Research Article

Pathogenic Process-Associated Transcriptome Analysis of Stemphylium lycopersici from Tomato

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Tomato (Solanum lycopersicum) gray leaf spot disease is a predominant foliar disease of tomato in China that is caused mainly by the necrotrophic fungal pathogen Stemphylium lycopersici. Little is known regarding the pathogenic mechanisms of this broad-host-range pathogen. In this study, a comparative transcriptomic analysis was performed and more genetic information on the pathogenicity determinants of S. lycopersici during the infection process in tomato were obtained. Through an RNA sequencing (RNA-seq) analysis, 1,642 and 1,875 genes upregulated during the early infection and necrotrophic phases, respectively, were identified and significantly enriched in 44 and 24 pathways, respectively. The induction of genes associated with pectin degradation, adhesion, and colonization was notable during the early infection phase, whereas during the necrotrophic phase, some structural molecule activity-related genes were prominently induced. Additionally, some genes involved in signal regulation or encoding hemicellulose- and cellulose-degrading enzymes and extracellular proteases were commonly upregulated during pathogenesis. Overall, we present some putative key genes and processes that may be crucial for S. lycopersici pathogenesis. The abilities to adhere and colonize a host surface, effectively damage host cell walls, regulate signal transduction to manage infection, and survive in a hostile plant environment are proposed as important factors for the pathogenesis of S. lycopersici in tomato. The functional characterization of these genes provides an invaluable resource for analyses of this important pathosystem between S. lycopersici and tomato, and it may facilitate the generation of control strategies against this devastating disease.

1. Introduction

Tomato (Solanum lycopersicum) gray leaf spot disease is considered a major damaging, even devastating, disease of cultivated tomatoes and has threatened tomato-growing areas worldwide [1]. As a foliar disease, it usually occurs and develops under warm air temperature and high-humidity conditions [2], and the leaf damage reduces fruit quality and yield [3, 4].

Stemphylium lycopersici and Stemphylium solani are both causative agents of gray leaf spot disease on tomato [5, 6], and they have been widely reported in many countries. As two closely related species, S. lycopersici and S. solani often cause almost indistinguishable symptoms. More importantly, as necrotrophic parasitic fungi, they have wide host ranges that include important vegetable crops, such as tomato [7, 8], pepper [9], and eggplant [3]. In recent years, several new hosts, such as lettuce [10], physali [11], asparagus [12], and watermelon [13], have been discovered and reported in certain areas. Therefore, the pathogenic mechanisms of the two species of Stemphylium against plants need to be well studied to effectively control the diseases that they cause.

For the past few years, research on the fungal pathogens S. lycopersici and S. solani has mainly focused on biological characteristics [14], histology [15], genetic and virulence variability levels [16], and phytotoxins [17]. Compared with other filamentous fungal pathogens, studies on the molecular mechanisms underlying the interactions of S. lycopersici and S. solani with their host, as well as on cloning of virulence-associated genes, are still limited. The
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to understand the molecular mechanisms of plant pathogenic diseases [18, 19]. Zeng et al. [20] have predicted S. lycopersici-secreted proteins, but the correlation between the virulence and secreted proteins has not been validated. Furthermore, little is known about the pathogenic molecular mechanisms, such as those involved in the infection process, disrupting host cells and reproduction in the host.

Pathogen infection and the colonization of host plants are complicated dynamic processes. High-throughput transcriptomic sequencing is a suitable approach for investigating the complex interplay between gene expression and regulation under specific conditions or phases [21] that will facilitate systematically revealing the complicated regulatory mechanisms behind pathogenesis and the relationships between the pathogens S. lycopersici and S. solani and the host.

In this study, S. lycopersici strain SL1216 isolated from diseased leaves of tomato was investigated. Using an RNA-Seq technique, gene expression during the infection and colonization phases of S. lycopersici was determined and compared. These research results provide valuable reference information to help reveal the molecular mechanisms of S. lycopersici pathogenicity, as well as interactions between tomato and S. lycopersici.

