Host Transcriptional Response of Sclerotinia sclerotiorum Induced by the Mycoparasite Coniothyrium minitans

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Mycoparasite Coniothyrium minitans parasitizes specifically the mycelia or sclerotia of Sclerotinia sclerotiorum, a worldwide spread plant fungal pathogen causing serious diseases on crops. The interaction of C. minitans with S. sclerotiorum remains reciprocal and complex and little is known, especially on the side of the host (S. sclerotiorum). In this study, the early transcriptional response of S. sclerotiorum to the mycoparasitism by C. minitans was explored and the differentially expressed genes (DEGs) were analyzed. Based on GO ontology, KEGG pathway and fungal categories database, 887 up-regulated DEGs were enriched in the growth related function (i.e., rRNA processing, ribosome biogenesis, binding and transport), while the 546 down-regulated DEGs were enriched in the stress-related functions (i.e., oxidoreductase, response to stress and heat and the chorismate biosynthetic process). The expression of shikimate pathway and the biosynthesis of phenylalanine involving genes was significantly suppressed. Furthermore, 581 unenriched DEGs were explored in the parasitizing process and were mapped on the Pfam domains of redox enzymes, Alpha/Beta hydrolase, haloacid dehalogenase, and other universal conserved domain containing proteins. Thirty-two DEGs encoding candidate effectors, with 16 up-regulated and 16 down-regulated, were observed with diverse function. SS1G_11912 (encoding SsNEP2) was significantly up-regulated and may function in the parasitism. The involving of the shikimate pathway of phenylalanine biosynthesis and effector candidates were discussed. The results provide a basal understand on the interaction of S. sclerotiorum and C. minitans.

Keywords: Sclerotinia sclerotiorum, Coniothyrium minitans, shikimate pathway, effector, necrosis- and ethylene-inducing peptide 1, mycoparasitism

INTRODUCTION

Sclerotinia sclerotiorum is a worldwide distributed destructive plant fungal pathogen, attacks more than 400 plant species (Boland and Hall, 1994) and causes huge economic losses (Schwartz and Singh, 2013). Coniothyrium minitans, a mycoparasitic fungus specific to some species in Sclerotinia (Campbell, 1947; Boland and Hall, 1994), parasitizes hypha and sclerotia of S. sclerotiorum efficiently and reduces the sclerotia inoculated in the soil (Campbell, 1947; Huang, 1977;
results

Identification of Differentially Expressed Genes (DEGs) of S. sclerotiorum Induced by C. minitans

In order to identify the gene expression profile of S. sclerotiorum, mycelial samples of S. sclerotiorum were collected 0, 4, and 12 h after co-cultured with hypha of C. minitans and three transcripted RNA libraries named SsCm0h, SsCm4h, and SsCm12h were constructed accordingly. More than 9.92 million clean reads were generated from each library, with approximately 5.74, 4.56, and 4.76 million were mapped to the genome of S. sclerotiorum in each library. The unique match of clean reads were 5.53, 4.42, and 4.53 million for each library, respectively, reflecting a high quality of the effective sequencing data (Supplementary Table S1). The mapped metadata files were uploaded into sequence read archive (SRA) with SRA accession SRR10436181, SRR10436182, SRR10436183 for SsCm0h, SsCm4h, and SsCm12h, respectively.

11125, 10843, and 11083 expressed genes in S. sclerotiorum were detected in libraries of SsCm0h, SsCm4h, and SsCm12h, respectively. The gene expression of S. sclerotiorum was compared to each parasitizing stage and a total of 1368 DEGs were detected during all the early stages of mycoparasitism process. During 0–4 h, 171 stage-specific DEGs were up-regulated and 237 were down-regulated; 237 up-regulated DEGs and 98 down-regulated DEGs were identified during 0–12 hpi; 154 up-regulated DEGs and 34 down-regulated DEGs were identified during 4–12 hpi; 227 up-regulated DEGs and 151 down-regulated DEGs were shared at the two stages of 4 or 12 hpi comparing to 0 hpi (Figure 1).

Functional Classification of DEGs in S. sclerotiorum

During 0–4 hpi, for the up-regulated DEGs, GO terms related to microbial growth including ‘rRNA processing (GO: 0006364),’ ‘transmembrane transport (GO: 0055085),’ and ‘macromolecule biosynthetic process (GO:0009059)’ were enriched (Figure 2A and Supplementary Table S2). While the down-regulated DEGs were enriched into the terms ‘oxidation-reduction process (GO: 0055114),’ ‘aromatic amino acid family biosynthetic process (GO: 0009073),’ ‘chorismate biosynthetic process (GO: 0009423),’ ‘protein folding (GO: 0006457),’ ‘response to stress (GO: 0006950),’ and ‘response to heat (GO: 0009408)’ (Figure 2B and Supplementary Table S2). The same catagories could be detected during 0–12 hpi for the down-regulated DEGs, while the term ‘carbohydrate metabolic process (GO:0005975)’ involved in the term ‘hydrolase activity (GO: 0016787)’ was also enriched for the up-regulated DEGs (Figures 2C,D and Supplementary Table S2).

Sclerotinia sclerotiorum DEGs were mapped onto Fungi Category (FunCat) database to identify categories that were significantly (p > 0.05) regulated when parasitized by C. minitans. FunCat category “rRNA processing (FCID: 11.04.01),” “cellular import (FCID: 20.09.18),” “non-vesicular cellular import (FCID: 20.09.18.07),” “C-compound and carbohydrate transport (FCID: 20.01.03),” and “polysaccharide metabolism (FCID: 01.05.03)” were enriched among the up-regulated DEGs (Figure 3 and Supplementary Table S3). While “protein folding and stabilization (FCID: 14.01),” “unfolded protein response (FCID: 32.01.07),” “heat shock response (FCID: 32.01.05),” “stress response (FCID: 32.01),” “metabolism of the cysteine-aromatic group (FCID: 01.01.09),” “metabolism of phenylalanine (FCID: 01.01.09.04),” and “metabolism of derivatives of dehydroquinic acid, shikimic acid, and chorismic acid (FCID: 01.20.15)” were significantly enriched among the down-regulated DEGs during the early parasitized process of S. sclerotiorum by C. minitans (Figure 3 and Supplementary Table S3).

