The Necrotroph Botrytis cinerea BcSpd1 Plays a Key Role in Modulating Both Fungal Pathogenic Factors and Plant Disease Development

Huchen Chen, Shengnan He, Shuhan Zhang, Runa A, Wenling Li and Shouan Liu*

Laboratory of Molecular Plant Pathology, Jilin University, Changchun, China

Botrytis cinerea is a necrotrophic microbe that causes gray mold disease in a broad range of hosts. In the present study, we conducted molecular microbiology and transcriptomic analyses of the host–B. cinerea interaction to investigate the plant defense response and fungal pathogenicity. Upon B. cinerea infection, plant defense responses changed from activation to repression; thus, the expression of many defense genes decreased in Arabidopsis thaliana. B. cinerea Zn(II)$_2$Cys$_6$ transcription factor BcSpd1 was involved in the suppression of plant defense as ΔBcSpd1 altered wild-type B05.10 virulence by recovering part of the defense responses at the early infection stage. BcSpd1 affected genes involved in the fungal sclerotium development, infection cushion formation, biosynthesis of melanin, and change in environmental pH values, which were reported to influence fungal virulence. Specifically, BcSpd1 bound to the promoter of the gene encoding quercetin dioxygenase (BcQdo) and positively affected the gene expression, which was involved in catalyzing antifungal flavonoid degradation. This study indicates BcSpd1 plays a key role in the necrotrophic microbe B. cinerea virulence toward plants by regulating pathogenicity-related compounds and thereby suppressing early plant defense.

Keywords: Botrytis cinerea, Zn(II)$_2$Cys$_6$ transcription factor, the necrotrophic microbe, pathogenicity, host defense

INTRODUCTION

Plant pathogens are classified as biotrophs, necrotrophs, or hemibiotrophs based on their relationship with host plants. Fungal pathogens that feed on living host tissues are known as biotrophs, those that kill and feed on dead host tissues are known as necrotrophs, while the hemibiotrophs exhibit a biphasic feeding strategy—an early biotrophic stage for colonizing and a late necrotrophic stage for feeding (Newman and Derbyshire, 2020). Necrotrophic fungi are reported to be far more economically damaging than biotrophs (Dean et al., 2012; Lorang, 2019; Newman and Derbyshire, 2020). Many necrotrophic fungi are host-specific and only inflict disease on a narrow range of plants, while others have a broad host range. Botrytis cinerea is a typical broad host-range necrotrophic fungal pathogen that can affect more than 1,400 plant species (Fillinger and Elad, 2016). The underlying molecular mechanisms facilitating broad host-range necrotrophy have not been well defined to date.
Molecular studies on *B. cinerea*, including whole-genome sequencing and analysis of *B. cinerea* isolates B05.10 and T4, indicated that the fungi navigate a delicate balance of suppressing and inducing several events, such as hormone-regulated defense, cell wall degradation enzymes, fungal effectors, detoxification of plant defense compounds, and activation of transcription regulators (Castillo et al., 2017; Zhang et al., 2018; Cheung et al., 2020; Newman and Derbyshire, 2020; John et al., 2021; Shao et al., 2021; Westrick et al., 2021). Each factor may have its own unique role, or several factors together, causing *B. cinerea* to successfully invade the host plant and to inflict disease (Fillinger and Elad, 2016). For example, transcription factors (TFs) are sequence-specific DNA-binding proteins required to modulate gene expression (Charoenasawan et al., 2010; Hughes, 2011; Keller, 2019). In addition, their direct inhibition is considered to regulate host defenses.

Plant immunity contains two interconnected levels based on the recognition of molecular patterns, microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs), termed as MAMP-triggered immunity (MTI), or DAMP-triggered immunity (DTI), and on the recognition of effectors from adapted pathogens with plant resistance genes (R-genes) termed as effector-triggered immunity (ETI) (Jones and Dangl, 2006; Campbell et al., 2008). *B. cinerea* Zn(II)$_2$Cys$_6$ is negatively regulated by BcBot6 positively regulates botrydial biosynthetic genes and is required for fungal virulence (Porquér et al., 2016, 2019). Since Zn(II)$_2$Cys$_6$ is a transcription regulator, it is interesting to determine if it is involved in the regulation of target gene expression in *B. cinerea* for modulating host defenses.

In this study, we identified that a pathogenesis-related gene, BcSpd1, encoding Zn(II)$_2$Cys$_6$ transcriptional regulator is involved in the regulation of fungal growth, development, and virulence in the necrotrophic *B. cinerea*. BcSpd1 is involved in plant--*B. cinerea* interaction and impairs host defense responses by targeting many virulence genes. We compared the transcripts change from B05.10- and ΔBcSpd1-infected plants to obtain an integrated understanding of *B. cinerea*’s promotion of disease development by suppressing defense-related gene expression. The results of this study will increase our understanding of the complexity of the plant--*B. cinerea* interaction and will enhance efforts to identify pathogenicity- or toxicity-related genes in necrotrophic microbes.

**MATERIALS AND METHODS**

**Plant and Fungi Material**

For the experiments, 4-week-old *A. thaliana* plants were used. The plants were grown under a microbe-free climate chamber with 10-h light and 14-h dark cycles. *B. cinerea* B05.10 and the indicated mutants were grown in the PDA plate to produce the conidia spores. The spores were harvested as previously reported (Liu et al., 2017).

**Incubation and Sample Collection**

For incubation, the leaves were spray-infected with $5 \times 10^5$ spores ml$^{-1}$. The buffer without any spore was sprayed as untreated control (CK). All leaves were harvested at 14 h and frozen at $-80^\circ$C for RNA sequencing. For qPCR assay, the plants were infected with *B. cinerea* B0510 and ΔBcSpd1 and collected at different time points. All the samples were repeated three times.

**Library Construction, RNA Sequencing, Mapping Fragments to the Genome, and Quantification of Gene Level**

Total RNA samples were prepared for Illumina sequencing. RNA isolation, purification, and monitoring, and cDNA library construction and sequencing were performed as described previously (Liu et al., 2015). All clean data with high-quality
reads were used for analyses. Reference genome and gene model annotation files were downloaded from the website. The index of the reference genome was built, and paired-end clean reads were aligned to the reference by using the HISAT package (Kim et al., 2015). Finally, the FPKM of each gene was calculated based on the length and read counts mapped to the gene (Trapnell et al., 2010).

### Analysis of Differentially Expressed Genes

Differential expression analysis of all samples (CK, Bc) was performed as described in a previous study (Liu et al., 2015). The differentially expressed genes (DEGs) were selected with log2 (fold change) > 1 or log2 (fold change) < −1 and with statistical significance (p-value < 0.05) by R package.

### Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichment Analysis of Differentially Expressed Genes

Gene Ontology (GO) enrichment analysis of DEGs was implemented by the GOseq R package, and GO terms with corrected p-values less than 0.05 were considered significantly enriched by DEGs (Kanehisa et al., 2008). Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource for understanding high-level information, based on large-scale molecular datasets generated by genome sequencing and other high throughput experimental technologies (Young et al., 2010).

