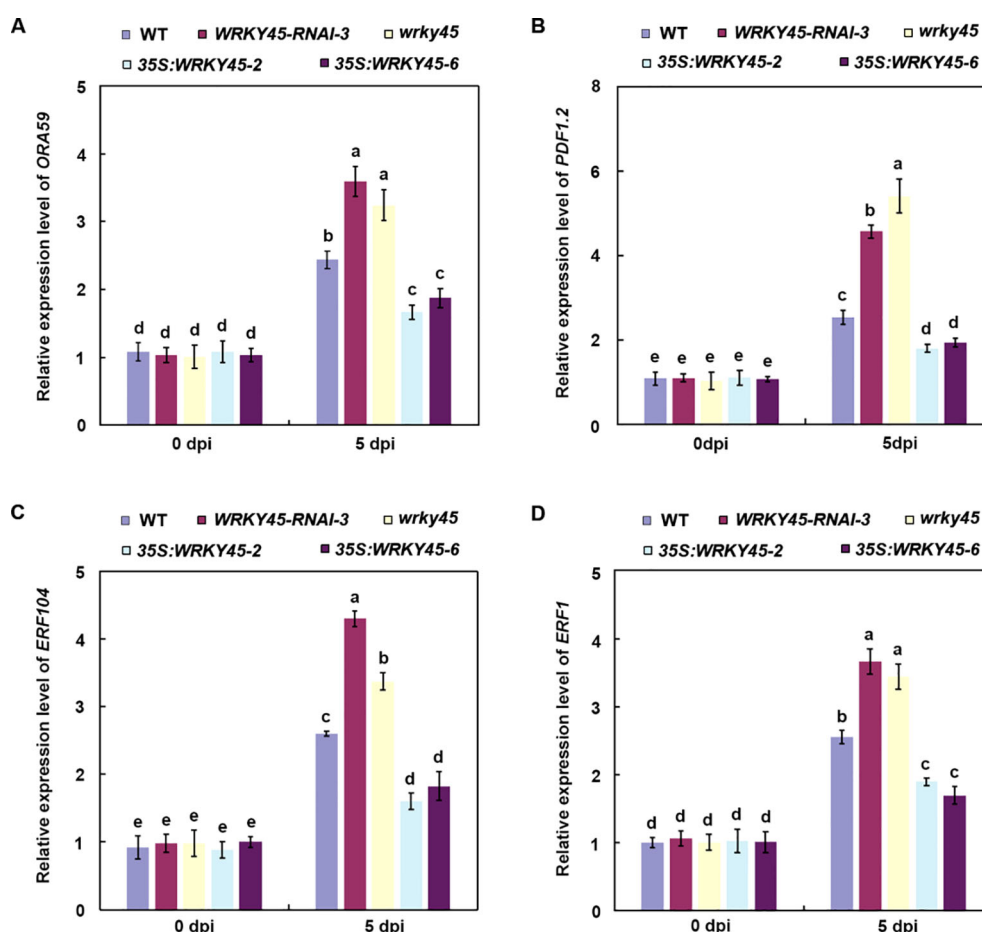


FIGURE 5

WRKY45 represses the expression of JA/ET-dependent defense genes. (A–D) Relative expression levels of *ORA59* (A), *PDF1.2* (B), *ERF104* (C), and *ERF1* (D) were quantified by qRT-PCR in wild-type, *wrky45* knockout mutants, *WRKY45-RNAi* lines, and *WRKY45* overexpression plants at 0 and 5 days post-inoculation (dpi) with *B. cinerea*, using *ACTIN2* as the internal reference gene. Data are presented as the mean \pm SD ($n = 3$ independent biological replicates). Statistical significance was determined using two-way ANOVA followed by Tukey's test. Different letters above the bars indicate significant differences ($P < 0.05$).

To assess the functional relevance of this binding, transient dual-luciferase assays were performed in *Nicotiana benthamiana* leaves. Co-expression of WRKY45 with the *ORA59* promoter-driven LUC reporter significantly reduced luminescence compared to the control, indicating that WRKY45 suppresses *ORA59* transcriptional activity (Figures 2D, E). Additionally, transient assays using the promoters of *PDF1.2*, *ERF104*, and *ERF1* revealed that WRKY45 consistently repressed their transcriptional activity (Supplementary Figure 2). Taken together, these findings demonstrate that WRKY45 functions as a transcriptional repressor by directly binding to the *ORA59* promoter and suppressing JA/ET-dependent immune genes, thereby attenuating *Arabidopsis* resistance to *B. cinerea*.

