# Comparative transcriptome analysis reveals the response mechanism of Cf-*16*-mediated resistance to *Cladosporium fulvum* infection in tomato

Dongye Zhang[1], Yufang Bao[1], Yaoguang Sun[1], Huanhuan Yang[1], Tingting Zhao[1], Huijia Li[1], Chong Du[1], Jingbin Jiang[1], Jingfu Li[1], Libo Xie[2] and Xiangyang Xu[1*]

## Abstract

**Background:** Leaf mold disease caused by *Cladosporium fulvum* is a serious threat affecting the global production of tomato. Cf genes are associated with leaf mold resistance, including *Cf-16*, which confers effective resistance to leaf mold in tomato. However, the molecular mechanism of the Cf-*16*-mediated resistance response is largely unknown.

**Results:** We performed a comparative transcriptome analysis of *C. fulvum*-resistant (cv. Ontario7816) and *C. fulvum*-susceptible (cv. Moneymaker) tomato cultivars to identify differentially expressed genes (DEGs) at 4 and 8 days post inoculation (dpi) with *C. fulvum*. In total, 1588 and 939 more DEGs were found in Cf-*16* tomato than in Moneymaker at 4 and 8 dpi, respectively. Additionally, 1350 DEGs were shared between the 4- and 8-dpi Cf-*16* groups, suggesting the existence of common core DEGs in response to *C. fulvum* infection. The up-regulated DEGs in Cf-*16* tomato were primarily associated with defense processes and phytohormone signaling, including salicylic acid (SA) and jasmonic acid (JA). Moreover, SA and JA levels were significantly increased in Cf-*16* tomato at the early stages of *C. fulvum* infection. Contrary to the previous study, the number of up-regulated genes in Cf-*16* compared to Cf-*10* and Cf-*12* tomatoes was significantly higher at the early stages of *C. fulvum* infection.

**Conclusion:** Our results provide new insight into the Cf-mediated mechanism of resistance to *C. fulvum*, especially the unique characteristics of Cf-*16* tomato in response to this fungus.

**Keywords:** Comparative transcriptome, *Cladosporium fulvum*, Cf-16 tomato, Molecular mechanism

## Background

Tomato (*Solanum lycopersicum* L.) is the second most important horticultural crop worldwide [1, 2] and an important model plant for fleshy fruit development and plant-pathogen interactions. Leaf mold disease caused by *Cladosporium fulvum* is considered to be one of the most devastating diseases in tomato. *C. fulvum* is a non-obligate, abiotrophic pathogenic fungus that infects foliage and occasionally petioles and stems [3–5]. Leaf mold

has long been prevalent in many countries and caused serious economic loss, especially under high-temperature and high-humidity conditions [6]. Currently, the most effective way is to cultivate *C. fulvum*-resistant tomato varieties with resistance genes.

From a coevolutionary perspective, plants recognize and respond to pathogens in several phases. In the first phase, pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) in plants, inducing PAMP-triggered immunity (PTI) and preventing pathogen colonization [7, 8]. In the second phase, successful pathogens bypass PTI and secrete effectors into plant cells, and the effector-triggered susceptibility response (ETS) ensues. In the third phase, plants

* Correspondence: xxy709@126.com
[1]Laboratory of Genetic Breeding in Tomato, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Northeast Region), Ministry of Agriculture and Rural Affairs, College of Horticulture and Landscape Architecture, Northeast Agricultural University, Harbin 150030, China
Full list of author information is available at the end of the article

gradually evolve to produce NB-LRR (nucleotide-binding site and leucine-rich repeat) proteins that directly or indirectly recognize specific pathogen effectors, and induce effector-triggered immunity (ETI). Finally, pathogens successfully infect plants and induce ETS by inhibiting or altering effectors that may be recognized by the plant and generating new effectors that cannot be recognized by the plant NB-LRRs. Simultaneously, plants again induce ETI by generating new R genes that encode proteins capable of identifying new effectors [9–11].

With respect to plant-pathogen interactions, different pathogens carry avirulence (AVR) genes corresponding to plant R genes and encode proteins that are recognized by effector proteins [12]. These proteins are secreted into the apoplastic space during infection and induce either compatible or incompatible interactions between fungi and infected plants [13]. Incompatible interactions (chlorosis) lead to the hypersensitive response (HR) when plants resist pathogens; compatible interactions occur when the pathogens can grow and ramify, causing necrosis in infected cells [14–17]. The tomato-C. fulvum interaction follows a typical gene-for-gene relationship, and the products of C. fulvum-resistance genes (Cf genes) in tomato specifically recognize the products encoded by the AVR genes in C. fulvum, leading to HR [18, 19]. At least 24 Cf genes have been reported since the discovery of the Cf-1 gene in the 1930s [20, 21], and these genes have been introduced into cultivated tomatoes [22–30].