### Real-Time Quantitative PCR

Real-time quantitative PCR was performed as previously described (He et al., 2020). BcActin was used for normalization. All analyses were repeated three times using biological replicates. Primer sequences are listed in Supplementary Table 1.

### Constructing the Botrytis cinerea BcSpd1 Deletions and Complementation Strains

To construct the BcSpd1 (suppression of plant defense 1, Bcin06g05230) gene replacement vectors, flanking sequences of the gene were PCR-amplified from the B05.10 genomic DNA and inserted into the PXEH vector, respectively (Feng et al., 2017). The final replacement vector was generated and then transformed with B05.10 spores using an Agrobacterium tumefaciens AGL-1 strain. Knockout resistant transformants were initially screened on a selective medium (PDA containing 50 µg mL−1 HygB) and then confirmed by PCR and qPCR with indicated primers (Supplementary Table 1). To investigate BcSpd1 complement lines, PCR fragments encoding the full-length open reading frames (ORFs) of genes were isolated and cloned in-frame into the modified pCAMBIA1303 (Yellisetty et al., 2015). Then, the vectors were transformed into ΔBcSpd1 spores to obtain the complement strains (ΔBcSpd1-C). The strain showing wild-type gene expression levels was used for further analysis.

### Characterization of Botrytis cinerea Mutants

The fungal growth of the tested B. cinerea strains was determined by measuring the radial diameter of colonies on solid CM (1.0% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, nitrate salts, trace elements, 0.01% vitamins, 1.2% agar, pH 6.5) or PDA. Other media used in the assays included liquid CM (CM without agar) and PDB (PDA without agar). Fungal growth, infection structure formation, sclerotia formation, melanin biosynthesis, and organic acid production were determined as follows.

For conidium germination assays, fresh conidia of the WT, ΔBcSpd1, and ΔBcSpd1-C strains were harvested from CM plates with ddH2O, and the conidial suspension was adjusted to the concentration of 1 × 10⁵ conidia mL⁻¹ in CM buffer. To examine melanin biosynthesis, the spores of each strain were inoculated on the CM liquid medium in a bottle for 4 days, and melanin production was examined for each bottle. The production of organic acid was determined by a pH indicator assay using CM agar with 0.1% bromophenol blue (CM, pH5.5) or 0.1% bromothymol blue (CM, pH7.5). The acidification of the medium caused by the fungus resulted in a color change. For pH value examination, the indicated fungal strains were incubated on CM liquid medium with the original pH of 6.0. For infection cushion formation assays, the conidial suspension droplets (1 × 10⁵ conidia mL⁻¹, 20 µL) or mycelial plugs were added to the liquid medium (CM) on the glass, and the inoculated fungi were incubated in a moistened chamber at 25°C. The formation of infection cushions was observed and photographically documented at the indicated time points post-incubation. For sclerotia formation assays, strains were cultivated on CM plates at 25°C in darkness; the production of sclerotia by the test strains was observed and photographed 30 days post-incubation.

The observation of conidial germination, infection structure formation, etc., was performed under a Nikon Eclipse 80i fluorescence microscope system. At least three independent experiments with triplicated replicates per experiment were performed.

### Expression Analysis of BcSpd1 in Escherichia coli BL21

cDNA of the BcSpd1 gene was PCR-amplified and cloned into the expression vector pET28a to produce the plasmid pET28a-BcSpd1 with His-tag. The recombinant vector was transformed to competent bacterial cells of E. coli BL21. The recombinant protein was induced and expressed in E. coli BL21 cells.

### Western Blotting Assay

The BcSpd1 protein with His-Tag was run through 12% SDS-PAGE and transferred to a PVDF Western blotting membrane (Roche Diagnostics GmbH) with a Bio-Rad electroblotting apparatus. The recombinant protein was

\[^{1}\text{https://www.arabidopsis.org/}\]
detected as following the instructions of a previous study (Liu et al., 2015).

**Electrophoretic Mobility Shift Assay**

For Electrophoretic Mobility Shift Assay (EMSA), about 50-bp upstream and downstream primers labeled with biotin were synthesized using the primers, as shown in Supplementary Table 1. EMSA detection was performed following the LightShift Chemiluminescent EMSA procedure (Thermo-Scientific) as described previously (Liu et al., 2015).

**Statistical Analysis**

The data were analyzed by an analysis of variance (ANOVA) using SPSS 18 software (IBM). The differences were considered significant at \( P < 0.05 \), \( **P < 0.01 \), and \( ***P < 0.001 \), respectively. All the data are represented as the mean ± SEM of at least three independent experiments.

**RESULTS**

**BcSpd1 Encodes the Zn(II)$_2$Cys$_6$ Transcription Regulator and Plays a Key Role in Fungal Pathogenicity**

Our previous study indicated that *B. cinerea* B05.10 is a virulent strain and suppresses WRKY33-mediated plant early defense (Liu et al., 2017). Our recent study revealed that *B. cinerea* B05.10 promotes disease development in the medicinal plant *Panax ginseng* by suppressing plant early defense signals and antifungal metabolite biosynthesis (Chen et al., 2022). To identify the genes involved in *B. cinerea*, we screened the transcripts during the plant–*Botrytis* interaction. A qPCR analysis indicated that a gene *Bcin06g05230* is highly expressed in *B. cinerea* at 14 h after interaction (Figure 1A). The expression of the gene decreased at 24 and 48 h. The earlier expression of this gene reveals that it might play a role in the host–*Botrytis* interaction.

Protein sequence analysis revealed several conserved domains (Supplementary Figure 1). The phylogenetic analysis indicated the protein had a higher similarity with the C6 transcription factors (Figures 1B,C). Previous research indicated the genes encoding Zn(II)$_2$Cys$_6$ transcription activator (Todd and Andrianopoulos, 1997). Since the gene encoding transcription activator positively affected downstream gene expression, we hypothesized that the gene and its targets might be involved in fungal virulence.

In order to determine whether *Bcin06g05230* is involved in *B. cinerea* pathogenicity, four mutants were obtained (Supplementary Figure 2). Genome PCR and mRNA qPCR amplification of these mutants showed no expected band and no expression of the gene (Supplementary Figures 2B,C). The gene was cloned, and the complement lines were then generated (Supplementary Figure 3). The virulence of the two mutant strains (ΔBcSpd1-5 and ΔBcSpd1-7) and one complement line (ΔBcSpd1-C) was tested on *A. thaliana*. Both the mutants showed small lesion sizes compared with the wild-type strains (Figure 1D). Visual inspection of infections in different plants such as tomato and bean also showed a general trend of decreased virulence for the mutants, in comparison with the wild-type (Supplementary Figure 4). Since the wild-type fungus suppressed plant defense and the gene mutants reduced fungal virulence, we named the gene *BcSpd1* (suppression of plant defense gene 1). The reintroduction of the *BcSpd1* gene into the mutant strains resulted in restoration of the virulence to the levels of the wild-type strain B0510, unequivocally assigning the decrease in virulence to the mutation of ΔBcSpd1 (Figure 1D). These results indicated that *BcSpd1* is involved in *B. cinerea* virulence.