Based on the KEGG pathway assessment, the “metabolism pathway” was the most significantly enriched pathway.
FIGURE 1 | Differentially expressed genes (DEGs) in S. sclerotiorum 0, 4, or 12 h after interacting with C. minitans. (A) Up-regulated DEGs of different comparison groups; (B) Down-regulated DEGs. Comparison groups were conducted between the different interaction time points. SsCm0h-vs-SsCm4h, SsCm0h-vs-SsCm12h and SsCm4h-vs-SsCm12h, the comparison groups of co-culture 4 h was versus to 0, 12 versus to 0 and 12 h compared to 4 h, respectively. (Supplementary Table S4). The fatty acid metabolism (map 01212) and biosynthesis (map 00061), biosynthesis of unsaturated fatty acids (map 01040), steroid biosynthesis (map 00100), starch and sucrose metabolism (map 00500) and propanoate metabolism (map 00640) and ribosome biogenesis in eukaryotes (map 03008) were significantly enriched among the up-regulated DEGs; while the pathway phenylalanine, tyrosine, and tryptophan biosynthesis (map 00400) was enriched pathway among the down-regulated DEGs.

Altogether, functional enrichment of the DEGs showed that physiologically related genes in S. sclerotiorum induced by C. minitans were enriched and up-regulated during the early stages, while stress-stimulus related genes were enriched and down-regulated.

The Shikimate Pathway Was Suppressed in S. sclerotiorum Challenged by C. minitans

The expression of five DEGs involved in the biosynthesis of phenylalanine was significantly suppressed (Figure 4). Gene SS1G_13550 is deduced to encode a multifunctional protein, including dehydroquininate synthase (EC 4.2.3.4) (DHQS), 3-dehydroquinate dehydratase (EC 4.2.1.10) (DHQD), shikimate dehydrogenase (E.C. 1.1.1.25) (SKDH), shikimate kinase (EC 2.7.1.71) (SHK) and 5-O-(1-Carboxyvinyl)-3-phosphoshikimate synthase (EC 2.5.1.19) (EPSPS), catalyzing the five steps from DAHP (7P-2-Dehydro-3-deoxy-D-arabino-heptonate) to EPSP (5-Enolpyruvylshikimate 3-phosphate) of the shikimate pathway. The first key step is catalyzed by SS1G_12793 encoding DAHP synthase (E.C. 2.5.2.15) and the final step to form chorismate was catalyzed by the enzyme chorismate synthase (E.C. 4.2.3.5) encoded by SS1G_03887. These three genes were significantly down-regulated during the early mycoparasitism stages (Figure 4). A gene SS1G_08569 encoding chorismate mutase (E.C. 5.4.99.5), catalyzing the conversion of chorismate to prephenate, was significantly down-regulated at 4 hpi (log$_2$Ratio = −3.29) and 12 hpi (log$_2$Ratio = −3.64) than 0 hpi (Figure 4). Followed closely, the step converting prephenate to phenylpyruvate is catalyzed by prephenate dehydratase, which was predicted to be encoded by SS1G_00612 in S. sclerotiorum, and the gene expression was suppressed at the early mycoparasitism stages by C. minitans (Figure 4). The results showed that the shikimate pathway might play an important role in the defense of S. sclerotiorum to C. minitans.

Expression of Effector Encoding Genes Was Significantly Regulated in S. sclerotiorum Challenged by C. minitans

There are 695 secretory proteins identified in the genome of S. sclerotiorum strain 1980, among which 304 proteins were predicted as conventional effector candidates. During the early stages in S. sclerotiorum challenged by C. minitans, 129 DEGs encoded secretory proteins, accounting for 9.4% of total detected DEGs (Supplementary Table S5). Totally 32 DEGs encoded candidate effectors, with 16 up-regulated and the other 16 down-regulated (Figure 5A). Among these genes, SS1G_1912, encoding a predicted effector with a domain of necrosis-inducing protein 1 (NPP1), was significantly up-regulated (4.91-fold of 12 to 0 hpi) at the early stages of mycoparasitism (Figure 5B).

Analysis of Unclassified DEGs in S. sclerotiorum

During the early mycoparasitic process, 580 DEGs in S. sclerotiorum could not be classified into any terms of GO ontology, KEGG pathway or FunCat category. Almost all the unclassified DEGs were hypothetical proteins without predicted function based on blast against the non-redundant protein (Nr)
FIGURE 2 | Gene Ontology (GO) classification of S. sclerotiorum DEGs. Genes were annotated in three categories: biological process (BP), molecular function (MF) and cellular component (CC). Y-axis represents the DEG number of a specific category. (A,B) Represent S. sclerotiorum up-regulated DEGs and down-regulated DEGs in 0–4 hpi, respectively. (C,D) Represent S. sclerotiorum up-regulated DEGs and down-regulated DEGs in 0–12 hpi, respectively. The detailed information was shown in Supplementary Table S2.

database (Supplementary Table S6). Proteins encoded by 163 DEGs have been annotated with functional domains against Pfam database, including 38 proteins with domain of unknown function, 125 proteins with similar functional domains reported in other microorganisms (Supplementary Table S6). The top five clan families were NADP_Rossmann (CL0063), AB_hydrolase (CL0028), HAD (CL0137), and Beta_propeller (CL0186) (Figure 6A and Supplementary Table S6). Some unclassified DEGs were identified with domains related to the function of redox enzymes, Alpha/Beta hydrolase, halococid dehalogenase, and some with universal conserved-domains (i.e., CL0029 and CL0186) (Murzin, 1992; Ollis et al., 1992; Koonin and Tatusov, 1994; Dunwell, 1998; Dunwell et al., 2004). SS1G_05913, SS1G_09620, and SS1G_05291 encoding proteins with domain of Glycosyltransferase family 2 (GT2) (PF00535), DJ-1/PfpI family (PF01965) and NmrA-like family (PF05368) respectively, were highly expressed and significantly up-regulated (log2Ratio > 3.8 and FDR ≤ 0.001) at 12 hpi induced by C. mimitans and the express were further confirmed by qRT-PCR (Figure 6B). Combined with the functional analysis of the DEGs, the results hint an intricate response of S. sclerotiorum to the parasitism by C. mimitans.