Transcriptome sequencing (RNA-Seq) has strongly accelerated research on host-pathogen interactions in plants such as rice [31], maize [32], cucumber [33], watermelon [34] and strawberry [35]. Avr4/Cf-4- and Avr9/Cf-9-dependent defense gene expression has been confirmed by cDNA-AFLP (cDNA-amplified fragment length polymorphism) analysis [36]. The Avr5 gene has been cloned through a combined bioinformatic and RNA-Seq-based transcriptome sequencing approach [18]. Cf-19-, Cf-12- and Cf-10-mediated resistance to C. fulvum in tomato has been characterized using cDNA-AFLP and RNA-Seq analyses [37–39]. However, few transcriptomic studies have examined Cf-16-mediated resistance.

In this study, in addition to performing a transcriptomic analysis, we measured the endogenous hormone levels of resistant and susceptible tomato cultivars in response to C. fulvum infection. Our results are not only useful for understanding the mechanism of Cf-mediated resistance to C. fulvum infection but also providing a basis for cloning of the Cf-16 gene.

## Results

### Microscopic analysis of C. fulvum invasion in two tomato cultivars

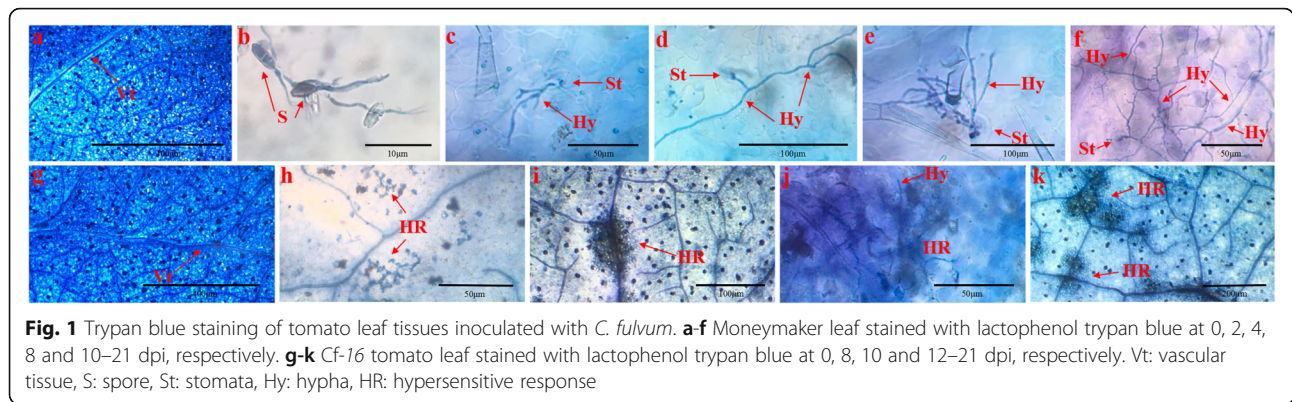The C. fulvum infection process in Cf-16 tomato or Moneymaker leaves was observed by light microscopy

(Fig. 1). As shown in Fig. 1a and g, no difference was found between Cf-16 tomato and Moneymaker at 0 dpi. Our results showed that conidiospores germinated at 2–3 dpi (Fig. 1b), with hyphae growing into the stomata in both Moneymaker and Cf-16 tomato leaves at 4 dpi (Fig. 1c). The hyphae then emerged through the stomata of Moneymaker leaves at 8 dpi (Fig. 1d), with the growth and number of emergent hyphae continuing to increase through 10 dpi, and the last few infected cells starting to undergo necrosis at 10–21 dpi (Fig. 1e, f). In contrast, a small number of HR areas appeared at 8 dpi in Cf-16 tomato (Fig. 1h), which had gradually grown at 10 dpi (Fig. 1i). In addition, hyphal growth was restricted to necrotic areas (Fig. 1j) until more necrotic lesions appeared in both mesophyll cells and leaf veins between 12 and 21 dpi (Fig. 1k). Obviously, plants carrying the Cf-16 resistance gene showed a strong HR after infection with C. fulvum, whereas the susceptible plants (i.e., Moneymaker) showed continuous hyphal growth. Based on these observations, we collected samples from each treatment at 4 and 8 dpi for RNA-Seq and qRT-PCR (quantitative real-time PCR) analyses.