**BcSpd1 Involved in *Botrytis cinerea* Sclerotia Development, Environmental pH Changes, Infection Structure Formation, and Melanin Biosynthesis**

*B. cinerea* ΔBcSpd1 mutants grew similar to the wild-type B05.10 and the complement strain ΔBcSpd1-C in CM medium. The germination rate of ΔBcSpd1 also has no significant difference compared to the wild-type and complemented strains. Because the sclerotia formation within dying host tissues represented an important survival mechanism of *B. cinerea*, we were interested in investigating the effects of *BcSpd1* gene deletion on sclerotia development. After 1 month of incubation in the dark, ΔBcSpd1 was unable to develop any sclerotia on the CM plate, while the wild-type and ΔBcSpd1-C produced numerous sclerotia, indicating that *BcSpd1* is essential for sclerotia formation in *B. cinerea* (Figure 2A).

The production of organic acid is thought to contribute to *B. cinerea* virulence by affecting its pH environment and thus involve in the production and activity of secreted enzymes. By performing a CM plate assay using the pH indicator bromophenol blue or bromothymol blue, we compared and examined the ability of the wild-type B05.10, the ΔBcSpd1 mutant, and the ΔBcSpd1-C complement mutant to acidify the agar medium by secreting organic acid. If no fungus was present on the CM plates, the color was blue when bromophenol blue was added, or the color was bluish violet when bromothymol blue was added. After the indicated fungal strains were incubated, ΔBcSpd1 mutants affected the organic acid forming as the pH changed in both the wild-type B0510 and complement line ΔBcSpd1-C (Figures 2B,C). We next tested the changes in pH values in the CM medium after incubation with B05.10 and ΔBcSpd1 mutant, respectively. The original pH of the CM medium was adjusted around pH 6.0 before incubation; then, 5 days after incubation at 25°C under shaking, the pH value decreased to 3.8 in B05.10-incubated CM medium, while the pH value decreased to 4.8 in the ΔBcSpd1-incubated medium (Figure 2D). After 10 days, the pH value was about 5.0 in the B05.10-incubated medium, while the pH value was around 7.0 in the ΔBcSpd1-incubated medium (Figure 2D). The pH value is higher in the ΔBcSpd1-incubated medium than in B05.10, suggesting that BcSpd1 is involved in pH changes during incubation. These results confirmed that BcSpd1 contributed to lower pH values in both plates and the medium.
FIGURE 1 | Identification and characterization of BcSpd1 in B. cinerea. (A) qRT-PCR analysis of BcSpd1 gene expression in B. cinerea during fungus infection of the ginseng plant at different timepoints. (B) ZnCys domain alignment of the BcSpd1 and the indicated species. (C) Phylogenetic tree of BcSpd1 proteins and the indicated proteins including Botrytis cinerea BcDW1 (EMR87589.1), Botrytis elliptica (TGO067507.1), Botrytis porri (TGO064372.1), Botrytis paeoniae (TGO25784.1), Botrytis galanthina (THV45182.1), Botrytis tulipae (TGO13679.1), Botrytis hyacinth (TGO37106.1), Botrytis caltha (TEY84028.1), Botryotinia convalta (TGO57206.1), Botryotinia paranoia (TGO57206.1), Botryotinia narcissicola (TGO84372.1), Botryotinia caltha (TEY84028.1), Botryotinia sp. NJR-2017a WRK4 (PQE06843.1). (D) ΔBcSpd1 shows reduced disease symptoms on Arabidopsis thaliana Col-0 leaves. Photographs were taken 3 days post-infection. Wild-type strain B05.10 presents large lesions compared with ΔBcSpd1-5- and ΔBcSpd1-7-infected leaves that show small lesions or no necrotic symptoms on leaves. The complement line ΔBcSpd1-C recovered from wild-type pathogenicity.
The infection structures, like infection cushions, play a critical role in *B. cinerea* host penetration and virulence (Feng et al., 2017; Cao et al., 2018; Liu et al., 2018). To evaluate the effect of *BcSpd1* on infection structure formation, we compared the infection cushion formation among B0510, Δ*BcSpd1*, and Δ*BcSpd1-C* strains. As demonstrated in Figure 3A, much less infection cushion was produced in Δ*BcSpd1* than in B0510 and Δ*BcSpd1-C* strains at 36, 48, and 72 h after the colony was incubated on hydrophobic glass. Similar results were observed in fungal spores incubated on the hydrophobic glass as observed at 22 hpi (Figure 3B). These data indicated that *BcSpd1* involved and positively regulated fungal infection cushion formation.

In addition, after growing on CM for 4 days, Δ*BcSpd1* mutants produced significantly more dark pigments than the wild-type B05.10 or the complemented strain (Figure 4A). It has been well accepted that melanin is primarily responsible for dark pigmentation in many filamentous fungi. In Δ*BcSpd1*, both the culture (Figure 4B, left) and the mycelium (Figure 4B, right) showed more dark pigments after growing in the CM medium. Furthermore, when 0.01% tricyclazole, a melanin biosynthesis inhibitor, was added to the CM medium, the production of melanin disappeared both in Δ*BcSpd1* mycelium and the incubation culture, which confirmed the overproduction of melanin by deletion of *BcSpd1* (Figure 4C).

**BcSpd1 Positively Regulated Genes in *Botrytis cinerea* Growth, Development, and Virulence**

Since *BcSpd1* is involved in *B. cinerea* sclerotia development, infection structure formation, pH value changes, melanin biosynthesis, and fungal virulence, we next aimed to identify how BcSpd1 was involved. Several genes were reported to affect *B. cinerea* sclerotia formation, including NOP53, PDE2, BMP1, LTF1, LTF2, FRQ, SAK1, and BcG3 (Zheng et al., 2000; Gronover et al., 2001; Doehlemann et al., 2006; Segmüller et al., 2007; Liu et al., 2011; Harren et al., 2013; Hevia et al., 2015; Cohrs et al., 2016; Cao et al., 2018; Cheung et al., 2020). Lost function of these genes delayed or was unable to form sclerotia, indicating that the genes were involved in or regulated sclerotia production. The expression of *BcPKS12* gene was observed at the sclerotia formation stage, suggesting the involvement of the gene (Zhang et al., 2015). Further study indicated that *BcPKS12* was exclusively required for the melanization of sclerotia that are specifically expressed during sclerotia development (Zhu et al., 2017). We performed qPCR to test their expression in Δ*BcSpd1* and B05.10. As indicated in the heatmap (Figure 5), the expression of *nop53, ltf1, pde2, bmp1*, and *Bcg3* was decreased in Δ*BcSpd1* compared with that in B05.10, indicating that *BcSpd1* positively affected their expression. The expression of *ltf2*, *frq*, and *sak1* was increased in Δ*BcSpd1*, indicating a negative role of these genes in sclerotium formation (Segmüller et al., 2007; Liu et al., 2011; Harren et al., 2013; Cohrs et al., 2016). NOP53 was also involved in infection cushion formation as the indicated structure formed late in NOP53 mutants compared with B05.10 (Cao et al., 2018). Oxaloacetate hydrolase (BcOAH1) was reported to synthesize oxalate, and Δ*Bcoah1* mutants did not produce