Expression of NPP1 Related Genes in S. sclerotiorum Challenged by C. mimitans

In order to discover the function of NPP1 related genes in the interaction of S. sclerotiorum and C. mimitans, NPP1 homologs were analyzed in the genome of S. sclerotiorum, its parasitic fungus C. mimitans and a pathogenic fungus Botrytis cinerea B05.10. Two homologs existed in each of the three genome.
In *S. sclerotiorum*, SsNEP2 was approximately induced 4.9-fold at 12 hpi compared with the initial contacting stage of 0 hpi with *C. minitans* checked by RNA-seq and qRT-PCR (Figure 2), while SsNEP1 kept at a relatively low level during the parasitism process (Supplementary Figure S1a). In *B. cinerea*, the most closely phylogenetic fungus of *S. sclerotiorum* and a non-host fungus of *C. minitans*, expression of BcNEP1 and BcNEP2 were not influenced by contacting with *C. minitans* (Supplementary Figures S1b,c). The results suggest that SsNEP2 may be involved in the interaction of *S. sclerotiorum* and *C. minitans*.

**DISCUSSION**

It is generally accepted that the pathogenic microbes induce host plant responses and inhibit the immune stress-related gene expression (Jones and Dangl, 2006; Irieda et al., 2019). Similarly, as a host, *S. sclerotiorum* may response to the parasitizing of parasite *C. minitans*. In this research, the transcriptional responses of *S. sclerotiorum* 4 and 12 h after contacting with *C. minitans* were monitored by RNA-seq.

Shikimate pathway presents in plants, bacteria, fungi, and certain protozoans including apicomplexan parasites, while is absent in animals (Keeling et al., 1999; Roberts et al., 2002). Shikimate pathway is the common aromatic biosynthetic pathway that involves seven enzymatic steps for the conversion of phosphoenolpyruvate and erythrose 4-phosphate to chorismate, providing the precursor of the three aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and many aromatic secondary metabolites (Herrmann and Weaver, 1999; Macheroux et al., 1999; Derrer et al., 2013; Mir et al., 2015). Increased enzyme activity of the shikimate pathway in plants enhances the resistance to pathogens (Roberts et al., 1998; Guillermo and Miller, 2004; Rippert et al., 2004). The metabolite level of the shikimate pathway was induced in the *Magnaporthe oryzae*-challenged rice roots and the resistance to pathogen infection was increased (Xu et al., 2015). Shikimate dehydrogenase (SKDH) is an active site of the multifunction amino acid protein and is considered to be the most promising component related to the plant resistance to *S. sclerotiorum* (Enferadi et al., 2011). Overexpression of barley chorismate mutase 1 (*HvCM1*) or chorismate synthase (*HvCS*) significantly increased the resistance to *Blumeria graminis* penetration in barley, while gene silencing increased susceptibility (Pingshu et al., 2009).

Shikimate pathway is also important for development and pathogenicity in fungi. Seven enzymes participating in the pathway were usually designed as antimicrobial and anti-parasitic drug targets (Roberts et al., 1998; Keeling et al., 1999; Aditya et al., 2012; Tzin et al., 2012; Mir et al., 2015). The chorismate mutase (*Cmu1*) secreted by *Ustilago maydis* was a virulence factor and deletion mutants of the gene led to virulence attenuation on maize, with correspondingly up-regulated during biotrophic development of *U. maydis* (Djamei et al., 2011). *SS1G_14320* encoding a chorismate mutase was expressed when cultured on PDB and throughout the infection process and predicted to be an important function in *Brassica napus* infection (Derbyshire et al., 2017). In this research, *SS1G_14320* was also up-regulated, while another chorismate mutase encoding gene *SS1G_08569* was detected down-regulated in *S. sclerotiorum* challenged by *C. minitans*. These may suggest that *S. sclerotiorum* recuits different genes to encode chorismate mutase in the interaction with host plant or *C. minitans*. Five genes encoding...
enzymes involved in the shikimate pathway and phenylalanine biosynthesis were significantly suppressed in *S. sclerotiorum* at the early stage of mycoparasitism process by *C. minitans*, namely SS1G_12793, SS1G_13550, SS1G_03887, SS1G_08569, and SS1G_00612. *C. minitans* probably decreases the resistance of *S. sclerotiorum* by inhibiting the shikimate pathway and the phenylalanine biosynthesis to profit its parasitization.

As a typical necrotrophic fungal pathogen, *S. sclerotiorum* is also reported to secrete effectors to manipulate host cells. 363 small secretory proteins with less than 300 amino acids in size were predicted in the genome of *S. sclerotiorum* (Amselem et al., 2011). Using different criteria, 79 and 70 effector candidates with diverse patterns of expression were identified to be involved in a wide range of functions, including chitin binding, proteases and protease inhibitors during the interaction with host plants (Amselem et al., 2011; Guyon et al., 2014; Heard et al., 2015; Derbyshire et al., 2017; Seifbarghi et al., 2017; Westrick et al., 2019). Recently, Westrick et al. (2019) found 57 DEGs encoded putative secreted effectors in *S. sclerotiorum* when infecting *Glycine max*. Out of these genes, 18 were differentially regulated at the late infection stage (96 hpi) compared to the early stages (average of 24 and 48 hpi) (Westrick et al., 2019). In this study, 13 pathogenic effector candidate coding genes of *S. sclerotiorum in planta* were also expressed when challenged by *C. minitans* with five up-regulated and the other eight down-regulated. For example, SS1G_03611, encoding a CFEM domain with proposed roles in fungal pathogenesis or conserved fungal effector domains, was induced during *S. sclerotiorum* infection on plants (Kulkarni et al., 2003; Heard et al., 2015) and was also up-regulated (1.84-fold) when challenged by *C. minitans*. Similar expression patterns were also observed for other four genes, SS1G_11912 (SsNEP2), SS1G_12778 (encoding a necrosis-inducing effector), SS1G_05073 (encoding a phospholipase C-like enzyme), and SS1G_11673 (encoding a putative pathogenic effector) (Bashi et al., 2010; Guyon et al., 2014; Derbyshire et al., 2017; Seifbarghi et al., 2017; Westrick et al., 2019). Eight pathogenic effector required for virulence on plant host and highly induced on plant were inhibited or undetectable during the early mycoparasitic stages of *S. sclerotiorum* by *C. minitans*, including SS1G_07404 (Ss-Rhs1,
rearrangement hotspot repeat 1), SS1G_01754, SS1G_00849, SS1G_07836, SS1G_09982, SS1G_08706, SS1G_08163, and SS1G_07027 (Guyon et al., 2014; Derbyshire et al., 2017; Seifbarghi et al., 2017; Westrick et al., 2019). Nine effectors of ssv263 (Liang et al., 2013), SsCutA (Bashi et al., 2012), SsPG1 (Bashi et al., 2012), SSITL (Zhu et al., 2013), SsECP6 (Heard et al., 2015), SsCP1 (Yang et al., 2018), SsSSVP1 (Lyu et al., 2016), SsCVNH (Lyu et al., 2015), and SsSm1 (Pan et al., 2018) were confirmed to be required for full virulence on plant host and expression of the encoding genes were up-regulated in plant infection, while the gene expressions did not show any difference during the early parasitism stages. The phenomenon suggests that S. sclerotiorum responds in a different manner to plant hosts and to C. minitans.