### Analysis of hormone response to C. fulvum infection

To explore hormone response to C. fulvum infection, HPLC-MS/MS (high-performance liquid chromatography-tandem mass spectrometry) was used to measure SA and JA levels. As shown in Fig. 2a, the SA content increased rapidly in Cf-16 tomato after inoculation, peaking from 4 to 8 dpi before decreasing to the original level at 8–16 dpi. Moreover, the SA content of Moneymaker gradually increased after inoculation, with a higher value than that of its control group at 0–3 dpi before decreasing to the minimum level observed at 12 dpi. In Cf-16 tomato, the JA content rapidly increased after inoculation, peaking from 0 to 3 dpi, and then rapidly decreased between 4 and 21 dpi; however, the JA level was generally higher than that detected for the control groups of Cf-16 tomato and Moneymaker after infection (from 2 to 16 dpi). In Moneymaker, the JA content increased after infection to a maximum at 3 dpi and then gradually decreased (Fig. 2b). Overall, the SA and JA levels in Moneymaker were greater after infection than those observed in its control group at 0–3 dpi. These results suggest that SA and JA levels rapidly increase during the early stages of infection and that these hormones play important roles in regulating the plant response to the pathogen and enhancing the defense of Cf-16 tomato infected with C. fulvum.

### RNA sequencing and transcript identification

To obtain transcriptome profiles of Cf-16 tomato and Moneymaker following C. fulvum infection, we performed RNA-Seq analysis at 4 and 8 dpi, with three biological replicates performed at each time point for each

**Fig. 1** Trypan blue staining of tomato leaf tissues inoculated with *C. fulvum*. **a-f** Moneymaker leaf stained with lactophenol trypan blue at 0, 2, 4, 8 and 10–21 dpi, respectively. **g-k** Cf-*16* tomato leaf stained with lactophenol trypan blue at 0, 8, 10 and 12–21 dpi, respectively. Vt: vascular tissue, S: spore, St: stomata, Hy: hypha, HR: hypersensitive response

treatment. In this study, an average of ~ 6.87 Gb of clean data were generated for each sample using the BGISEQ-500 platform (Additional file 9: Figure S1, Additional file 1: Table S1). As shown in Additional file 1: Table S1, the quality scores of more than 98% of the reads were ≥ 20%, and more than 91% of the clean reads had quality scores of ≥30%. After the reads were filtered, 64.15–72.64 million clean reads were generated, and at least 93.29% of these reads were mapped to the tomato reference genome, among which more than 78.26% were aligned to unique locations. Ultimately, 18,514 novel transcripts were obtained, with 12,790 unknown splicing events in known genes, 2047 novel coding transcripts without any known features, and 3677 transcripts for long noncoding RNAs.

### DEGs in response to *C. fulvum*

DEGs from Cf-*16* tomato and Moneymaker in response to *C. fulvum* at 4 and 8 dpi were identified based on an adjusted *P*-value of ≤0.001 and a $\log_2$ fold change of ≥2. FPKM (fragments per kilobase of exon per million fragments mapped) values for all genes and the fold changes and adjusted *P*-values for DEGs are shown in Additional file 2: Table S2 and Additional file 3: Table S3, respectively.

In the control groups, 3298 and 2464 DEGs were observed between Cf-*16* tomato and Moneymaker at 4 and 8 dpi, respectively. Among the samples collected after infection with *C. fulvum* at 4 and 8 dpi, 2242 and 3095 DEGs were identified between Cf-*16* tomato and Moneymaker, respectively (Table 1). Compared with the respective control groups, 8526 DEGs were identified in Cf-*16* tomato (including 5110 up-regulated and 3416 down-regulated genes) at 4 dpi, 6938 in Moneymaker (including 4213 up-regulated and 2725 down-regulated genes) at 4 dpi, 3711 in Cf-*16* tomato (including 1609 up-regulated and 2102 down-regulated) at 8 dpi, and 2772 in Moneymaker (including 757 up-regulated and 2015 down-regulated genes) at 8 dpi.

Numerous DEGs were detected at 4 dpi in both cultivars (Table 1); however, we also noted a number of DEGs between the control groups for both cultivars (CK_MM_4dpi-vs-CK_Cf_4dpi and CK_MM_8dpi-vs-CK_Cf_8dpi). Furthermore, 707 DEGs were shared by the control and inoculation groups of Moneymaker and Cf-*16* tomato (Fig. 3c). Thus, some of the DEGs in each comparison may not be associated with leaf mold resistance. Notably, 306 DEGs overlapped between Cf-*16* tomato and Moneymaker only at 4 dpi (CK_Cf_4dpi-vs-Cf_4dpi and MM_4dpi-vs-Cf_4dpi) (Fig. 3a), whereas