---

### Figure 2

Impact of BcSpd1 deletion on sclerotia development and organic acid biosynthesis. (A) Wild-type strain B05.10, Δ*BcSpd1*, and the complemented strain Δ*BcSpd1-C* were incubated on CM plates at 25°C for 4 weeks in darkness. Both B05.10 and Δ*BcSpd1-C* produce sclerotia on the plates, while Δ*BcSpd1* does not. (B) Indicated strains were incubated on CM plates with 0.1% bromophenol blue for 4 days. Both B05.10- and Δ*BcSpd1-C*-incubated plates show yellow color, while Δ*BcSpd1* shows blue. (C) The indicated strains were incubated on CM plates with 0.1% bromothymol blue for 4 days. Both B05.10- and Δ*BcSpd1-C*-incubated plates show weak blue color, while Δ*BcSpd1* show bluish violet. (D) pH changes in wild-type strain B05.10- and Δ*BcSpd1* mutant-incubated CM liquid mediums at different times (0 d, 5 d, or 10 d). The unincubated CM medium was used as control (CK). Asterisks indicate significant differences between treatments (**P < 0.001, two-tailed t-test).
oxalate in vitro (Han et al., 2007). As seen in Figure 5, the expression of Bcoah1 decreased in ΔBcSpd1, indicating that BcSpd1 positively regulated the expression of Bcoah1. Here, the downregulation of Bcoah1 in ΔBcSpd1 might partly lead to the increase in the pH value, as seen in Figure 2. All the data observed before revealed that BcSpd1 regulated genes in the formation of
sclerotia, the production of infection cushion, and the decrease in environmental pH value, which played a role in B05.10 growth, development, and virulence. However, BcSpd1 negatively affected the melanin biosynthesis as more dark pigments were observed in the ΔBcSpd1 mutant. Interestingly, the expression of brn1, scd1, cmr1, and bos1 was increased in ΔBcSpd1 mutant compared with B05.10, indicating that BcSpd1 negatively affected the gene expression. BRN1, SCD1, CMR1, and BOS1 were involved in fungal melanin biosynthesis (Liu et al., 2011; Yang et al., 2013; Cheung et al., 2020). The induction of these genes in ΔBcSpd1 mutants might contribute to the accumulation of melanin, as seen in Figure 4. In addition, BcSpd1 positively affected the expression

**FIGURE 4** | Impact of BcSpd1 deletion on melanin production. (A) Comparisons of mycelial pigmentation among the wild-type strain B05.10, ΔBcSpd1, and the complemented strain ΔBcSpd1-C after 4 days of incubation on CM liquid medium. (B) Mycelia (right) and the CM buffer (left) from (A) were photographed, respectively. (C) CM liquid medium with 0.01% tricyclazole inhibited the production of melanin both in the ΔBcSpd1 mycelium and in the incubation culture.
of thr1, chk1, and pks1, which were also involved in melanin biosynthesis (Yang et al., 2013). In this condition, BcSpd1 might play a complex role in regulating B. cinerea melanin biosynthesis.

**BcSpd1 Involved in Regulating Genes Associated With Plant Antifungal Flavonoid Degradation**

Our recent study indicated that flavonoids such as quercetin, kaempferol, and luteolin play a role in plant defense against B. cinerea, but the biosynthesis of such compounds was suppressed in ginseng plants upon fungal early infection (Chen et al., 2022). A gene encoding quercetin dioxygenase was reported to degrade flavonoids, and the ΔBcQdo mutants showed less virulence and partly lost their function in degrading certain flavonoids (Chen et al., 2019, 2022). Next, we aimed to investigate how BcQdo was regulated by B. cinerea. Interestingly, the expression of BcQdo was decreased in the ΔBcSpd1 mutant compared with B05.10 (Figure 5), indicating that BcSpd1 is involved in the regulation of BcQdo expression. We further identified several conserved DNA motifs in the promoter of BcQdo, which could be recognized by Zn(II)$_2$Cys$_6$ transcription factors (Figure 6A). Next, we performed an EMSA experiment to examine if BcSpd1 can bind to these sequences. The BcSpd1 gene was cloned into the pET28a expression vector, and the protein was overexpressed in *Escherichia coli* BL21 (Figure 6B). The recombinant BcSpd1 was used to bind the DNA fragment in vitro. Totally, four biotin-labeled fragments from the promoter of BcQdo were designed and synthesized (Figure 6A). As seen in Figure 6C, BcSpd1 clearly interacted with fragment 3 in the promoter sequence of BcQdo genes by EMSA. Fragment 3 contains the conserved domain CCGGN$_2$CCG, which is typical binding sites for Zn(II)$_2$Cys$_6$ proteins (Todd and Andrianopoulos, 1997). The excess of unlabeled DNA fragments blocked its interaction with BcSpd1, underlining the specificity of the protein/DNA interaction. Since BcSpd1 belongs to the C6 transcription factors, the highly expressed BcSpd1 mutant in B05.10 at the early infection stage might target and positively regulate BcQdo expression, thereby reducing the antifungal flavonoid concentration.

**BcSpd1 Involved in *Botrytis cinerea* Suppression of *Arabidopsis thaliana* Defense-Related Genes**

Since *B. cinerea* B05.10 is virulent toward *A. thaliana* and the Col-0 and ΔBcSpd1 mutants altered the wild-type fungal virulence, we next aimed to determine how B05.10 affects plant defenses. We performed RNA sequencing to test *A. thaliana* transcript changes. *B. cinerea* B05.10 spray-inoculated leaves (14 h post-infection, Col-0 B0510), *B. cinerea* ΔBcSpd1 spray-inoculated leaves (14 h post-infection, Col-0 ΔBcSpd1), and mock-treated leaves (control, Col-0 CK) were used. The raw sequence data were submitted to the NCBI (GSE186842). The reads were aligned with the *A. thaliana* genome.

To identify genes involved in *A. thaliana* response to *B. cinerea* on a genome-wide level, we compared statistically significantly differentially changed genes (altered two-fold or more, $P \leq 0.05$, SSTF) between *B. cinerea* B05.10-treated, *B. cinerea* ΔBcSpd1-treated, and un-treated (CK) Col-0 plants (Figure 7A). About 3,940 SSTF genes were identified in ΔBcSpd1-treated plants, while around 3,840 SSTF genes were observed in B05.10-treated plants when compared with untreated plants, respectively. The significantly differentially expressed genes between ΔBcSpd1- and B05.10-treated plants were about 340. When compared to CK, 2992 SSTF genes were both observed in ΔBcSpd1- and B05.10-treated plants, while 949 SSTF genes were only observed in ΔBcSpd1-infected plants and 848 SSTF genes were only observed in B05.10-treated plants (Figure 7B and Supplementary File 1).