NPP1 is a conversed peptides triggering the transcript accumulation of pathogenesis-related (PR) genes, production of ROS and ethylene, callose apposition, and HR-like cell death in plants (Fellbrich et al., 2002; Qutob et al., 2006; Oome et al., 2014). Fusarium oxysporum NEP1 induced rapid structural changes, including the thinning of the cuticle and disruption of chloroplasts in spotted knapweed, dandelion, and Arabidopsis (Keates et al., 2003). S. sclerotiorum NEPs caused necrosis on tobacco leaves (Bashi et al., 2010). However, there is no direct proof to support the effects of SsNep2 expression on virulence though SsNep2 was expressed within 6 h and peaked at 24 h after inoculated on B. napus leaves (Bashi et al., 2010). Here we found that SsNEP2 was significantly up-regulated in S. sclerotiorum challenged by C. minitans, while the expression of homolog BcNEP2 was relatively stable in the non-host B. cinerea challenged by C. minitans (Supplementary Figures S1b,c). Therefore, the role of SsNEP2 in the parasitic system of S. sclerotiorum and C. minitans need further investigation.

Coniothyrium minitans has been used as a biological agent to control diseases caused by S. sclerotiorum and S. minor. The foliar application of C. minitans conidia on bean plants during the early bloom to mid-bloom period reduced the proportion of plants infected by an average of 56% (p < 0.001) (Huang et al., 2000). Hyphal extension of S. sclerotiorum was inhibited by 68% when germinated conidia of C. minitans were spread on leaves of oilseed rape (Shi et al., 2004). Approximately 76% decrease of disease lesions was developed when flower petals of Brassica spp. were treated with C. minitans + S. sclerotiorum (Li et al., 2006). When contact with each other on oilseed rape, during parasitizing on the hypha, C. minitans may suppress the shikimate pathway in S. sclerotiorum and alter effector-like proteins to attenuate the virulence of S. sclerotiorum, and finally control the Sclerotia stem rot of crops.

In this study, we have only surveyed the transcriptional response of the host S. sclerotiorum induced by C. minitans during the early mycoparasitic stages. The interaction of
S. sclerotiorum parasitized by C. minitans is a two-way process, and a considerable number of reads mapped to the genome of C. minitans were also included in the interactive RNA_seq libraries. The responses of C. minitans need further study in order to elucidate the two-way interaction and a great opportunity would be provided to understand the mycoparasitism mechanism.

MATERIALS AND METHODS

Strain and Growth Conditions

Coniothyrium minitans strain ZS-1 was used to activate the response of S. sclerotiorum strain 1980 or B. cinerea strain B05.10 with hyphal contact. All the strains were grown on potato dextrose agar plates (PDA) (BD Biosciences, Franklin Lakes, NJ, United States) at 20°C.

RNA-seq Preparation and Sequencing

Mycelia of strain 1980 were cultured on the sterile cellophane membrane, which was placed on the PDA plates (Φ = 9 cm); 12 h later, the conidia of C. minitans strain ZS-1 were shaken at 20°C in PDB at 150 rpm for 36 h, washed with sterilized water for three times and re-suspended in water to $1.0 \times 10^6$ conidia mL$^{-1}$. The conidial suspension was spread on the 48-h-old colony (1 mL for each plate) of S. sclerotiorum. The mycelial mixtures were sampled at three interactional points of 0 h (immediately after coating), 4 h (co-culture for 4 h) and 12 h (co-culture for 12 h), and total RNA was extracted using RNA reagent (NewBio Industry, Tianjin, China) following the instructions. mRNA was enriched by using the oligo(dT) magnetic beads for the first strand cDNA synthesis by random hexamer-primer. Then buffer, dNTPs, RNase H, and DNA polymerase I were added to synthesize the second strand and the double strand cDNA was purified with QiaQuick PCR extraction kit (Qiagen, Mainz, Germany). Finally, fragments from the double strand cDNA were ligated with sequencing adaptors to construct the sequencing library. The library products were sequenced via an Illumina HiSeq 2000 at 49 bps of single-end read at BGI. One library was constructed for each interactional point and qRT-PCR was used to confirming the interactional transcriptome.

Analysis of Differentially Expressed Genes (DEGs) at Different Interaction Time Points

Adaptors, reads with more than 10% unknown bases and low-quality reads (quality value ≤ 5 of a read) were removed.
from the raw reads to obtain the clean reads. The clean reads were mapped to the genome of *S. sclerotiorum* using SOAP aligner/soap2 (Li et al., 2009). Mismatches of no more than two bases were allowed in the alignment. The gene expression level was calculated using the RPKM method (reads per kb per million bases were allowed in the alignment. The gene expression level of the three RNA-seq libraries based on the genome of *S. sclerotiorum*. SsCm0h, SsCm4h, and SsCm12h represented the gene expression of *S. sclerotiorum* at 0, 4, and 12 h mycoparasitism stages induced by *C. minitans*, respectively. Gene expression data of *S. sclerotiorum* in SsCm0h, SsCm4h, and SsCm12h with clean reads ≥ 10 in one of the time points were retained. Based on the gene RPKM value data, a series of comparison groups were conducted to analyze the mycoparasitism-related genes among the early interaction stages. We examined the DEGs at different interaction stages, and three comparisons were conducted: 0–4 hpi, gene expression of *S. sclerotiorum* in CmSs4h versus CmSs0h; 0–12 hpi, CmSs12h versus CmSs0h; and 4–12 hpi, CmSs12h versus CmSs4h.