4 Discussion

Gray mold, caused by the fungal pathogen *B. cinerea*, poses a significant threat to a wide range of crop species. WRKY transcription factors are crucial regulators of plant growth,

development, and stress adaptation. In *Arabidopsis*, the functional characterization of individual WRKY members under both biotic and abiotic stresses has been key to uncovering the transcriptional networks involved in plant immunity. Here, we provide mechanistic insights into how WRKY45 modulates resistance to *B. cinerea* using *Arabidopsis* as a model.

Our phenotypic and physiological analyses show that *WRKY45* loss-of-function plants exhibit enhanced resistance to *B. cinerea*, while overexpression lines display increased susceptibility. These findings align with previous reports that several WRKY proteins act as immune repressors. For example, WRKY7, WRKY11, and WRKY17 suppress basal defense by downregulating JA and SA associated signaling (Fuenzalida-Valdivia et al., 2024). Notably, the phenotypes of *WRKY45* mutants are similar to those of the *wrky70 wrky54* double mutant, which exhibits enhanced resistance to necrotrophs, including *B. cinerea*, and shows strong induction of JA/ET- and SA-responsive genes (Li et al., 2017). These similarities support the conclusion that WRKY45 is part of a broader group of WRKY repressors that maintain immune homeostasis. However, unlike these previously described WRKY proteins, WRKY45

directly targets *ORA59*, a central integrator of JA/ET signaling, suggesting a more specialized role at a critical signaling node.

Transcriptomic profiling further revealed that *WRKY45* deficiency leads to the strong activation of hallmark JA/ET-responsive genes, including *ORA59*, *PDF1.2*, *ERF104*, and *ERF1*. This transcriptional profile contrasts with that of *wrky33* mutants, where *WRKY33* primarily regulates camalexin biosynthesis and ROS homeostasis (Birkenbihl et al., 2012; Liu et al., 2015). In contrast, this pattern more closely resembles the *WRKY57*-mediated repression of *B. cinerea* resistance described by Jiang and Yu, which acts through the JA signaling pathway (Jiang and Yu, 2016). These distinctions underscore the functional diversification within the *WRKY* family, where distinct members either activate or repress defense pathways depending on physiological and environmental contexts.

Mechanistically, we show that *WRKY45* directly binds multiple W-box motifs in the *ORA59* promoter and suppresses its transcriptional activity. Since *ORA59* integrates JA and ET signals, its repression by *WRKY45* provides a highly efficient mechanism for modulating downstream immune activation. This direct suppression at the promoter level distinguishes *WRKY45* from *WRKY11* and *WRKY17*, which modulate defense indirectly through JA signaling cascades (Journot-Catalino et al., 2006). Moreover, *WRKY45*'s ability to repress the promoters of *PDF1.2*, *ERF104*, and *ERF1* suggests that it may coordinate a broader transcriptional repression module that limits JA/ET-mediated immunity.

Despite these findings, several important questions remain. Whether *WRKY45* is involved in the hormonal interplay between JA, SA, and ET is still unclear. Additionally, *B. cinerea* infection is characterized by dynamic shifts from biotrophic-like to necrotrophic growth (Reboledo et al., 2020). High-resolution temporal transcriptomics, chromatin accessibility profiling, or single-cell RNA-seq could provide further insights into how *WRKY45* activity changes during these distinct infection phases.

In conclusion, our results establish *WRKY45* as a transcriptional repressor that attenuates JA/ET-mediated defense against *B. cinerea*. By directly suppressing *ORA59* and downstream immune genes, *WRKY45* fine-tunes host defense responses. These findings enhance our understanding of *WRKY*-mediated transcriptional repression and open new avenues for translational research to enhance disease resistance in crops.

5 Conclusion

In this study, we identified *WRKY45* as a transcriptional repressor that negatively regulates *Arabidopsis* defense against *B. cinerea* by suppressing JA/ET-mediated immune signaling. Loss of *WRKY45* function enhanced resistance, reduced oxidative damage, and better preserved cellular integrity under pathogen challenge, whereas overexpression of *WRKY45* increased susceptibility to *B. cinerea*. Mechanistically, *WRKY45* directly binds to W-box motifs in the *ORA59* promoter inhibits its transcriptional activation, thereby attenuating the expression of downstream defense genes

such as *PDF1.2*, *ERF104*, and *ERF1*. These findings not only enhance our understanding of the complex transcriptional networks governing plant immunity but also highlight the dual, context-dependent roles of *WRKY* transcription factors in fine-tuning stress responses. Given its conserved regulatory motifs, *WRKY45* may serve as a promising target for molecular breeding and genome editing strategies aimed at enhancing crop tolerance to necrotrophic fungal pathogens.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1390436>.