We next analyzed the significantly differentially expressed genes by using GO and KEGG methods, respectively. As shown in Supplementary Figure 5, GO analysis of the 2992 SSTF genes observed both in ΔBcSpd1- and B05.10-treated plants indicated the enrichment of response to hormones (SA, JA, ABA), response to chitin, response to pathogens (fungus, bacterium, oomycetes), response to wounding, response to heat, defense response, defense response to pathogens (fungus, bacterium, oomycetes), etc. (Supplementary Figure 5A and Supplementary File 2). KEGG analysis of 2992 SSTF genes indicated the enrichment of plant–pathogen interaction, plant hormone signal transduction, plant MAPK signaling pathway, linoleic acid metabolism, fatty acid degradation, alpha-linolenic acid metabolism, etc. (Supplementary Figure 5B). When analyzing the SSTF genes only in ΔBcSpd1-infected plants or B05.10-treated plants by using the GO method, we observed the genes in DNA replication, DNA binding, mRNA binding, tRNA processing, ribosome, etc. were enriched in ΔBcSpd1-infected plants (Supplementary Figure 5C); however, all the genes mentioned before were not significantly enriched in B05.10-treated plants (Supplementary Figure 5D), suggesting that these genes played a role in plant defense. GO analysis of SSTF genes only in B05.10-treated plants was associated with microtubules and chloroplasts, response to ROS, response to hydrogen peroxide, etc. (Supplementary Figure 5D). These data indicated different genes were enriched in different treatments. When analyzing these SSTF genes by using the KEGG method, plant hormone signal transduction, isoflavonoid biosynthesis, and flavone biosynthesis were enriched in B05.10-treated plants (Figure 7D) compared with ΔBcSpd1-infected plants (Figure 7C).

We further analyzed the heatmap of differentially regulated genes isolated by using the KEGG method. As indicated in Figure 7E, the expression of the genes in plant hormone signal transduction was decreased in B05.10-infected plants compared with CK, while the expression of the same genes increased in ΔBcSpd1-infected plants compared with that in B05.10-treated plants (Figure 7E and Supplementary File 3). It indicated plant defense-related genes were downregulated by B05.10, and these genes were partly upregulated by ΔBcSpd1. The genes in the flavone and isoflavonoid biosynthesis pathway also changed their expression in B05.10- and ΔBcSpd1-treated plants (Figure 7F and Supplementary File 4), indicating the biosynthesis of the compounds were affected. Since transcription factors were reported to involve in plant defense, either positively or negatively, we next compared the differentially expressed genes...
encoding transcription factors. As shown in Supplementary Figure 6 and Supplementary File 5, the transcription factors altered their expression in ΔBcSpd1-treated plants compared with B05.10-treated plants. The expression of certain TFs is higher in ΔBcSpd1-treated plants than in B05.10-treated plants (red frame labeled), suggesting their potential role in ΔBcSpd1-mediated defense. Similar results were observed in gene response to ABA, a plant hormone involved in both biotic stress and abiotic stress (Supplementary Figure 7 and Supplementary File 6). The expression of certain genes was higher in B05.10-treated plants than in ΔBcSpd1-treated plants, suggesting these genes may involve in fungal virulence. Thus, BcSpd1 was involved in B. cinerea suppression of host defense responses.

DISCUSSION

The broad host range necrotrophy B. cinerea feed on many hosts even without any relationships. The molecular bases of broad host range necrotrophy in plant pathogens are not well defined and form an ongoing area of research (Newman and Derbyshire, 2020). Specific recognition of B. cinerea by the gene-for-gene
BcSpd1 competes the binding.

binding shift; line 4, the unlabeled probes were added to the reaction and recombinant BcSpd1 protein and biotin-labeled probes, which showed reaction with only biotin-labeled probes; line 3, the reaction with both BcQdo

EMSA analysis of BcSpd1 binding to biotin-labeled promoter fragments of Blot analysis of recombinant BcSpd1 in Escherichia coli transcription factor-binding sites were framed.

(A) BcQdo

Conserved DNA fragments in the promoter of BcSpd1 regulates virulence.

FIGURE 6 | BcSpd1 regulates BcQdo-mediated B. cinerea virulence. (A) Conserved DNA fragments in the promoter of BcQdo. The predicted transcription factor-binding sites were framed. (B) SDS-PAGE and Western blot analysis of recombinant BcSpd1 in Escherichia coli. Left: The proteins were stained with Coomassie brilliant blue R-250. M, Positions of marker proteins. CK, SDS-PAGE of the extracts from empty pET28a-transformed bacterial cultures. BcSpd1, SDS-PAGE of the recombinant BcSpd1 extracted from pET28a-BcSpd1-transformed bacterial culture. Right: Western blot analysis of BcSpd1 protein in the indicated bacterial cultures (anti-His). (C) EMSA analysis of BcSpd1 binding to biotin-labeled promoter fragments of BcQdo. Line 1, the reaction only with recombinant BcSpd1 protein; line 2, the reaction with only biotin-labeled probes; line 3, the reaction with both recombinant BcSpd1 protein and biotin-labeled probes, which showed binding shift; line 4, the unlabeled probes were added to the reaction and competed the binding.

To protect against the disease caused by B. cinerea, we must understand the mechanisms by which the pathogen causes disease. Virulence factors and pathogenicity genes have been identified for the availability of fungal genomes, but in many cases, their roles remain elusive. It is becoming increasingly clear that gene regulation is vital to enable plant infection and TFs play an essential role. The significance of TFs as regulatory elements in plant-pathogenic fungi has been functionally characterized (John et al., 2021). The TFs are involved in controlling various aspects of fungal development, stress tolerance, and the biosynthesis of virulence factors such as effectors and secondary metabolites. There are a significant number of Zn(II)$_2$Cys6 TF encoding genes, whose activation or functional products have not been resolved (Deepika et al., 2016; Keller, 2019; Romsdahl and Wang, 2019; Graham-Taylor et al., 2020). Here, we reported a new Zn(II)$_2$Cys6 TF, BcSpd1, that played a key role in B. cinerea. Loss of BcSpd1 function in ΔBcSpd1 reduced B. cinerea virulence, and the fungal structures or secondary metabolites associated with BcSpd1 would contribute to fungal pathogenicity. BcSpd1 positively regulated genes involved in the sclerotium development and the infection cushion formation and decreased the environment pH values, while BcSpd1 negatively affected the melanin biosynthesis (Figure 8A). Similar results were reported in several recent studies. For example, the cucurbit pathogen Colletotrichum orbiculare Zn(II)$_2$Cys6 TF Mtf4 was shown to control the development of the appressorium, which played a role in host penetration (Kodama et al., 2019). In Verticillium dahliae, Vendf1 was identified as a Zn(II)$_2$Cys6 TF that is required for full virulence on cotton (Zhang et al., 2018). Zn(II)$_2$Cys6 TPC1 is involved in the early stage of plant infection by M. oryzae. TPC1 is required for polarized growth and virulence in M. oryzae by regulated synthesis of reactive oxygen species and the MAPK pathway during host invasion (Galhano et al., 2017). In Alternaria brassicicola, AbPf2 was dispensable for normal growth but crucial for virulence (Cho et al., 2013). Gene deletion of Pf2 orthologs in Parastagonospora nodorum and Pyrenophora triticirepentis resulted in downregulation of key effector genes including ToxA and Tox3, leading to the loss of host-specific virulence in wheat (Rybak et al., 2017). In Zymoseptoria tritici, the putative Pf2 ortholog Zt107320 was reported to mediate virulence and sporulation during infection (Habig et al., 2020). Fundamental knowledge of TF regulation provides avenues to identify novel virulence factors in plant–fungus interaction and improves the understanding of the regulatory networks linked to pathogen evolution, while TFs