2. Materials and Methods

2.1. Biological Material and Inoculation Assays. We isolated S. lycopersici strain SL1216 from infected tomato leaves obtained from a commercial tomato greenhouse in Shouguang, Shandong Province, China, and it was highly virulent in tomato. Tomato plants (S. lycopersicum "Jinpeng No. 1") grown individually in plastic pots (10 cm diameter × 8 cm height) were placed in a growth chamber set at 25°C under a 12 h light:12 h dark photoperiod.

The S. lycopersici spores were harvested from fungal growth on V8 juice agar plates [22] at 25°C for 14 d under a 12 h light:12 h dark photoperiod and then adjusted to 1 × 10^5 spores/mL with sterile ddH_2O. The spore suspension was dropped on isolated tomato leaves, and the leaves were cultured in an artificial climate incubator at 25°C with 100% relative humidity under a 12 h light:12 h dark photoperiod. The symptoms of infected leaves were observed at 0, 100% relative humidity under a 12 h light:12 h dark photoperiod.

For RNA sequencing, the expression levels of eight randomly selected differentially expressed genes (DEGs) were collected and processed as described in Section 2.1 for the control sample. The test was conducted using triplicate biological replicates for every treatment (Con, 36 hpi, and 84 hpi). Nine samples were therefore collected. Total RNA was quantified and assessed for purity to meet the sequencing requirements.

2.3. RNA Sequencing and Read Assembly. Equal quantities of high-quality RNA for each sample were used to construct the sequencing libraries with a VAHTS mRNA-seq v2 Library Prep Kit (Illumina, Inc., San Diego, CA). Each transcriptome was sequenced on an Illumina HiSeq X Ten (PE 150) instrument at Novogene Corporation, Beijing, China, in accordance with the manufacturer's standard protocol. Raw reads were processed using Trimmomatic [23]. The reads containing ploy-N and the low-quality reads were removed to obtain the clean reads. The clean reads were assembled into expressed sequence tag clusters (contigs) and de novo assembled into the transcript by using Trinity [24] (version: 2.4) in the paired-end method. The TGIF clustering tools [25] were used to cluster assembly sequences and remove redundancies to produce a unigene dataset. The unigenes were mapped to the S. lycopersici genome to further exclude contaminating sequences. The resulting transcript dataset was taken as a S. lycopersici reference transcriptome for subsequent analyses.

2.4. Transcriptome Annotation and Differential Expression Analysis. The assembled unigenes were annotated on the basis of the top hits of a BLASTX search against various protein databases. The Blast2GO [26] program was employed to obtain a functional annotation of all the unigenes using the Gene Ontology (GO) database. The clean reads were aligned to the assembled unigenes by SOAP2 [27]. The expression levels based on the number of reads uniquely mapped to a unigene were normalized using the fragments per kb per million reads method. Differentially expressed genes (DEGs) were analyzed using the DESeq2 package [28] based on the negative binomial distribution test. The thresholds of DEGs were set as false discovery rate (FDR) ≤ 0.05 and |log 2 fold change| ≥ 1 in this paper. The GO enrichment analysis of DEGs was implemented with a perl module (GO::TermFinder) [29]. The statistical enrichment of the DEGs among the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was tested using R functions (phyper and qvalue). For GO terms and KEGG pathways, corrected p values less than 0.05 were considered to be significantly enriched among the differentially expressed unigenes.

2.5. RNA-seq Validation by Quantitative Real-Time PCR (qRT-PCR). To validate the expression profiles obtained by RNA-seq, the expression levels of eight randomly selected DEGs were analyzed by performing qRT-PCR. Specific primer pairs were designed for the eight DEG sequences in https://sg.idtdna.com/Primerquest/Home/Index. The actin gene was used as the internal control gene to obtain more accurate quantitative results (Table S1). The synthesis of first-strand cDNA from each sample was performed
using EasyScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal, TransGen, China) in accordance with the manufacturer’s instructions. qRT-PCR was performed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus, TaKaRa) on a Mastercycler Ep Realplex system (Eppendorf, Hamburg, Germany). The amplifications were performed in 20 µL reactions containing 10 µL of 2x TransStart® Top Green qPCR SuperMix (TransGen), 0.4 µL of each primer (10 µM), 2 µL diluted cDNA (1–100 ng), and 7.2 µL of nuclease-free water. The samples were incubated at 94°C for 30 s as an initial denaturation, followed by 40 amplification cycles at 94°C for 5 s, 60°C for 15 s, and 72°C for 20 s. The relative expression quantification of each DEG was determined using the 2−ΔΔCt method [30]. These qRT-PCR assays were performed with three biological replicates.