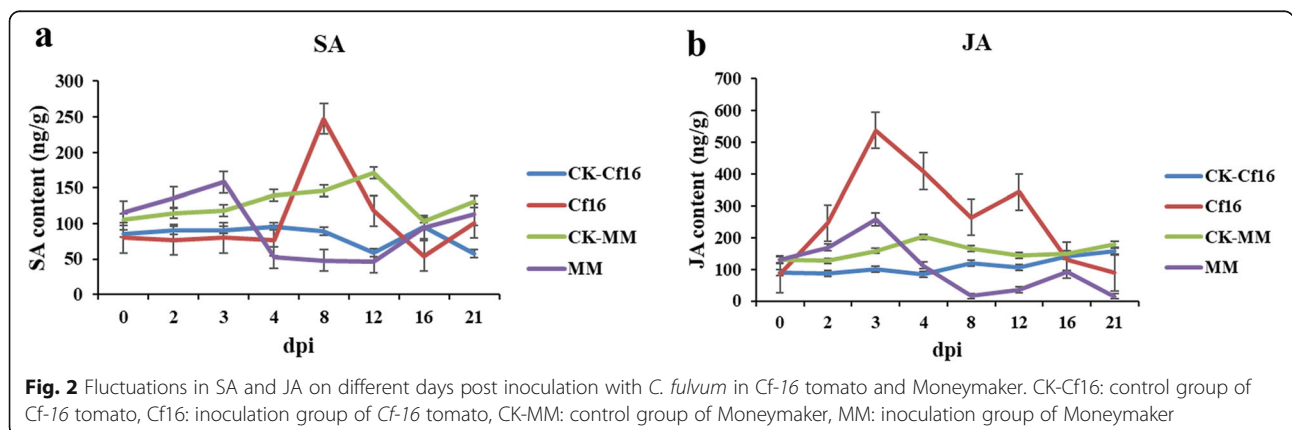**Fig. 2** Fluctuations in SA and JA on different days post inoculation with *C. fulvum* in Cf-*16* tomato and Moneymaker. CK-Cf16: control group of Cf-*16* tomato, Cf16: inoculation group of *Cf-16* tomato, CK-MM: control group of Moneymaker, MM: inoculation group of Moneymaker

**Table 1** DEGs identified from different comparisons

| DEG set | Total DEGs | Upregulated | Downregulated |
|---|---|---|---|
| CK_Cf_4dpi-vs-Cf_4dpi | 8526 | 5110 | 3416 |
| CK_Cf_8dpi-vs-Cf_8dpi | 3711 | 1609 | 2102 |
| CK_MM_4dpi-vs-MM_4dpi | 6938 | 4213 | 2725 |
| CK_MM_8dpi-vs-MM_8dpi | 2772 | 757 | 2015 |
| CK_MM_4dpi-vs-CK_Cf_4dpi | 3298 | 1922 | 1376 |
| CK_MM_8dpi-vs-CK_Cf_8dpi | 2464 | 1361 | 1103 |
| MM_4dpi-vs- Cf_4dpi | 2242 | 1211 | 1031 |
| MM_8dpi-vs- Cf_8dpi | 3095 | 2043 | 1052 |