resistant mechanism has not been well studied so far (Lorang, 2019). No major R-gene has been identified with resistance to B. cinerea. Plant immunity toward this fungus appears to be under complex poorly understood genetic control (Rowe and Kliebenstein, 2008). The broad host range necrotrophic plant pathogens have evolved diverse, and sometimes convergent, responses to similar selective regime governed by interactions with a highly heterogeneous host landscape (Newman and Derbyshire, 2020). Here, we reported that B. cinerea BcSpd1 played a key role in regulating fungal growth, development, and virulence, and the gene presented as the pathogenic factor which repressed plant defense responses (Figure 8).

A new Zn(II)$_2$Cys6 TF, BcSpd1, was identified in B. cinerea (Chen et al., 2021). The gene was expressed in the different fungal growth stages and under various environmental conditions. Its expression was increased in B. cinerea-infected host tissues compared to uninfected controls. BcSpd1 is a small, 246-amino-acid protein containing a Zn(II)$_2$Cys6 motif, which is characteristic of Zn(II)$_2$Cys6 TFs. The protein is predicted to be localized in the nucleus, suggesting its role in transcriptional regulation. To investigate the function of BcSpd1, we generated a ΔBcSpd1 strain and compared its growth and virulence traits to those of the wild-type (WT) strain. The ΔBcSpd1 strain exhibited delayed growth, reduced virulence, and altered gene expression patterns compared to the WT strain. These results indicate that BcSpd1 is essential for B. cinerea virulence.

Several BcSpd1-regulated genes were identified using transcriptome analysis. A significant number of genes encoding proteins involved in various biological processes were found to be differently expressed in the ΔBcSpd1 strain compared to the WT strain. These genes included those involved in fungal metabolism, stress response, and development. Furthermore, we performed EMSA analysis to investigate the binding of BcSpd1 to the promoter regions of these genes. The results showed that BcSpd1 binds to the promoter regions of these genes, confirming its role in transcriptional regulation.

In summary, our study provides insights into the role of BcSpd1 in B. cinerea virulence. The gene is essential for fungal growth, development, and virulence, and its expression is regulated by environmental factors. The identification of BcSpd1-regulated genes suggests potential targets for developing strategies to control B. cinerea infections.

FIGURE 6 | BcSpd1 regulates BcQdo-mediated B. cinerea virulence. (A) Conserved DNA fragments in the promoter of BcQdo. The predicted transcription factor-binding sites were framed. (B) SDS-PAGE and Western blot analysis of recombinant BcSpd1 in Escherichia coli. Left: The proteins were stained with Coomassie brilliant blue R-250. M, Positions of marker proteins. CK, SDS-PAGE of the extracts from empty pET28a-transformed bacterial cultures. BcSpd1, SDS-PAGE of the recombinant BcSpd1 extracted from pET28a-BcSpd1-transformed bacterial culture. Right: Western blot analysis of BcSpd1 protein in the indicated bacterial cultures (anti-His). (C) EMSA analysis of BcSpd1 binding to biotin-labeled promoter fragments of BcQdo. Line 1, the reaction only with recombinant BcSpd1 protein; line 2, the reaction with only biotin-labeled probes; line 3, the reaction with both recombinant BcSpd1 protein and biotin-labeled probes, which showed binding shift; line 4, the unlabeled probes were added to the reaction and competed the binding.
FIGURE 7 | Transcription analysis of *B. cinerea* B05.10- and ΔBcSpd1-infected *A. thaliana* Col-0 plants. (A) Number of differentially expressed genes (≥ 2-fold; \( P \leq 0.05 \)) in Col-0 at 14 h after mock treatment (CK) or B05.10 or ΔBcSpd1 spray inoculation. The total number of genes between treatments or fungal strains is indicated. (B) Gene analysis of the overlap genes between B05.10-affected and ΔBcSpd1-affected differentially regulated genes. (C) KEGG analysis of differentially expressed genes only observed in B05.10-affected plants. (D) KEGG analysis of differentially expressed genes only observed in ΔBcSpd1-affected plants. (E) A heatmap analysis of differentially expressed genes in the plant hormone transduction pathway among CK, B05.10-, and ΔBcSpd1-treated Col-0 plants. (F) A heatmap analysis of differentially expressed genes in the flavone and isoflavonoid biosynthesis pathways among CK, B05.10-, and ΔBcSpd1-treated Col-0 plants.
(e.g., BcSpd1) can themselves be specifically targeted for disease management. The development of inhibitors or fungicides, which could suppress BcSpd1 function in *B. cinerea*, would help to control this pathogen in the future.

From the comparative analysis of host response toward the *B. cinerea* virulent strain B05.10 and the ΔBcSpd1 mutant in this study, it appears that the outcome of the interaction between the hosts and the two fungal pathogens is much different. The difference is mainly determined by qualitative and quantitative differences in the ΔBcSpd1-dependent activation of similar defense response in the same plant (Figure 8B). In one aspect, the plants inoculated with strain B05.10 were impaired in the accumulation of hormone pathway genes compared with plants infected with the mutant strain at the early stage, and this impairment seemed to be causal to disease development. The strain B05.10 actively suppressed host hormones and signaling was the consequence of early expression of *BcSpd1*. Similar results were reported in the *A. thaliana* wrky33 mutant, which was highly susceptible to *B. cinerea* 2100, while the wrky33nced3nced5 triple mutants were deficient in ABA biosynthesis restored Col-0 resistance by recovering from the expression of plant defense-related genes such as TFs and hormone signaling (Liu et al., 2015). The ABA signaling acted as the susceptible factor in plants not only toward *B. cinerea* isolate 2100 but also to the isolate B05.10 (Liu et al., 2015, 2017). Quite interestingly, the gene responses to ABA were partly reduced in ΔBcSpd1-infected Col-0 plants and additionally indicated that the susceptibility of plants toward B0510 was very likely by BcSpd1-modulated host ABA signaling. In other aspects, our data suggested that the transcripts of some TFs were affected by B05.10 infection. Interestingly, compared with B05.10, the ΔBcSpd1-infected plants partly recovered from the expression of these genes at an early stage, suggesting their potential role in plant defense. Here, BcSpd1 is involved in such repression and contributes to *B. cinerea* virulence. Our study further indicates that *B. cinerea* BcSpd1 is involved in suppressing certain flavones as the compounds play a role in host defense (Chen et al., 2022). On the one hand, BcSpd1 positively regulated *BcQdo* expression, which is involved in the degradation of certain flavones. On the other hand, many genes in the flavone and isoflavonoid biosynthetic pathway are activated by the ΔBcSpd1 mutants, suggesting that BcSpd1 repressed their expression levels...
at early infection stages, which could also explain the reduction of antifungal metabolisms in ginseng plants upon B05.10 infection (Chen et al., 2022). Our study proves that *B. cinerea* BcSpd1 plays a key role in pathogenicity by suppression of plant early defense responses.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: National Center for Biotechnology Information (NCBI) BioProject database under accession number GSE186842.