**Functional Enrichment Analyses of DEGs**

Differentially expressed genes in *S. sclerotiorum* were enriched based on the functional categorization of *S. sclerotiorum* in FungiFun (Pribe et al., 2015). The significant level of the three analyses of GO ontology (GO), KEGG pathway (KEGG) and Fungi Categories (FungiFun) were cut at 0.05 with Fisher's exact test. The protein families (Pfam) annotations were conducted using HMMER software (version 3.2.1) with the hmmscan algorithm based on the database of Pfam (version 32.0) at default parameters (Eddy, 2011; El-Gebali et al., 2019).

**Effectors Prediction and Annotation**

We used complete genome and predicted proteomes of *S. sclerotiorum* strain 1980 downloaded from the NCBI (Amselem et al., 2011) to speculate the putative effectors. The presence of secretion signals was predicted with SignalP v.4 (Nielsen, 2017), transmembrane helices and GPI anchor sequence were predicted with TMHMM (Krogh et al., 2001) and GPlsom (Fankhauser and Maser, 2005), respectively. Effectors were set as under 300 amino-acids in length. For the identification of genes expressed in *S. sclerotiorum* challenged by *C. minitans*, RNA-seq data for gene induction fold at 0, 4, and 12 hpi were used. The predicted proteins were annotated using Blast2GO (Conesa et al., 2005) and PFAM². Pfam domains were annotated using HMMER3 searches against the PFAM 32.0 database e-value < 0.01 (El-Gebali et al., 2019).

**Quantitative RT-PCR (qRT-PCR)**

The RNA samples were prepared as described in Section “RNA-seq Preparation and Sequencing” and additional treatment with *B. cinerea* instead of *S. sclerotiorum* was set as the non-host control set. The level of gene expression was determined on a Bio-Rad CFX Real-Time System (Bio-Rad, Berkeley, CA, United States). The cDNA was synthesized using oligo(d)T primer in the EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen biotechnology, Beijing, China). Each PCR reaction contained 7.5 µL of 2 × iTaq Universal SYBR Green Supermix (Bio-Rad, Berkeley, CA, United States), 0.2 µL of cDNA template, 0.3 µL of each primer and 6.7 µL of ddH2O. The program was as follows: 95°C for 2 min, followed by 42 cycles of 95°C for 15 s, 57°C for 15 s and 72°C for 15 s, and a cycle with 0.5°C per second from 65 to 95°C to remove the influence of primer dimer. Total cDNA abundance in the samples was standardized against the *S. sclerotiorum* β-tubulin gene. The primers used to obtain an amplicon of approximately 100–150 bp from each target gene are listed in Supplementary Table S4. All samples were amplified in triplicate. Three independent repeats of the experiment were performed in the same way.

**Statistical Analysis**

The significant value of the differences in our analyses was evaluated with ANOVA program in the software SAS9.2 at the significant level of *p* = 0.01.

**CONCLUSION**

Although the biocontrol mechanism of *C. minitans* to *S. sclerotiorum* has been studied since *C. minitans* was first reported in Campbell (1947), knowledge about the mycoparasitic process is still at its infancy. In this paper, based on the transcriptome data, we identified “host fungus” response signatures in *S. sclerotiorum* (the host) induced by *C. minitans* (the mycoparasite) that the up-regulated transcripts were enriched mainly on function of lifestyle, while down-regulated transcripts were enriched on responses to stimulus. Genes involved in the shikimate pathway were inhibited and effector encoding genes were regulated. Collectively, our data indicate that *S. sclerotiorum* deployed different genes or different expression patterns to infect plants or respond to the parasitism of *C. minitans*.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study can be found in the mapped metadata files were uploaded into sequence read archive (SRA) with SRA accessions SRR10436181, SRR10436182, SRR10436183 of SsCm0h, SsCm4h, and SsCm12h, respectively.
AUTHOR CONTRIBUTIONS

DJ and YF designed the research. HZ and YF wrote the manuscript. HZ and TZ executed the experiments. HZ, JC, and JX performed the data and bioinformatics analyses. All authors read and approved the final manuscript.

FUNDING

The research was financially supported by the National Key R&D Program of China (2017YFD0200400), the National Natural Science Foundation of China (Grant No. 31572048), and the earmarked fund for China Agriculture Research System (CARS-13).

ACKNOWLEDGMENTS

We thank the reviewers for their kind suggestions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00183/full#supplementary-material

FIGURE S1 | The relative expression of NEP related genes of S. sclerotiorum and B. cinerea. Mycelia of strain 1980 or B05.10 were cultured on sterile cellophane membrane for 12 h and covered with conidial suspension (1.0 × 10⁶ conidia mL⁻¹) of C. minitans. The mycelial mixtures were sampled at 0 hpi (immediately after coating), 4 hpi (co-culture for 4 h), or 12 hpi (co-culture for 12 h), and RNA was extracted for qRT-PCR. The gene changes in transcript abundance were normalized with RPKM. For qRT-PCR, the gene expression was normalized against that of α-tubulin gene in S. sclerotiorum. The qRT-PCR valued the gene expression at 0 hpi set as 1. (a) SsNEP1; (b) BcNEP1; (c) BcNEP2. The primers of qRT-PCR for SsNEP1, BcNEP1, and BcNEP2 were listed in Supplementary Table S7.

TABLE S1 | The statistics of RNA_seq mapped to the genome of Sclerotinia sclerotiorum. The clean reads with mismatches of no more than two bases allowed in the alignment were mapped to the genome of S. sclerotiorum using SOAP aligner/soap2.