541 DEGs overlapped only at 8 dpi (CK_Cf_8dpi-vs-Cf_ 8dpi and MM_8dpi-vs-Cf_8dpi) (Fig. 3b). Based on these stricter criteria, these DEGs are likely the most promising candidates involved in leaf mold resistance; accordingly, these DEGs were investigated further.
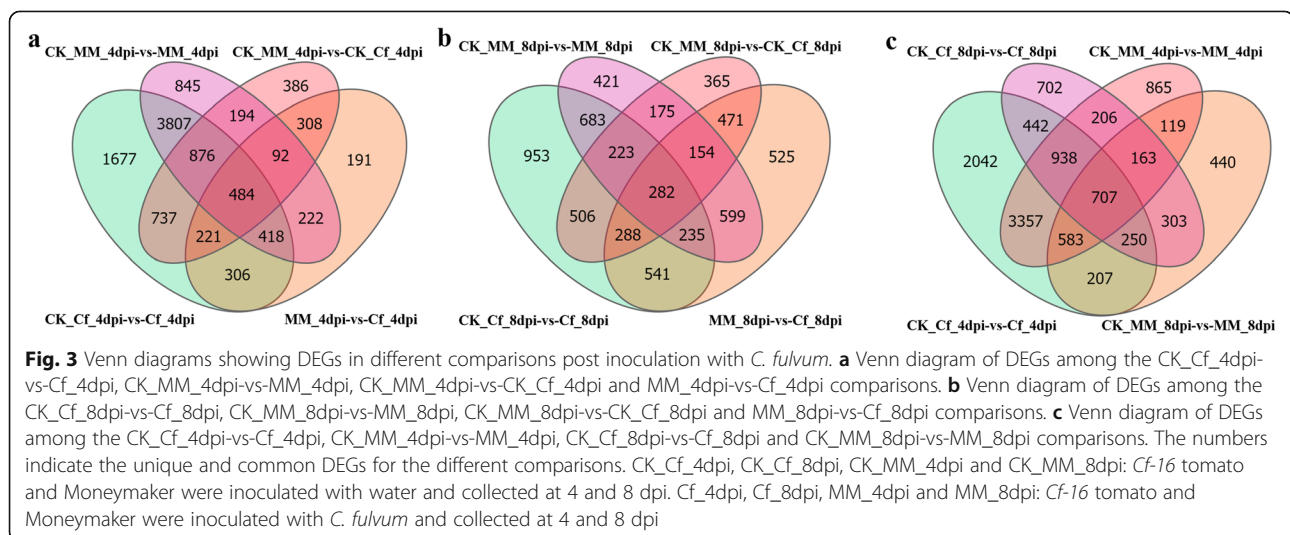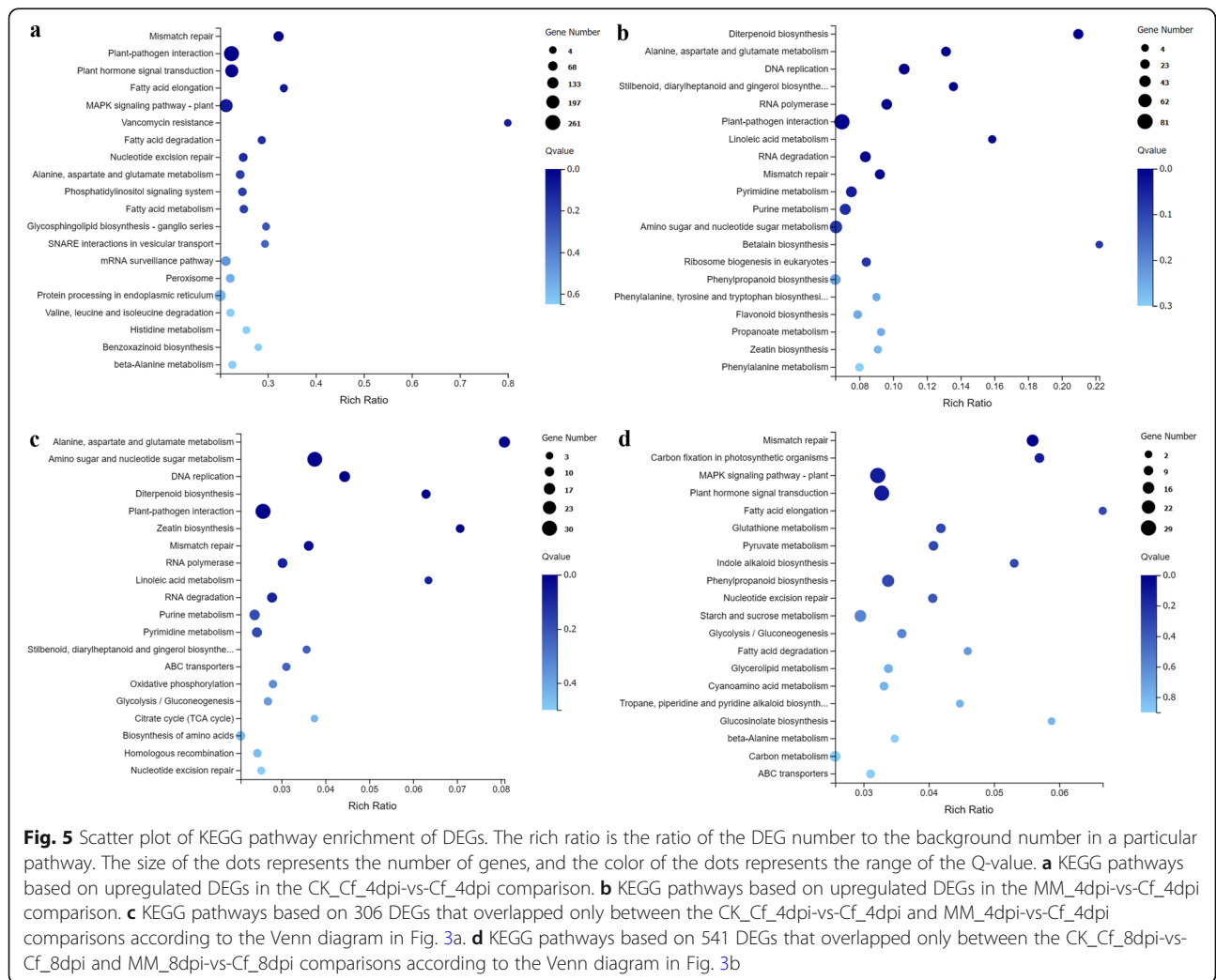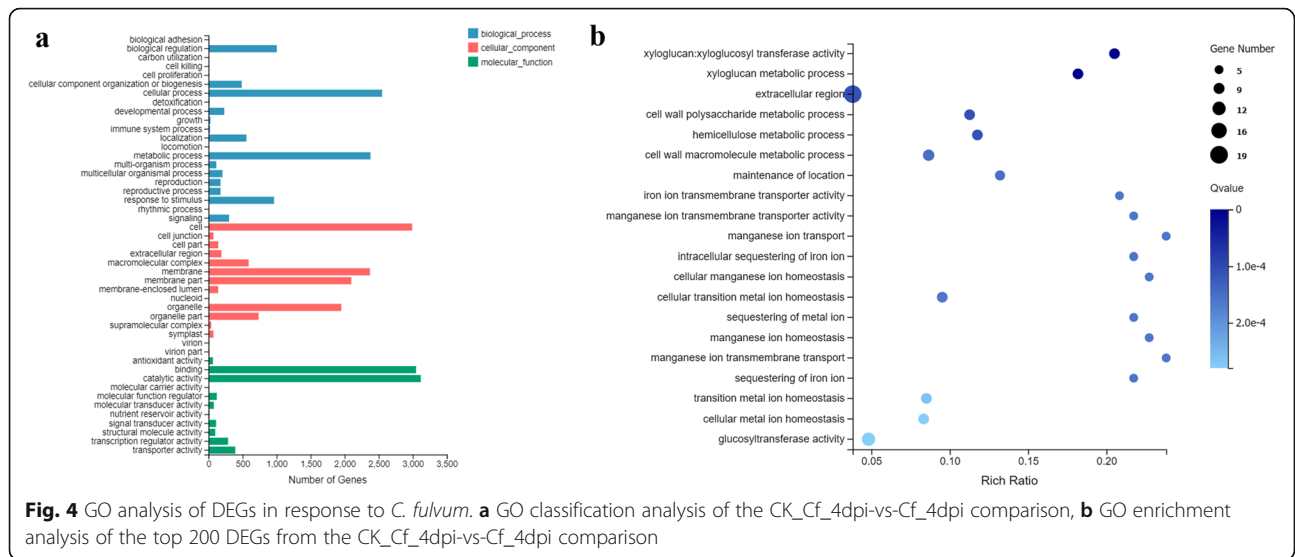
### GO and KEGG enrichment analyses of DEGs