### 3. Results

#### 3.1. Time Course of S. lycopersici Infection on Tomato Leaves

Isolated tomato leaves were inoculated with a spore suspension, and the symptom development process was observed (Figure 1). At 48 hpi, brown spots began to appear at the leaf inoculation sites. However, we observed significant local leaf cellular necrosis by 96 hpi. Because pathogenesis-related gene expression tended to precede symptom onset, we considered 36 hpi with *S. lycopersici* as the early infection phase, whereas 84 hpi was considered the necrotrophic phase. Samples were taken at these two time points for transcriptome sequencing to investigate the dynamics of pathogenesis-related genes during *S. lycopersici* infection of tomato.

#### 3.2. De Novo Assembly of the S. lycopersici Transcriptome

Using RNA-seq technology, nine transcriptomic datasets were generated from control (Con), 36 hpi, and 84 hpi samples to better understand the pathogenicity of *S. lycopersici*. We obtained 66.15 Gb raw bases and 60.08 Gb clean bases. An overview of the transcriptome assembly statistics is shown in Table 1. After removing low-quality and adapter sequences, a total of 137.23 M, 141.27 M, and 140.78 M clean reads were obtained for Con, 36 hpi, and 84 hpi samples, respectively. The average Q30 values were greater than 92.50%, and the average GC values of the Con, 36 hpi, and 84 hpi samples were 54.97%, 55.33%, and 55.28%, respectively.

#### 3.3. DEGs of S. lycopersici

The global distribution of DEGs in 36 hpi-vs-Con and 84 hpi-vs-Con was determined using a volcano plot (Figures 2(a) and 2(b)). A total of 3,012 and 3,630 DEGs were identified at 36 hpi and 84 hpi, respectively, compared with the Con (p value < 0.05 and |log2 fold change| > 1). Overall, the majority of the DEGs (1,642, 54.52%) in 36 hpi-vs-Con were upregulated, whereas 1,875 (51.65%) DEGs were upregulated in 84 hpi-vs-Con (Figure 2(c)). Among the DEGs, 2,850 were common to 36 hpi-vs-Con and 84 hpi-vs-Con, whereas 927 and 1,545 DEGs were unique in 36 hpi-vs-Con and 84 hpi-vs-Con, respectively (Figure 2(d)). The presence of unique DEGs in different comparison groups suggested that different physiological activities were performed in *S. lycopersici* during the various infection stages.

#### 3.4. S. lycopersici Transcriptome Annotation

##### 3.4.1. Enrichment Analysis of GO Terms for the DEGs

The DEGs were subjected to a GO analysis to understand their functional differences. A corrected p value below 0.05 indicated that the function was enriched. Using Blast2GO software, the GO terms were organized into three ontologies: biological process, cellular component, and molecular function (Figure 3). The GO enrichment classification revealed that biological processes were dominated by cellular, metabolic, and single-organism processes. For the cellular component category, the DEGs assigned to the cell, cell part, and organelle were the most abundant. In the molecular function category, the DEGs related to catalytic activity and binding were the most abundant in both phases. The
3.4.2. KEGG Metabolic Pathway Enrichment Analysis of DEGs. To elucidate the significantly enriched biochemical pathways of DEGs in *S. lycopersici*, an enrichment analysis by comparing both 36 hpi and 84 hpi with Con was performed using the KEGG database. The DEGs in 36 hpi-vs-Con and 84 hpi-vs-Con clustered into four categories, with the most represented classification being metabolic categories (Figure 4). Carbohydrate metabolism (161 and 181 DEGs, respectively), amino acid metabolism (132 and 154 DEGs, respectively), and lipid metabolism (111 and 135 DEGs, respectively) were the main metabolic pathways. In the genetic information processing categories, the most significantly enriched KEGG pathways in 36 hpi-vs-Con and 84 hpi-vs-Con were translation (97 and 174 DEGs, respectively) and folding, sorting, and degradation (103 and 119 DEGs, respectively). The KEGG cellular processing categories included two main pathways in 36 hpi-vs-Con and 84 hpi-vs-Con, including transport and catabolism (86 and 96 DEGs, respectively) and cell growth and death (59 and 72 DEGs, respectively). Additionally, the most abundant subcategory in KEGG environmental information processing was signal transduction (94 and 104 DEGs, respectively).