TABLE S2 | GO categories analysis of the DEGs of S. sclerotiorum during mycoparasitism process induced by C. minitans (p ≤ 0.05). The GO classification was conducted on the online website of FungiFun (https://sbi.hki-jena.de/fungifun/fungifun.php) based on GO ontology database of the fungi species of S. sclerotiorum.

TABLE S3 | Enrichment of FunCat categories of the DEGs in S. sclerotiorum (p ≤ 0.05). The classification of FunCat categories was conducted on the online website of FungiFun (https://sbi.hki-jena.de/fungifun/fungifun.php) based on FunCat classification database of the fungi species of S. sclerotiorum.

TABLE S4 | The significantly enriched KEGG pathway of the DEGs of S. sclerotiorum during the early mycoparasitism stages (p ≤ 0.05). The KEGG pathway analysis was conducted on the online website of FungiFun (https://sbi.hki-jena.de/fungifun/fungifun.php) based on KEGG pathway classification database of the fungi species of S. sclerotiorum.

TABLE S5 | The secretory proteins in S. sclerotiorum and the related DEGs in S. sclerotiorum induced by C. minitans.

TABLE S6 | Gene list of the unclassified DEGs in S. sclerotiorum.

TABLE S7 | Primers involved this research.

REFERENCES

Aditya, D., Satya, T., Shivendra, P., and Pravindra, K. (2012). Structure and function of enzymes of shikimate pathway. Curr. Bioinform. 7, 374–391. doi: 10.2174/1574989312803900983

Amselem, J., Cuomo, C. A., van Kan, J. A., Viaud, M., Benito, E. P., Couloux, A., et al. (2011). Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLoS Genet. 7:e1002230. doi: 10.1371/journal.pgen.1002230

Audic, S. P., and Claverie, J.-M. (1997). The significance of digital gene expression profiles. Genome Res. 7, 985–995. doi: 10.1101/gr.7.10.986

Bashi, D. Z., Hegedus, D. D., Buchwaltd, L., Rimmer, S. R., and Borhan, M. H. (2010). Expression and regulation of Sclerotinia sclerotiorum necrosis and ethylene-inducing peptides (NEPs). Mol. Plant Pathol. 11, 43–53. doi: 10.1111/j.1364-3703.2009.00571.x

Bashi, D. Z., Rimmer, S. R., Khachatourians, G. G., and Hegedus, D. D. (2012). Factors governing the regulation of Sclerotinia sclerotiorum cutinase A and polygalacturonase 1 during different stages of infection. Can. J. Microbiol. 58, 605–616. doi: 10.1139/cj-2012-031

Boland, G. J., and Hall, R. (1994). Index of plant hosts of Sclerotinia sclerotiorum. Can. J. Plant Pathol. 16, 93–108. doi: 10.1080/0706069409050766

Budge, S. P., McQuilken, M. P., Fenlon, J. S., and Whipp, J. M. (1995). Use of Coniothyrium minitans and Gliocladium virens for biological control of Sclerotinia sclerotiorum in glasshouse Lettuce. Biol. Control 5, 513–522. doi: 10.1006/bcon.1995.1061

Budge, S. P., and Whipp, J. M. (1991). Glasshouse trials of Coniothyrium minitans and Trichoderma species for the biological control of Sclerotinia sclerotiorum in celery and lettuce. Plant Pathol. 40, 59–66. doi: 10.1111/j.1365-3059.1991.tb02293.x

Campbell, W. A. (1947). A new species of Coniothyrium parasitic on sclerota. Mycologia 39, 190–195. doi: 10.2307/3755006

Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M., and Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674–3676. doi: 10.1093/bioinformatics/bti610

Derringer, M., Denton-Giles, M., Hegedus, D., Seibergary, S., Rollins, J., van Kan, J., et al. (2017). The complete genome sequence of the phytotrophic fungus Sclerotinia sclerotiorum reveals insights into the genome architecture of broad host range pathogens. Genome Biol. Evol. doi: 10.1093/gbe/evx030 [Epub ahead of print].

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., et al. (2011). The Pfam protein families database in 2019. Nucleic Acids Res. 39:D427–D432. doi: 10.1093/nar/gky995

Elsheshtawi, M., Elkhaky, M. T., Sayed, S. R., Bahkali, A. H., Mohammed, A. A., Gambhir, D., et al. (2017). Integrated control of white rot disease on beans.
Annu. Rev. Herrmann, K. M., and Weaver, L. M. (1999). The shikimate pathway.

Huang, H. C. (1977). Importance of

Huang, H. C., and Kokko, E. G. (1988). Penetration of hyphae of

Jones, D., Gordon, A. H., and Bacon, J. S. (1974). Co-operative action by endo-

Kamal, M. M., Savocchia, S., Lindbeck, K. D., and Ash, G. J. (2016). Biology

Guyon, K., Balagué, C., Roby, D., and Raffaele, S. (2014). Secretome analysis reveals

Fankhauser, N., and Maser, P. (2005). Identification of GPI anchor attachment

Jiang, D., Li, G., Yi, X., and Wang, D. (1996). Characterization of the sclerotial

Zhao et al.

application of fungal biocontrol agents for the control of white mold of dry Bean

doi: 10.1139/b77-039

of Sclerotinia sclerotiorum

doi: 10.1139/j14-0031-y

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., and Wold, B. (2008).

Pan, Y., Chuanchun, Y., Hengxue, R., Junjun, W., and Zhimou, G. (2018). SsSm1, a cerato-platanin family protein, is involved in the hyphal development and

caused by Sclerotinia sclerotiorum using Contans and reduced fungicides application. Saudi J. Biol. Sci. 24, 405–409. doi: 10.1016/j.sjbs.2016.01.038

Enferadi, S. T., Rabiei, Z., Vannozzi, G. P., and Akbari, G. A. (2011). Shikimate dehydrogenase expression and activity in sunflower genotypes susceptible and resistant to Sclerotinia sclerotiorum (Lib.) de Bary. J. Agric. Sci. Technol. 13, 493–502.