To determine the functions of DEGs involved in the response to *C. fulvum*, we performed GO (Gene Ontology) classification and KEGG (Kyoto Encyclopedia of Gene and Genomes) functional enrichment analyses using the Phyper function in R software [40]. For DEGs detected in Cf-*16* tomato, significant GO terms were primarily enriched in "biological regulation", "cellular process", "metabolic process" and "response to stimulus" in the biological process category, and these terms are associated with disease resistance. In the cellular component ontology, "cell", "membrane", "membrane part" and "organelle" were the most abundant categories that were specific to the resistant tomato cultivar. Genes involved in "binding", "catalytic activity", "transcription regulator activity" and "transporter activity" were enriched in the molecular function category. Notably, the terms "binding" and "catalytic activity" are known to play important roles in plant hormone signal transduction (Fig. 4a).

Subsequently, the top 200 DEGs between *Cf-16* tomato and its control group at 4 dpi (CK_Cf_4dpi-vs-Cf_4dpi) were selected for GO enrichment analysis to better understand the Cf-*16*-mediated resistance response to *C. fulvum*. As shown in Fig. 4b, the most highly enriched GO terms were those associated with the organization of the cell wall or the metabolism of its components, including "xyloglucan: xyloglucosyl transferase activity", "xyloglucan metabolic process", "cell wall polysaccharide metabolic process" and "hemicellulose metabolic process". As the first barrier to invasion, the cell wall is the first obstacle for most pathogens [41, 42]. Therefore, DEGs associated with these significant terms may play important roles against *C. fulvum* infection in Cf-*16* tomato.

KEGG pathway enrichment analysis was also performed to investigate the biological pathways underlying the incompatible interaction. As shown in Fig. 5a, the pathways "Plant hormone signal transduction" and "Plant-pathogen interaction" were significantly enriched (in the figure, the color of each dot indicates the Q-value, and the standard for significant enrichment is Q-value ≤0.01). In addition, "Fatty acid metabolism" and "Phosphatidylinositol signaling system" were found to be related to the Cf-*16* tomato response to *C. fulvum* infection. Furthermore, 34 disease-resistance genes (Additional file 6: Table S6) and 32 DEGs (Additional file 7: Table S7) were identified in the significantly enriched KEGG pathways "Plant-pathogen interaction" and "Plant hormone signal transduction", respectively. In summary, the most highly enriched pathways, "Plant hormone signal transduction" and "Plant-pathogen interaction", may be the major metabolic pathways involved in the Cf-*16*-mediated resistance response to *C. fulvum*.