The upregulated DEGs in 36 hpi-vs-Con and 84 hpi-vs-Con were significantly enriched in 44 and 24 pathways,
respectively (p value < 0.05). The KEGG enrichment analysis revealed that the greatest numbers of upregulated genes were involved in the starch and sucrose metabolic pathway (ko: 00500) and purine metabolic pathway (ko: 00230), with 26 upregulated DEGs enriched in both pathways in 36 hpi-vs-Con, followed by the spliceosome (ko: 03040, 25 upregulated DEGs), ribosome biogenesis in eukaryotes (ko: 03008, 24 upregulated DEGs), mitogen-activated protein kinase (MAPK) signaling pathway (ko: 04011, 22 upregulated DEGs), and pentose and glucuronate interconversion pathway (ko: 00040, 19 upregulated DEGs) (Figure 5). Compared with the 36 hpi-vs-Con group, there were some changes in the enriched pathways in 84 hpi-vs-Con. The six pathways having the most DEGs (at no less than 30) were the ribosome (ko: 03010, 56 upregulated DEGs), biosynthesis of amino acids (ko: 01230, 43 upregulated DEGs), ribosome biogenesis in eukaryotes (ko: 03008, 41 upregulated DEGs), spliceosome (ko: 03040, upregulated DEGs), RNA transport...
3.6. Genes Associated with Pathogenesis

3.6.1. Genes Associated with Cell Wall-Degrading Enzymes (CWDEs). In plant pathogenic fungi, CWDEs have important roles in penetrating and colonizing their hosts. In this study, a number of CWDE-encoding genes displayed differential expression during the early infection (36 hpi-vs-Con group) and necrotrophic (84 hpi-vs-Con group, Table S1) phases. Nonsupervised clustering analyses were performed, and a gene expression heatmap was constructed to obtain a more precise overview (Figure 7). In tomato leaves, eight genes associated with pectin-degrading enzymes were significantly enriched during the early infection phase, and seven showed nondifferential expression or were downregulated during the necrotrophic phase. The top two upregulated genes were TW65_01775, TW65_02246, and TW65_005488, TW65_005489, TW65_005490, TW65_005491, TW65_005492, TW65_005493, and TW65_005494. Among the 17 upregulated genes during the necrotrophic phase, nine genes showed higher expression levels than during the early infection phase. There were 13 upregulated genes during the early infection phase that functioned to degrade hemicellulose, but almost all of them showed relatively lower expression levels during the necrotrophic phase, except for TW65_07268.

On the whole, a number of S. lycopersici genes associated with CWDEs had diverse expression patterns during the pathogenic process on tomato leaves. Their combined expression may help impair plant cell structures and provide nutrients for pathogen growth. Pectin-degrading enzyme-related genes were upregulated mainly during the early stages of infection. The upregulated expression levels of hemicellulose- and cellulose-degrading enzyme-coding genes were maintained for relatively long times. In addition, different cellulose-degrading enzyme-coding genes were induced during different interaction periods.

3.6.2. Genes Involved in Adhesion and Colonization. Genes related to adhesion and colonization also play important roles in pathogenesis [31]. The KEGG analysis showed that seven upregulated genes (TW65_01775, TW65_02246, TW65_04588, TW65_07048, TW65_07418, TW65_08271, and TW65_08475) were significantly enriched in the focal adhesion pathway (ko: 04510) in 36 hpi-vs-Con (p < 0.05). The network map of the metabolic pathways suggested that in 36 hpi-vs-Con, six important pathways significantly enriched in the KEGG analysis were strongly associated with the focal adhesion pathway (p < 0.05). These six pathways included the actin cytoskeleton pathway (ko: 04610) and five signaling pathways, PI3K-Akt...
Our study showed that six of these seven closely related pathways were uniquely enriched in 36 hpi-vs-Con, indicating that the expression of related genes was associated with pathogen-host interactions during the early infection phase.