Fankhauser, N., and Maser, P. (2005). Identification of GPI anchor attachment

signals by a Kohonen self-organizing map. Bioinformatics 21, 1846–1852. doi: 0.1093/bioinformatics/bti299

Fellbrich, G., Romanski, A., Varet, A., Blume, B., Brunner, F., Engelhardt, S., et al. (2002). NPP1, a phytophthora-associated trigger of plant defense in parsley and Arabidopsis. Plant J. 32, 375–390. doi: 10.1046/j.1365-313X.2002.01454.x

Guillermo, B. V., and Miller, J. (2004). Combining ability for resistance to Sclerotinia head rot in sunflower. Crop Sci. 44, 1542–1545. doi: 10.2135/ cropsci002004.1542

Guo, Y. (2008). Cloning and Functional Analysis of Conidiation Associated Gene CMPE2X of Coniothyrium Minitans. Master’s thesis, Huazhong Agricultural University, Wuhan.

Guyon, K., Balagué, C., Roby, D., and Raffaele, S. (2014). Secretome analysis reveals effector candidates associated with broad host range necrotrophy in the fungal plant pathogen Sclerotinia sclerotiorum. BMC Genomics 15:336. doi: 10.1186/1471-2164-15-336

Hamid, M. I., Zeng, F., Cheng, J., Jiang, D., and Fu, Y. (2013). Disruption of heat shock factor 1 reduces the formation of conidia and thermostolerance in the mycoparasitic fungus Coniothyrium minitans. Fungal Genet. Biol. 53, 42–49. doi: 10.1016/j.fgb.2012.12.002

Heard, S., Brown, N. A., and Hammond-Kosack, K. (2015). An interspecies comparative analysis of the predicted secretomes of the necrotrophic plant pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLoS One 10:e0130534. doi: 10.1371/journal.pone.0130534

Herrmann, K. M., and Weaver, L. M. (1999). The shikimate pathway. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 473–503. doi: 10.1146/annurev.arplant.50.1.473

Huang, H. C. (1977). Importance of Coniothyrium minitans in survival of sclerotia of Sclerotinia sclerotiorum in wilted sunflower. Can. J. Bot. 55, 289–295. doi: 10.1139/b77-039

Huang, H. C., Bremer, E., Hynes, R. K., and Erickson, R. S. (2000). Filar application of fungal biocontrol agents for the control of white mold of dry Bean caused by Sclerotinia sclerotiorum. Biol. Control 18, 270–276. doi: 10.1006/bcon.2000.0829

Huang, H. C., and Kokko, E. G. (1988). Penetration of hyphae of Sclerotinia sclerotiorum by Coniothyrium minitans without the formation of appressoria. J. Phytopathol. 123, 133–139. doi: 10.1111/j.1439-0434.1988.tb04460.x

Irieda, H., Inoue, Y., Mori, M., Yamada, K., Oshikawa, Y., Saitoh, H., et al. (2019). Conserved fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. Proc. Natl. Acad. Sci. U.S.A. 116, 496–505. doi: 10.1073/pnas.1807297116

Jiang, D., Li, G., Yi, X., and Wang, D. (1996). Characterization of the sclerotial parasite Coniothyrium minitans II: comparison of the cultural characteristics and the abilities to parasitize the sclerotia of Sclerotinia sclerotiorum. J. Huazhong Agric. Univ. 15, 229–232. doi: 10.13330/cj. cnki.hnlxb.1996.03.006

Jones, D., Gordon, A. H., and Bacon, J. S. (1974). Co-operative action by endo-

and exo-beta(1 leads to 3)-glucanases from parasitic fungi in the degradation of cell-wall glucans of Sclerotinia sclerotiorum (Lib.) de Bary. Biochem. J. 140, 493–952.

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

Kamal, M. M., Savocchia, S., Lindbeck, K. D., and Ash, G. J. (2016). Biology and biocontrol of Sclerotinia sclerotiorum (Lib.) de Bary in oilseed Brassicas. Australas. Plant Pathol. 45, 1–14. doi: 10.1007/s13313-015-0391-2

Keates, S. E., Kostman, T. A., Anderson, J. D., and Bailey, B. A. (2003). Altered gene expression in three plant species in response to treatment with Nep1, a fungal protein that causes necrosis. Plant Physiol. 132, 1610–1622. doi: 10.1104/pp.102.019386

Keeling, P. J., Palmer, J. D., Donald, R. G. K., Roos, D. S., Waller, R. F., and McFadden, G. I. (1999). Shikimate pathway in apicomplexan parasites. Nature 397, 219–220. doi: 10.1038/16618

Koonin, E. V., and Tatusov, R. L. (1994). Computer analysis of bacterial haloacid dehydrogenases defines a large superfamily of hydrolases with diverse specificity: application of an iterative approach to database search. J. Mol. Biol. 244, 125–132. doi: 10.1006/jmbi.1994.1711

Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001). Predicting transmembrane protein topology with a hidden markov model: application to complete genomes. J. Mol. Biol. 305, 567–580. doi: 10.1006/jmbi.2000.4315

Kulkarni, R. D., Kelkar, H. S., and Dean, R. A. (2003). An eight-cysteine-containing protein that causes necrosis.

Australas. Plant Pathol.