**AUTHOR CONTRIBUTIONS**

SL and HC designed the research plan and wrote the manuscript. SL, HC, SZ, SH, WL, and RA performed the research. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was partly supported by the National Natural Science Foundation of China (NSFC grant no. 32171801 to SL) and the Cross-Disciplinary Innovation Founding of Jilin University (No. JLUJKJC2020313 to SL).

**ACKNOWLEDGMENTS**

We thank Paul Tudzynski from the University of Münster, for kindly providing *Botrytis cinerea* strain B05.10.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.820767/full#supplementary-material

**REFERENCES**

Bahn, Y. S. (2015). Exploiting Fungal Virulence-Regulating Transcription Factors As Novel Antifungal Drug Targets. *PLoS Pathog.* 11:e1004936. doi: 10.1371/journal.ppat.1004936

Birkentbihl, R. P., Liu, S., and Somssich, I. E. (2017). Transcriptional events defining plant immune responses. *Curr. Opin. Plant Biol.* 38, 1–9. doi: 10.1016/j.pbi.2017.04.004

Boutetot, 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. doi: 10.1146/annurev-phyto-080614-120106

Campbell, R. N., Leverenz, M. K., Ryan, L. A., and Reece, R. J. (2008). Metabolic control of transcription: paradigms and lessons from *Saccharomyces cerevisiae*. *Biochem.* J. 414, 177–187. doi: 10.1042/BJ20080923

Cao, S. N., Yuan, Y., Qin, Y. H., Zhang, M. Z., de Figueiredo, P., Li, G. H., et al. (2018). The pre-rRNA processing factor Nop53 regulates fungal development and pathogenesis via mediating production of reactive oxygen species. *Environ. Microbiol.* 20, 1531–1549. doi: 10.1111/1462-2920.14082

Caramori, G., Ruggeri, P., Mumby, S., Atzeni, F., and Adcock, I. M. (2019). *Botrytis cinerea* BcSpd1 affects signal transduction among CK, B05.10, and ΔBcSpd1-treated plants compared with CK. (D) GO analysis of differentially expressed genes observed only in ΔBcSpd1 affected Col-0 plants compared with CK. (D) GO analysis of differentially expressed genes observed only in B05.10 affected Col-0 plants with CK.

**SUPPLEMENTARY FIGURE 1** | Protein blast analysis revealed conserved domains in BcSpd1. The amino acid of BcSpd1 was submitted to NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the conserved domain was indicated.

**SUPPLEMENTARY FIGURE 2** | Construction of the BcSpd1 gene deletion mutants (ΔBcSpd1). Replacement of BcSpd1 with a hygromycin gene (Hyg) cassette by homologous recombination (A) and PCR verification of transformants (B). The agarose gels depict (in order): PCR amplification of Hyg genes, PCR amplification of BcSpd1 genes (one heterozygotic line, four transformed homozygotic lines). (C) qPCR analysis of BcSpd1 genes in wild-type B0510 and the indicated ΔBcSpd1 mutants.

**SUPPLEMENTARY FIGURE 3** | Construction of the complemented lines of BcSpd1 (ΔBcSpd1-C).

**SUPPLEMENTARY FIGURE 4** | Phenotype of BcSpd1 deletion mutants showed less pathogenicity than wild-type *B. cinerea* B05/10.

**SUPPLEMENTARY FIGURE 5** | GO and KEGG analysis of differentially expressed genes. (A) GO analysis of differentially expressed genes observed in both B05.10 and ΔBcSpd1 affected Col-0 plants compared with CK. (B) KEGG analysis of differentially expressed genes observed in both B05.10 and ΔBcSpd1 affected Col-0 plants compared with CK. (C) GO analysis of differentially expressed genes observed only in ΔBcSpd1 affected Col-0 plants compared with CK. (D) GO analysis of differentially expressed genes observed only in B05.10 affected Col-0 plants with CK.

**SUPPLEMENTARY FIGURE 6** | Heatmap analysis of differentially expressed genes encoding transcription factors among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FIGURE 7** | Heatmap analysis of differentially expressed genes response to ABA among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FILE 1** | The top 100 differentially expressed genes among CK, B05.10, and ΔBcSpd1.

**SUPPLEMENTARY FILE 2** | GO enrichment pathway analysis of the differentially expressed genes observed only in ΔBcSpd1 affected Col-0 plants with CK.

**SUPPLEMENTARY FILE 3** | Differentially expressed genes involved in plant defense signals and antifungal metabolites degradation.

**SUPPLEMENTARY FILE 4** | GO and KEGG analysis of differentially expressed genes observed only in ΔBcSpd1 affected Col-0 plants compared with CK.

**SUPPLEMENTARY FILE 5** | Differentially expressed genes encoding transcription factors among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FILE 6** | Differentially expressed genes response to ABA among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FILE 7** | Phenotype of BcSpd1 deletion mutants showed less pathogenicity than wild-type *B. cinerea* B05/10.

**SUPPLEMENTARY FIGURE 8** | Construction of the complemented lines of BcSpd1 (ΔBcSpd1-C).

**SUPPLEMENTARY FIGURE 9** | Phenotype of BcSpd1 deletion mutants showed less pathogenicity than wild-type *B. cinerea* B05/10.

**SUPPLEMENTARY FIGURE 10** | Heatmap analysis of differentially expressed genes encoding transcription factors among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FIGURE 11** | Heatmap analysis of differentially expressed genes response to ABA among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FILE 12** | The top 100 differentially expressed genes among CK, B05.10, and ΔBcSpd1.

**SUPPLEMENTARY FIGURE 13** | GO enrichment pathway analysis of the differentially expressed genes observed only in ΔBcSpd1 affected Col-0 plants with CK.

**SUPPLEMENTARY FILE 14** | GO and KEGG analysis of differentially expressed genes observed only in B05.10 affected Col-0 plants with CK.

**SUPPLEMENTARY FIGURE 15** | Phenotype of BcSpd1 deletion mutants showed less pathogenicity than wild-type *B. cinerea* B05/10.

**SUPPLEMENTARY FIGURE 16** | Heatmap analysis of differentially expressed genes encoding transcription factors among CK, B05.10, and ΔBcSpd1 treated Col-0 plants.

**SUPPLEMENTARY FILE 17** | GO enrichment pathway analysis of the differentially expressed genes observed only in ΔBcSpd1 affected Col-0 plants with CK.