3.6.3. Genes Involved in Signal Regulation. Specific sensors/receptors and intricate coordination in cell signaling are considered to play major roles in pathogenic fungi associated with cell recognition and initial invasive structure formation when invading and infecting their plant hosts. In our study, one Pth11-like integral membrane protein (TW65_02983), a putative sensor/receptor, was found to have upregulated expression in 36 hpi-vs-Con, but no significant increase was seen in 84 hpi-vs-Con, indicating that it was required mainly during the early periods of the pathogenic process for cell identification (Table S3).

Protein kinases play a major role in cell signals (including MAPK, cAMP, and calcium), which function in some major signaling pathways and are associated with virulence in phytopathogenic fungi, such as affecting adhesion [31]. The transcriptional analysis suggested that the expression levels of several protein kinases, such as serine/threonine protein kinase (TW65_00823, TW65_01420, TW65_01775, TW65_05069, TW65_07479, and TW65_98149), histidine

Figure 6: Validation of the expression levels of DEGs in *S. lycopersici* by qRT-PCR. Relative expression of 4 genes was upregulated (a), while the other 4 genes were downregulated (b) at 36 hpi and 84 hpi.

Figure 7: A heatmap of differentially expressed genes associated with CWDEs. Each small square represents a CWDE-associated DEG, and the color represents the log2 fold change of gene differential expression. Red indicates genes with high expression levels in 36/84 hpi-vs-Con. Green indicates genes with low expression levels in 36/84 hpi-vs-Con.
kinase (TW65_01772, TW65_02044, and TW65_04432), phosphatidylinositol 3-kinase (TW65_06373), and inositol monophosphatase (TW65_08114), increased during the pathogenic process (Table S3).

MAPK cascades play pivotal roles in the infection-associated development of fungi [32]. Two genes (TW65_06425 and TW65_71836) encoding mitogen-activated protein kinases, which are vital in MAPK cascades, also showed higher expression levels during the pathogenic phase. Thus, these signal regulation-associated genes may play important roles during the pathogenic process.

3.6.4. Genes Associated with Fungal Proteases. Proteases are important for phytopathogenic fungi during different aspects of the infection process, such as adhesion, initial penetration, and colonization [33]. In this study, the upregulation of serine proteases (TW65_06425 and TW65_71836) encoding mitogen-activated protein kinases, which are vital in MAPK cascades, also showed higher expression levels during the pathogenic phase. Thus, these signal regulation-associated genes may play important roles during the pathogenic process.

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4. Discussion

S. lycopersici is a necrotrophic phytopathogenic fungus capable of infecting a wide range of plants and causing leaf spot disease [34], which has transitioned from minor to major disease, especially on facility-grown tomatoes over the last few years. Therefore, the pathogenic mechanisms of S. lycopersici against plants should be studied. Here, we reported the infection-related transcriptome of a S. lycopersici isolate sampled from tomato. Various candidate pathogenicity determinants of S. lycopersici were found during its pathogenesis on tomato. The expression dynamics of these genes at different periods illustrated their important roles in infection, colonization, pathogenicity, and survival in a hostile plant environment. These results may facilitate the study of the pathogenic mechanism of S. lycopersici in tomato.