doi: 10.1073/pnas.1103111111

Pan, Y., Chunuchun, Y., Hengxue, R., Junjun, W., and Zhimou, G. (2018). SsSm1, a cerato-platanin family protein, is involved in the hyphal development and
pathogenic process of Sclerotinia sclerotiorum. Plant Sci. 270, 37–46. doi: 10.1016/j.plantsci.2018.02.001
Phillips, A. J. L., and Price, K. (1983). Structural aspects of the parasitism of sclerotia of Sclerotinia sclerotiorum by Coniothyrium minitans. Can. J. Phytopathol. 107, 193–203. doi: 10.1111/j.1449-0434.1983.tb00373.x
Pingsha, H., Yan, M., and Roger, W. P. (2009). Functional contribution of chitinase synthase, anthranilate synthase, and chitinas mutase to penetration resistance in barley-Powdery Mildew interactions. Mol. Plant Microbe Interact. 22, 311–320. doi: 10.1094/mpmi-22-3-0311
Priebe, S., Kreisel, C., Horn, F., Guthke, R., and Linde, J. (2015). FungiFun2: a comprehensive online resource for systematic analysis of gene lists from fungal species. Bioinformatics 31, 445–446. doi: 10.1093/bioinformatics/btu627
Qutob, D., Kemmerling, B., Brunner, F., Kufner, I., Engelhardt, S., Gust, A. A., et al. (2006). Phytoalexin and innate immune responses induced by Nep1-like proteins. Plant Cell 18, 3721–3744. doi: 10.1105/tpc.106.044180
Ripper, P., Scimemi, C., Dubald, M., and Matringe, M. (2004). Engineering plant shikimate pathway for production of tocotrienol and improving herbicide resistance. Plant Physiol. 134, 92–100. doi: 10.1104/pp.103.032441
Roberts, C. W., Roberts, F., Lyons, R. E., Kirisits, M. J., Mui, E. J., Finnerty, J., et al. (2002). The shikimate pathway and its branches in apicomplexan parasites. J. Infect. Dis. 185(Suppl. 1), S25–S36. doi: 10.1086/338004
Schwartz, H. F., and Singh, S. P. (2013). Breeding common bean for resistance against Sclerotinia sclerotiorum. Crop Sci. 53, 1832–1844. doi: 10.2135/cropsci2013.02.0081
Seifibarghi, S., Borhan, M. H., Wei, Y., Coutu, C., Robinson, S. J., and Hegedus, D. D. (2017). Changes in the Sclerotinia sclerotiorum transcriptome during infection of Brassica napus. BMC Genomics 18:266. doi: 10.1186/s12864-017-3642-5
Shi, J., Li, Y., Qian, H., Du, G., and Chen, J. (2004). Pre-germinated conidia of Coniothyrium minitans enhances the foliar biological control of Sclerotinia sclerotiorum. Biotechnol. Lett. 26, 1649–1652. doi: 10.1023/s/10529-904-3515-2
Shukunami, R., Iwamoto, Y., Sugiura, S., Ikeda, K., Nakayashiki, H., and Ikeda, K. (2016). Field method to monitor the mycoparasitic fungus Coniothyrium minitans on aerial parts of plants by the hyperparasite Mycoparasitism by Trutmann, P., Keane, P. J., and Merriman, P. R. (1980). Reduction of sclerotial complexes of Sclerotinia sclerotiorum during infection of Glycine max on the road to pathogenesis. BMC Genomics 20:157. doi: 10.1186/s12864-019-5517-4
Whipples, J. M., Bennett, A., Challen, M., Clarkson, J., Coventry, E., Muthumeenakshi, S., et al. (2007). “Control of sclerotial pathogens with the mycoparasite Coniothyrium minitans,” in Novel Biotechnologies for Biocontrol Agent Enhancement and Management, eds M. Vurro, and J. Gressel, (Dordrecht: Springer), 223–241. doi: 10.1007/978-1-4020-5799-1_12
Wu, S. (2006). Partial Cloning Conidiation-Associated Gene of Coniothyrium Minitans and Improving Its Conidia Production in Shaken Liquid Medium. Master’s thesis, Huazhong Agricultural University, Wuhan.
Xu, X.-H., Wang, C., Li, S.-X., Su, Z.-Z., Zhou, H.-N., Mao, L.-J., et al. (2015). Friend or foe: differential responses of rice to invasion by mutualistic or pathogenic fungi revealed by RNAseq and metabolic profiling. Sci. Rep. 5:13624. doi: 10.1038/srep13624
Yang, G., Tang, L., Gong, Y., Xie, J., Fu, Y., Jiang, D., et al. (2018). A cerato-platanin protein ScCP1 targets plant PR1 and contributes to virulence of Sclerotinia sclerotiorum. New Phytol. 217, 739–755. doi: 10.1111/nph.14842
Yang, R., Han, Y. C., Li, G. Q., Jiang, D. H., and Huang, H. C. (2007). Suppression of Sclerotinia sclerotiorum by antifungal substances produced by the mycoparasite Coniothyrium minitans. Eur. J. Plant Pathol. 119, 411–420. doi: 10.1007/s10658-007-9174-0
Zeng, F., Gong, X., Hamid, M. I., Fu, Y., Jiatao, X., Cheng, J., et al. (2012). A fungal cell wall integrity-associated MAP kinase cascade in Coniothyrium minitans is required for conidiation and mycoparasitism. Fungal Genet. Biol. 49, 347–357. doi: 10.1016/j.fgb.2012.02.008
L. M., Zhang, J., Han, Y. C., Yang, L., Wu, M. D., Jiang, D. H., et al. (2014). Degradation of oxalic acid by the mycoparasite Coniothyrium minitans plays an important role in interacting with Sclerotinia sclerotiorum. Environ. Microbiol. 16, 2591–2610. doi: 10.1111/1462-2920.12409
Zeng, W., Wang, D., Kirk, W., and Hao, J. (2012). Use of Coniothyrium minitans and its effect on sclerotial germination. J. Phytopathol. 160, 261–268. doi: 10.1111/j.1439-0434.1984.tb00716.x
Zin, V., Galili, G., and Aharoni, A. (eds.) (2012). “Shikimate pathway and aromatic amino acid biosynthesis,” in Encyclopedia of Life Sciences, (Chichester: John Wiley & Sons, Ltd). doi: 10.1002/9780470015902.a0001315.pub2
Wei, W., Zhu, W., Cheng, J., Xie, J., Jiang, D., Li, G., et al. (2016). Nox complex signal and MAPK cascade pathway are cross-linked and essential for pathogenicity and conidiation of mycoparasite Coniothyrium minitans. Sci. Rep. 6:24325. doi: 10.1038/srep24325
Wei, W., Zhu, W., Cheng, J., Xie, J., Li, B., Jiang, D., et al. (2013). CmPEX6, a gene involved in peroxisome biogenesis, is essential for parasitism and conidiation by the sclerotial parasite Coniothyrium minitans. Appl. Environ. Microbiol. 79, 3658–3666. doi: 10.1128/AEM.00375-13
Westrick, N. M., Ranjan, A., Jain, S., Grau, C. R., Smith, D. L., and Kabbage, M. (2019). Gene regulation of Sclerotinia sclerotiorum during infection of Glycine max: on the road to pathogenesis. BMC Genomics 20:157. doi: 10.1186/s12864-019-5517-4
Zhao et al. Sclerotinia sclerotiorum Response to Mycoparasite