Author contributions

HR: Methodology, Formal Analysis, Software, Data curation, Writing – original draft, Conceptualization, Writing – review & editing, Validation. ZY: Writing – review & editing, Software, Validation. ZC: Validation, Software, Writing – review & editing. YM: Writing – review & editing, Software. SB: Writing – review & editing, Software. YC: Writing – review & editing, Methodology, Funding acquisition. MC: Funding acquisition, Methodology, Writing – review & editing. HZ: Funding acquisition, Writing – original draft, Formal Analysis, Methodology, Supervision, Writing – review & editing. WG: Validation, Supervision, Methodology, Writing – review & editing, Funding acquisition.

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Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

- AbuQamar, S., Moustafa, K., and Tran, L. S. (2017). Mechanisms and strategies of plant defense against *Botrytis cinerea*. *Crit. Rev. Biotechnol.* 37, 262–274. doi: 10.1080/07388551.2016.1271767
- Bajji, M., Kinet, J. M., and Lutts, S. (2002). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regul.* 36, 61–70. doi: 10.1023/A:1014732714549
- Barros, J. A. S., Cavalcanti, J. H. F., Pimentel, K. G., Medeiros, D. B., Silva, J. C. F., Condori-Apfata, J. A., et al. (2022). The significance of WRKY45 transcription factor in metabolic adjustments during dark-induced leaf senescence. *Plant Cell Environ.* 45, 2682–2695. doi: 10.1111/pce.14393
- Bi, K., Scalschi, L., Jaiswal, N., Mengiste, T., Fried, R., Sanz, A. B., et al. (2021). The *Botrytis cinerea* Crh1 transglycosylase is a cytoplasmic effector triggering plant cell death and defense response. *Nat. Commun.* 12, 2166. doi: 10.1038/s41467-021-22436-1
- Birkenbihl, R. P., Diezel, C., and Somssich, I. E. (2012). Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. *Plant Physiol.* 159, 266–285. doi: 10.1104/pp.111.192641
- Brunello, L., Kunkowska, A. B., Olmi, E., Triozzi, P. M., Castellana, S., Perata, P., et al. (2024). The transcription factor ORA59 represses hypoxia responses during *Botrytis cinerea* infection and reoxygenation. *Plant Physiol.* 197, kiae677. doi: 10.1093/plphys/kiae677
- Chen, L., Song, Y., Li, S., Zhang, L., Zou, C., and Yu, D. (2012). The role of WRKY transcription factors in plant abiotic stresses. *Biochim. Biophys. Acta (BBA)-Gene Regul. Mech.* 1819, 120–128. doi: 10.1016/j.bbagr.2011.09.002
- Chen, L., Xiang, S., Chen, Y., Li, D., and Yu, D. (2017). Arabidopsis WRKY45 interacts with the DELLA protein RGL1 to positively regulate age-triggered leaf senescence. *Mol. Plant* 10, 1174–1189. doi: 10.1016/j.molp.2017.07.008
- Chen, L., Zhang, L., Li, D., Wang, F., and Yu, D. (2013). WRKY8 transcription factor functions in the TMV-cg defense response by mediating both abscisic acid and ethylene signaling in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* 110, E1963–E1971. doi: 10.1073/pnas.1221347110
- Chen, L., Zhang, L., Xiang, S., Chen, Y., Zhang, H., and Yu, D. (2021). The transcription factor WRKY75 positively regulates jasmonate-mediated plant defense to necrotrophic fungal pathogens. *J. Exp. Bot.* 72, 1473–1489. doi: 10.1093/jxb/eraa529
- Cheng, Q., Dong, L., Gao, T., Liu, T., Li, N., Wang, L., et al. (2018). The bHLH transcription factor GmPIB1 facilitates resistance to *Phytophthora sojae* in Glycine max. *J. Exp. Bot.* 69, 2527–2541. doi: 10.1093/jxb/ery103
- Chen, X., Zhang, T., Wang, H., Zhao, W., and Guo, Z. (2025). Transcription factor WRKY complexes in plant signaling pathways. *Phytopathol. Res.* 7, 1–21. doi: 10.1186/s42483-025-00349-x
- Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* 13, 414–430. doi: 10.1111/j.1364-3703.2011.00783.x
- Du, P., Wang, Q., Yuan, D. Y., Chen, S. S., Su, Y. N., Li, L., et al. (2023). WRKY transcription factors and OBERON histone-binding proteins form complexes to balance plant growth and stress tolerance. *EMBO J.* 42, e113639. doi: 10.15252/emboj.2023113639
- Fuenzalida-Valdivia, I., Herrera-Vásquez, A., Gangas, M. V., Sáez-Vásquez, J., Álvarez, J. M., Meneses, C., et al. (2024). The Negative Regulators of the Basal Defence WRKY7, WRKY11 and WRKY17 modulate the jasmonic acid pathway and an alternative splicing regulatory network in response to *Pseudomonas syringae* in Arabidopsis thaliana. *Mol. Plant Pathol.* 25, 70044. doi: 10.1111/mpp.70044
- Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. doi: 10.1146/annurev.phyto.43.040204.135923
- Huang, Z., He, J., Xia, D., Zhong, X. J., Li, X., Sun, L., et al. (2016). Evaluation of physiological responses and tolerance to low-temperature stress of four Iceland poppy (*Papaver nudicaule*) varieties. *J. Plant Interact.* 11, 117–123. doi: 10.1080/17429145.2016.1217433
- Jiang, Y., Guo, L., Liu, R., Jiao, B., Zhao, X., Ling, Z., et al. (2016). Overexpression of poplar PtrWRKY89 in transgenic Arabidopsis leads to a reduction of disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. *PLoS One* 11, e0149137. doi: 10.1371/journal.pone.0149137
- Jiang, J., Ma, S., Ye, N., Jiang, M., Cao, J., and Zhang, J. (2017). WRKY transcription factors in plant responses to stresses. *J. Integr. Plant Biol.* 59, 86–101. doi: 10.1111/jipb.12513
- Jiang, Y., and Yu, D. (2016). The WRKY57 transcription factor affects the expression of jasmonate ZIM-domain genes transcriptionally to compromise *Botrytis cinerea* resistance. *Plant Physiol.* 171, 2771–2782. doi: 10.1104/pp.16.00747
- Journot-Catalino, N., Somssich, I. E., Roby, D., and Kroj, T. (2006). The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana. *Plant Cell* 18, 3289–3302. doi: 10.1105/tpc.106.044149
- Ju, S., Go, Y. S., Choi, H. J., Park, J. M., and Suh, M. C. (2017). DEWAX transcription factor is involved in resistance to *Botrytis cinerea* in Arabidopsis thaliana and Camelina sativa. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01210
- Kang, M., Wu, H., Liu, H., Liu, W., Zhu, M., Han, Y., et al. (2023). The pan-genome and local adaptation of Arabidopsis thaliana. *Nat. Commun.* 14, 6259. doi: 10.1038/s41467-023-42029-4
- Li, F., Deng, Y., Liu, Y., Mai, C., Xu, Y., Wu, J., et al. (2023). Arabidopsis transcription factor WRKY45 confers cadmium tolerance via activating PCS1 and PCS2 expression. *J. Hazard Mat* 460, 132496. doi: 10.1016/j.jhazmat.2023.132496
- Li, J., Zhong, R., and Palva, E. T. (2017). WRKY70 and its homolog WRKY54 negatively modulate the cell wall-associated defenses to necrotrophic pathogens in Arabidopsis. *PLoS One* 12, e0183731. doi: 10.1371/journal.pone.0183731
- Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes[M]//Methods in enzymology. *Acad. Press* 148, 350–382. doi: 10.1016/0076-6879(87)48036-1
- 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. *eLife* 4, e07295. doi: 10.7554/eLife.07295
- Liu, Q., Li, X., Yan, S., Yu, T., Yang, J., Dong, J., et al. (2018). OsWRKY67 positively regulates blast and bacterial blight resistance by direct activation of PR genes in rice. *BMC Plant Biol.* 18, 257. doi: 10.1186/s12870-018-1479-y

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1724180/full#supplementary-material>