Plant tissue penetration is a prerequisite for plant pathogenic fungi to infect and colonize a potential host [35], and CWDEs are particularly important, especially for necrotrophs and fungi that have no specialized penetration structures [36]. In addition, CWDEs are required for all phytopathogenic fungi during the late stages of host invasion [37]. In this study, a number of genes encoding CWDEs were induced during the pathogenesis of S. lycopersici. In total, 80% of upregulated pectin-degrading enzyme-associated genes were induced during the early infection phase. A total of 30 hemicellulose- and cellulose-degrading enzyme-related genes were induced during pathogenesis. Among them, 20 upregulated genes in each of the infection stages indicated their ubiquitous and indispensable roles during the progression of gray leaf spot disease in tomato. Pectinolytic enzymes are the first set of degradative enzymes secreted during infection, and they may assist in invasion and contribute to virulence in a wide range of pathogens [38]. Cellulases and hemicellulases may also contribute to virulence during infection. Van Vu et al. reported that the cellulases of Magnaporthe oryzae contribute to host penetration and further invasion [39]. Yang et al. showed that in Botrytis cinerea, the cell death activity of the xylanase BcXyl1 is independent of its xylanase activity, suggesting that BcXyl1 not only contributes to B. cinerea virulence but also induces plant defense responses [40]. Therefore, it is tempting to speculate that S. lycopersici is utilizing differential sets of CWDEs to promote pathogenesis by promoting cell wall damage under varying physiological conditions. The genes showing different expression dynamics may play different roles in pathogenesis. Here, pectin-degrading enzymes were
necessary during the early stages of the infection, and hemi-
cellulose- and cellulose-degrading enzymes were indispens-
able during the entire pathogenic period. It is worth noting
that we found one gene encoding the polygalacturonase
SIPG1 (TW65_07585), which was especially induced dur-
ing the early infection phase. An *Aspergillus flavus* strain
causing a deletion of the polygalacturonase *pecA* gene
reduces lesion development in cotton [41]. A single Bcpg1
is required for the full virulence of *B. cinerea* against dif-
ferent hosts [42]. However, the relevance of SIPG1 to the
infection and pathogenesis of *S. lycopersici* requires further
investigation.

The transcriptome analysis in this study revealed that a
number of adhesion pathway-related genes closely corre-
lated with the actin cytoskeleton and signal transduction
were mainly induced during the early infection phase. The
KEGG analysis showed that seven upregulated genes during
the early infection phase were significantly enriched in the
focal adhesion pathway (ko: 04510). Additionally, the KEGG
analysis revealed that during the early infection phase, there
were six important significantly enriched pathways strongly
associated with the focal adhesion pathway. These included
five important signaling pathways. Furthermore, two genes
encoding an activating transcription factor (TW65_00541)
and a transcription factor (TW65_06260), respectively, were
significantly induced. The function of adhesion is thought to
be essential for a fungal pathogen’s normal prepenetration
development and for successful infection [43]. First, firm
attachments enable spores to avoid being washed from the
host plant’s surface. Then, the proper recognition of the
topographic signals is necessary for properly oriented germ
tube growth and appressorial differentiation [43]. Further-
more, adhesion continues to be important for intercellular
development, cell wall penetration, and host tissue coloniza-
tion as the hyphae enter the substomatal chambers [43]. The
adhesion-deficient mutants of *Fusarium solani* f. sp. *cucurbi-
tae* have greatly reduced virulence levels on nonwounded
zucchini fruit, confirming the importance of adhesion as a
virulence factor [44]. In *Saccharomyces cerevisiae*, the FLO1
and FLO11 genes responsible for adhesion and initial surface
adhesion, respectively, are activated by the transcription
factor Flo8 under the control of the cAMP/PKA signal trans-
duction pathway [45, 46], which controls adhesion,
colonization, and pseudohyphal development [45, 47]. Thus,
we speculated that genes involved in the focal adhesion pathway
of *S. lycopersici* are conducive to spore adhesion, bud tube
directional growth, appressorial formation, and cell wall
contact in the substomatic cavity during the interaction
establishment phase. In addition, many important signaling
pathways are involved in regulating the expression of
adhesion-related genes.