**SUPPLEMENTARY FILE 18** | GO and KEGG analysis of differentially expressed genes observed only in B05.10 affected Col-0 plants with CK.
Doehlemann, G., Berndt, P., and Hahn, M. (2006). Different signalling pathways

Cho, Y., Ohm, R. A., Grigoriev, I. V., and Srivastava, A. (2013). Fungal-specific

Cook, D. E., Mesarich, C. H., and Thomma, B. P. (2015). Understanding plant

Cheung, N., Tian, L., Liu, X., and Li, X. (2020). The Destructive Fungal Pathogen

Habig, M., Bahena-Garrido, S. M., Barkmann, F., Haueisen, J., and Stukenbrock,

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and

Galhano, R., Illana, A., Ryder, L. S., Rodríguez-Romero, J., Demuez, M.,

Feng, H. Q., Li, G. H., Du, S. W., Yang, S., Li, X. Q., de Figueiredo, P., et al. (2017).

Harren, K., Brandhoff, B., Knodler, M., and Tudzynski, B. (2013). The High-

growth and virulence of the fungal wheat pathogen Zymoseptoria tritici.

E. H. (2020). The transcription factor Zt107320 affects the dimorphic switch,

Badaruddin, M., et al. (2017). Tpc1 is an important Zn(II)2Cys6 transcriptional

Genomics

Sclerotinia sclerotiorum

in silico analysis of secondary metabolite biosynthesis clusters in the genome

pathogens.

Mol. Plant Microbe Interact.

89:1598-019-52444-7

Jones, J. D., and Dangl, J. L. (2006). The plant immune system. Nature 444,

323–329.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., et al. (2008).
Porquiera, A., Morgant, G., Moraga, J., Dalmais, B., Luyten, I., Simon, A., et al. (2016). The botryidal biosynthetic gene cluster of *Botrytis cinerea* displays a bipartite genomic structure and is positively regulated by the putative Zn(II)Cys6 transcription factor BcBot6. *Fungal Genet. Biol.* 96, 33–46. doi: 10.1016/j.fgb.2016.02.003 

Romsdahl, J., and Wang, C. C. C. (2019). Recent advances in the genome mining of *Aspergillus* secondary metabolites (covering 2012–2018). *Medchemcomm* 10, 840–866. 

Rowe, H. C., and Kliebenstein, D. J. (2008). Complex genetics control natural variation in *Arabidopsis thaliana* resistance to *Botrytis cinerea*. *Genetics* 180, 2237–2250. doi: 10.1534/genetics.108.091439 

Rykab, K., See, P. T., Phan, H. T. T., Syme, R. A., Moffat, C. S., Oliver, R. P., et al. (2017). A functionally conserved Zn-Cys binuclear cluster transcription factor class regulates necrotrophic effector gene expression and host-specific virulence of two major Pleosporales fungal pathogens of wheat. *Mol. Plant Pathol.* 18, 420–434. 

Sang, H., and Kim, J. I. (2020). Advanced strategies to control plant pathogenic fungi by host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). *Plant Biotechnol. Rep.* 14, 1–8. doi: 10.3390/jims22157956 

Segmüller, N., Ellendorf, U., Tuzdyski, B., and Tuzdyski, P. (2007). BcSAK1, a stress-activated mitogen-activated protein kinase, is involved in vegetative differentiation and pathogenicity in *Botrytis cinerea*. *Eukaryotic Cell* 6, 211–221. doi: 10.1128/EC.00153-06 

Shao, D., Smith, D. L., Kabbage, M., and Roth, M. G. (2021). Effectors of Plant Necrotrophic Fungi. *Front. Plant Sci.* 12:687713. doi: 10.3389/fpls.2021.687713 

Tirjten, K., and Schreier, P. H. (2013). "New targets for fungicides," in Modern Methods in Crop Protection Research, eds P. Schüke, W. Kraemer, U. Schirmer, and M. Witschel (Hoboken, NJ: John Wiley & Sons Ltd), 197–216. 

Todd, R. B., and Andrianopoulos, A. (1997). Evolution of a fungal regulatory gene family: the Zn(II)2Cys6 binuclear cluster DNA binding motif. *Fungal Genet. Biol.* 21, 388–405. doi: 10.1006/fgb.1997.0993 

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* 28, 511–515. doi: 10.1038/nbt.1621 

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., and Katagiri, F. (2009). Network properties of robust immunity in plants. *PLoS Genet.* 5:e1000772. doi: 10.1371/journal.pgen.1000772 

Vidhyasekaran, P. (2014). "PAMP signaling in plant innate immunity," in PAMP Signals in Plant Innate Immunity. *Signaling and Communication in Plants*, Vol. 21. (Dordrecht: Springer). doi: 10.1007/978-0-470-7426-1_2 

Westrick, N. M., Smith, D. L., and Kabbage, M. (2021). Disarming the Host: detoxification of Plant Defense Compounds During Fungal Necrotrophy. *Front. Plant Sci.* 12:651716. doi: 10.3389/fpls.2021.651716 

Yang, Q., Chen, Y., and Ma, Z. (2013). Involvement of BcVeA and BcVelB in regulating conidiation, pigmentation and virulence in *Botrytis cinerea*. *Fungal Genet. Biol.* 50, 63–71. doi: 10.1016/j.fgb.2012.10.003 

Yellisetty, V., Reddy, L. A., and Mandapaka, M. (2015). In planta transformation of sorghum (*Sorghum bicolor* (L.) Moench) using TPS1 gene for enhancing tolerance to abiotic stresses. *J. Genet.* 94, 425–434. doi: 10.1007/s12041-015-0340-y 

Young, M. D., Wakefield, M. J., Smyth, G. K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol.* 11:R14. doi: 10.1186/gb-2010-11-2-r14 

Yuan, M., Ngou, B. P. M., Ding, P., and Xin, X. F. (2021). PTI-ETI crosstalk: an integrative view of plant immunity. *Curr. Opin. Plant Biol.* 62:102030. doi: 10.1016/j.pbi.2021.102030 

Zhang, C., He, Y., Zhu, P., Chen, L., Wang, Y., Ni, B., et al. (2015). Loss of bcbmr1 and bceps13 in *Botrytis cinerea* Not Only Blocks Melanization But Also Increases Vegetative Growth and Virulence. *Mol. Plant Microbe Interact.* 28, 1091–1101. doi: 10.1094/MPMI-04-15-0085-R 

Zhang, W. Q., Gui, Y. I., Short, D. P. G., Li, T. G., Zhang, D. D., Zhou, L., et al. (2018). *Verticillium dahliae* transcription factor VdFf1 regulates the expression of multiple secreted virulence factors and is required for full virulence in cotton. *Mol. Plant Pathol.* 19, 841–857. doi: 10.1111/mpp.12569 

Zheng, L., Campbell, M., Murphy, J., Lam, S., and Xu, J. R. (2000). The Bmp1 gene is essential for pathogenicity in the gray mold fungus *Botrytis cinerea*. *Mol. Plant Microbe Interact.* 13, 724–732. doi: 10.1094/MPMI.2000.13.7.724 

Zhu, P., Li, Q., Zhang, C., Na, Y., and Xu, L. (2017). Bepks12 gene inactivation substantiates biological functions of sclerotium melanization in *Botrytis cinerea*. *Physiol. Mol. Plant Pathol.* 98, 80–84. 

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. 

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher. 

Copyright © 2022 Chen, He, Zhang, A, Li and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.