**Fig. 3** Venn diagrams showing DEGs in different comparisons post inoculation with *C. fulvum*. **a** Venn diagram of DEGs among the CK_Cf_4dpi-vs-Cf_4dpi, CK_MM_4dpi-vs-MM_4dpi, CK_MM_4dpi-vs-CK_Cf_4dpi and MM_4dpi-vs-Cf_4dpi comparisons. **b** Venn diagram of DEGs among the CK_Cf_8dpi-vs-Cf_8dpi, CK_MM_8dpi-vs-MM_8dpi, CK_MM_8dpi-vs-CK_Cf_8dpi and MM_8dpi-vs-Cf_8dpi comparisons. **c** Venn diagram of DEGs among the CK_Cf_4dpi-vs-Cf_4dpi, CK_MM_4dpi-vs-MM_4dpi, CK_Cf_8dpi-vs-Cf_8dpi and CK_MM_8dpi-vs-MM_8dpi comparisons. The numbers indicate the unique and common DEGs for the different comparisons. CK_Cf_4dpi, CK_Cf_8dpi, CK_MM_4dpi and CK_MM_8dpi: Cf-*16* tomato and Moneymaker were inoculated with water and collected at 4 and 8 dpi. Cf_4dpi, Cf_8dpi, MM_4dpi and MM_8dpi: Cf-*16* tomato and Moneymaker were inoculated with *C. fulvum* and collected at 4 and 8 dpi

Zhang et al. BMC Plant Biology        (2020) 20:33

Page 5 of 16



Fig. 4 GO analysis of DEGs in response to *C. fulvum*. **a** GO classification analysis of the CK_Cf_4dpi-vs-Cf_4dpi comparison, **b** GO enrichment analysis of the top 200 DEGs from the CK_Cf_4dpi-vs-Cf_4dpi comparison



Fig. 5 Scatter plot of KEGG pathway enrichment of DEGs. The rich ratio is the ratio of the DEG number to the background number in a particular pathway. The size of the dots represents the number of genes, and the color of the dots represents the range of the Q-value. **a** KEGG pathways based on upregulated DEGs in the CK_Cf_4dpi-vs-Cf_4dpi comparison. **b** KEGG pathways based on upregulated DEGs in the MM_4dpi-vs-Cf_4dpi comparison. **c** KEGG pathways based on 306 DEGs that overlapped only between the CK_Cf_4dpi-vs-Cf_4dpi and MM_4dpi-vs-Cf_4dpi comparisons according to the Venn diagram in Fig. 3a. **d** KEGG pathways based on 541 DEGs that overlapped only between the CK_Cf_8dpi-vs-Cf_8dpi and MM_8dpi-vs-Cf_8dpi comparisons according to the Venn diagram in Fig. 3b

Zhang *et al. BMC Plant Biology*       (2020) 20:33

Page 6 of 16

Up-regulated genes involved in the "Plant-pathogen interaction" pathway were also significantly enriched between Moneymaker and Cf-16 tomato (Fig. 5b, Additional file 8: Table S8). Thus, we performed KEGG pathway analysis for 306 and 541 DEGs that overlapped between Cf-16 tomato and Moneymaker only at 4 and 8 dpi, respectively (Fig. 3a and b). Interestingly, the pathways "Plant-pathogen interaction" and "Plant hormone signal transduction" were significantly enriched (Fig. 5c and d). Some disease-resistance genes involved in "Plant-pathogen interaction" and six hormone-related genes involved in "Plant hormone signal transduction" are listed in Table 2 and Table 3, respectively. These DEGs may constitute the most promising candidates involved in leaf mold resistance. These results indicate once again that plant hormones play a key role in the Cf-16 tomato response to C. fulvum infection.

## Analysis of metabolism and regulatory pathways

To obtain an overview of the regulatory pathways induced by C. fulvum, DEGs were visualized via MapMan analysis. According to the results (Fig. 6a), the majority of DEGs were upregulated and functionally enriched in transcription factors (TFs), including receptor kinases. Other pathways, including "calcium regulation" and "light", were also upregulated or downregulated in response to C. fulvum infection. Most DEGs were related to hormones associated with the upregulation of IAA (indole-3-acetic acid), ABA (abscisic acid) and ethylene. In fact, the upregulated genes were classified as R genes, MAPKs, PR proteins, TFs and genes associated with the hormones ethylene, ABA, SA and JA, further supporting the importance of these pathways in the Cf-16-mediated

resistance response to C. fulvum infection (Fig. 6b, Additional file 4: Table S4).