During the establishment of an interaction between the
pathogen and the host plant, receptors and sensors are uti-
lized to sense and respond to the physicochemical cues of
the host surface, which trigger the initiation of pathogenic
development in fungi. Several intracellular signaling path-
ways, such as G-protein and cAMP/PKA, as well as MAPK
cascades that function downstream, regulate the formation
of infection-related structures required for host penetration
[32]. One gene for a predicted receptor/sensor PTH11-like
integral membrane protein (TW65_02983) was found to
have upregulated expression exclusively during the early
infection phase. *PTH11* was identified in *M. oryzae*, and it
may be required for completing appressorium morphogenesis.
A significant proportion (>85%) of the *pth11Δ* mutant
failed in appressorial formation [48]. Furthermore, Pth11 is
essential for pathogenesis as a bona fide G-protein-coupled
receptor that functions upstream of the cAMP-dependent
signaling pathway. Several histidine kinases and other pro-
tein kinases were upregulated or differentially expressed in
our study. Biochemical studies have revealed that histidine
kinases in phytopathogenic bacteria sense not only environ-
mental stimuli but also important host plant chemicals, sug-
going the existence of mutual communication between
phytopathogenic plants and bacteria [49]. The overexpres-
sion of the hybrid histidine kinase DRK1 in the yeast phase
of *Sporothrix schenckii* is involved in the regulation of the
mycelium-to-yeast transition and required for pathogenesis
[50]. Additionally, we found two MAPK-encoding genes
showing high expression levels during the pathogenic phase.
MAPK cascades play pivotal roles in appressorial formation
and pathogenicity in phytopathogenic fungi. MAPK cas-
cades comprise three kinds of conserved kinases, MAP
kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK),
and MAPK, which function downstream of G-
protein signaling and cAMP/PKA pathways [33]. In *M. ory-
zae*, the MAPKKK Mst11, the MAPKK Mst7, the MAPK
Pmk1, and the adaptor protein Mst50 function as an
Mst11-Mst7-Pmk1 cascade to regulate appressorial develop-
ment [51]. In *B. cinerea*, the deletion of BMP1, Ste7, Ste11, or
Ste50 causes defects in the development of infection-related
structures, confirming that BMP1 MAPK signaling is likely
regulated by the surface sensor Msb2 [52-54]. Similarly,
He et al. and Wang et al. demonstrated that the MAPK
CgMK1 and its upstream components MAPKKK CgStel1
and MAPKK CgStel7, as well as the putative adaptor protein
CgStel50, play critical roles in appressorial formation, inva-
sive growth, cellophane membrane penetration, and patho-
genicity of the hemibiotrophic pathogen *Colletotrichum
gloeosporioides* [55, 56]. Thus, *S. lycopersici* is likely to per-
ceive and interact with host cells through receptors/sensors,
histidine kinases, and other protein kinases. Then, MAPK
cascades function downstream of G-protein signaling and
cAMP/PKA pathways, thereby regulating appressorial for-
mation and pathogenicity on tomato.

A few fungal protease-encoding genes of *S. lycopersici*
were identified as being induced during pathogenesis on
tomato in our study. The correlation between proteolytic
activity and pathogenicity has been shown for several phyto-
pathogenic fungi in the establishment of disease [57, 58].
The subtilisin-like serine proteases FgPrb1 of *Fusarium gra-
minearum* and Aaprb1 of *Alternaria alternata* are required
for fungal pathogenesis [59, 60]. In addition, several fungal
secreted proteases and metalloproteases possess functions that
cleave specific plant chitinases. For example, both the secreted
metalloprotease FoMep1 and the serine protease FoSep1 dis-
covered from tomato pathogen *F. oxysporum* f. sp. *lycopersici*
are responsible for reducing the chitinase activity in tomato
This suggests that during pathogenesis, S. lycopersici may upregulate its arsenals to protect its hyphae from degradation by the host defensive cell wall-degrading enzymes.

5. Conclusions

In conclusion, here, the RNA-seq of S. lycopersici from tomato was presented, and a large number of candidate pathogenicity determinants of S. lycopersici were found that may play crucial roles during the establishment of tomato gray leaf spot disease. However, the functions of these genes need to be confirmed using homologous recombination, insertional mutagenesis, and RNAi-based gene silencing. Our results not only facilitate an understanding of the pathogenic molecular mechanisms of S. lycopersici in tomato but also lay a foundation for disease control.

Data Availability

The RNA-seq data has been deposited in China National GeneBank (CNGB, https://www.cngb.org/index.html) under project number CNP0002533.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Materials

Table S1: the upregulated genes involved in CWDEs. Table S2: the KEGG analysis of the upregulated genes enriched in metabolic pathways associated with the focal adhesion pathway in 36 hpi-vs-Con. Table S3: the upregulated genes involved in signal reception and regulation. Table S4: the upregulated genes associated with fungal proteases. (Supplementary Materials)

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