- Lorenzo, O., Piqueras, R., Sánchez-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. doi: 10.1105/tpc.007468
- Luo, D., Cai, J., Sun, W., Yang, Q., Hu, G., and Wang, T. (2024). Tomato SlWRKY3 negatively regulates Botrytis cinerea resistance via *TPK1b*. *Plants* 13, 1597. doi: 10.3390/plants13121597
- Penninckx, I. A., Eggermont, K., Terras, F. R., Thomma, B. P., De Samblanx, G. W., Buchala, A., et al. (1996). Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. *Plant Cell* 8, 2309–2323. doi: 10.1105/tpc.8.12.2309
- Pré, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C. M., and Memelink, J. (2008). The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. *Plant Physiol.* 147, 1347–1357. doi: 10.1104/pp.108.117523
- Rasool, K. G., Khan, M. A., Aldawood, A. S., Tufail, M., Mukhtar, M., and Takeda, M. (2015). Identification of proteins modulated in the date palm stem infested with red palm weevil (*Rhynchophorus ferrugineus* Oliv.) using two-dimensional differential gel electrophoresis and mass spectrometry. *Int. J. Mol. Sci.* 16, 19326–19346. doi: 10.3390/ijms160819326
- Reboledo, G., Agorio, A., Vignale, L., Batista-García, R. A., and Ponce De, León, I. (2020). Botrytis cinerea transcriptome during the infection process of the bryophyte Physcomitrium patens and angiosperms. *J. Fungi* 7, 11. doi: 10.3390/jof7010011
- Scholz, P., Chapman, K. D., Ischebeck, T., and Guzha, A. (2023). Quantification of Botrytis cinerea Growth in Arabidopsis thaliana. *Bio-protocol* 13, e4740. doi: 10.21769/BioProtoc.4740
- Song, H., Li, Y., Wang, Z., Duan, Z., Wang, Y., Yang, E., et al. (2022). Transcriptome profiling of Toona ciliata young stems in response to Hypsipyla robusta Moore. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.950945
- van Kan, J. A. (2006). Licensed to kill: the lifestyle of a necrotrophic plant pathogen. *Trends Plant Sci.* 11, 247–253. doi: 10.1016/j.tplants.2006.03.005
- Vatov, E., Ludewig, U., and Zentgraf, U. (2021). Disparate dynamics of gene body and cis-regulatory element evolution illustrated for the senescence-associated cysteine protease gene *SAG12* of plants. *Plants* 10, 1380. doi: 10.3390/plants10071380
- Wang, W., Cao, H., Wang, J., and Zhang, H. (2025). Recent advances in functional assays of WRKY transcription factors in plant immunity against pathogens. *Front. Plant Sci.* 15. doi: 10.3389/fpls.2024.1517595
- Wang, X., Guo, R., Tu, M., Wang, D., Guo, C., Wan, R., et al. (2017). Ectopic expression of the wild grape WRKY transcription factor VqWRKY52 in Arabidopsis enhances resistance to the biotrophic pathogen powdery mildew but not to the necrotrophic pathogen Botrytis cinerea. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.00097
- Wang, H., Xu, Q., Kong, Y. H., Chen, Y., Duan, J. Y., Wu, W. H., et al. (2014). Arabidopsis WRKY45 transcription factor activates *PHOSPHATE TRANSPORTER1;1* expression in response to phosphate starvation. *Plant Physiol.* 164, 2020–2029. doi: 10.1104/pp.113.235077
- Xiang, S., Wu, S., Zhang, H., Mou, M., Chen, Y., Li, D., et al. (2020). The PIFs redundantly control plant defense response against Botrytis cinerea in Arabidopsis. *Plants* 9, 1246. doi: 10.3390/plants9091246
- Zhang, H., Zhang, L., Ji, Y., Jing, Y., Li, L., Chen, Y., et al. (2022). Arabidopsis SIGMA FACTOR BINDING PROTEIN1 (SIB1) and SIB2 inhibit WRKY75 function in abscisic acid-mediated leaf senescence and seed germination. *J. Exp. Bot.* 73, 182–196. doi: 10.1093/jxb/erab391
- Zhou, X., Sun, Z., Huang, Y., He, D., Lu, L., Wei, M., et al. (2025). WRKY45 positively regulates salinity and osmotic stress responses in Arabidopsis. *Plant Physiol. Biochem.* 219, 109408. doi: 10.1016/j.plaphy.2024.109408
- Zhu, S., Fan, R., Xiong, X., Li, J., Xiang, L., Hong, Y., et al. (2022). *MeWRKY IIas*, subfamily genes of WRKY transcription factors from cassava, play an important role in disease resistance. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.890555