## Gene co-expression network analysis

Weighted gene co-expression network analysis (WGCNA) is a common algorithm used in transcriptomic studies [43]. Thirteen different modules were obtained using a gene dendrogram colored according to correlations between gene expression levels (Fig. 7a). Among them, the genes in MEred and MEgreenyellow were highly expressed in Cf-16 tomato at 4 dpi, whereas those in MEpurple exhibited relatively high expression in both Cf-16 tomato and Moneymaker at 4 dpi (Fig. 7b). We performed KEGG analysis for these three modules. For the MEred module, pathways related to "Plant-pathogen interaction", "Oxidative phosphorylation" and "Phenylalanine, tyrosine and tryptophan biosynthesis" were enriched; for MEgreenyellow, pathways related to "Pentose phosphate pathway", "Flavonoid biosynthesis", "Phenylpropanoid biosynthesis" and "Plant hormone signal transduction" were enriched (Additional file 10: Figure S2). Notably, some DEGs of the pathway "Plant-pathogen interaction" in the MEred module were also present in Additional file 8: Table S8 (DEGs in the significantly enriched KEGG pathway "Plant-pathogen interaction" between MM_4dpi-vs-Cf_4dpi and CK_Cf_4dpi-vs-Cf_4dpi). Therefore, these disease-resistance genes should be studied in greater depth in the future to elucidate their role in the Cf-16-mediated resistance response to C. fulvum infection in tomato.

## Validation of RNA-Seq data by qRT-PCR

To verify the RNA-Seq data, 16 DEGs were chosen for qRT-PCR; three biological replicates were performed.

**Table 2** DEGs in the significantly enriched KEGG pathway "Plant-pathogen interaction" based on 306 common DEGs that overlapped only between the CK_Cf_4dpi-vs-Cf_4dpi and MM_4dpi-vs-Cf_4dpi comparisons according to the Venn diagram in Fig. 3a

| Gene ID | Gene definition | $Log_2$ Fold-change | |
| --- | --- | --- | --- |
| | | CK_Cf_4dpi-vs-Cf_4dpi | MM_4dpi-vs-Cf_4dpi |
| 101,246,100 | putative ATPase | 2.22 | 4.99 |
| 101,251,989 | disease resistance protein | 1.44 | 2.36 |
| 101,253,178 | disease resistance protein RPM1 | 2.42 | 4.89 |
| 101,256,988 | glucosamine---fructose-6-phosphate aminotransferase (isomerizing) | 1.74 | 5.88 |
| 101,258,758 | LRR receptor-like serine/threonine-protein kinase FLS2 | 1.77 | 1.60 |
| 101,263,364 | putative ATPase | 3.26 | 7.32 |
| 101,263,890 | tubulin-folding cofactor B | 1.26 | 2.13 |
| 109,118,687 | disease resistance protein | 2.34 | 1.61 |
| 109,120,689 | disease resistance protein RPM1 | 2.94 | 5.15 |
| 109,121,092 | LRR receptor-like serine/threonine-protein kinase FLS2 | 3.45 | 2.36 |
| BGI_novel_G001085 | 5′-AMP-activated protein kinase, catalytic alpha subunit | 1.80 | 2.34 |
| BGI_novel_G001591 | disease resistance protein RPM1 | 2.83 | 2.76 |

**Table 3** DEGs in the significantly enriched KEGG pathway "Plant hormone signal transduction" based on 541 common DEGs that overlapped only between the CK_Cf_8dpi-vs-Cf_8dpi and MM_8dpi-vs-Cf_8dpi comparisons according to the Venn diagram in Fig. 3b

| Gene ID | Gene definition | Log$_2$ Fold-change | |
|---|---|---|---|
| | | CK_Cf_8dpi-vs-Cf_8dpi | MM_8dpi-vs-Cf_8dpi |
| 101,245,668 | xyloglucan:xyloglucosyl transferase TCH4 | 4.16 | 2.63 |
| 101,251,578 | aprataxin | 3.19 | 2.20 |
| 101,262,506 | arabidopsis histidine kinase 2/3/4 (cytokinin receptor) | 1.32 | 2.13 |
| 101,263,609 | disease resistance protein RPM1 | 1.70 | 1.54 |
| 101,264,326 | SAUR family protein | 1.75 | 3.76 |
| 104,649,076 | auxin responsive GH3 gene family | 4.19 | 2.62 |

These 16 genes were selected from significantly enriched KEGG pathways (such as "Plant hormone signal transduction", "Plant-pathogen interaction" and "Metabolic pathways"). The expression data obtained by qRT-PCR were consistent with the RNA-Seq results, indicating a similar trend between the transcriptome and qRT-PCR datasets (Fig. 8). Among the 16 DEGs, a significantly up-regulated gene with ID 101247936 (Fig. 8h) was predicted to encode a jasmonate-ZIM-domain-containing protein in the "Plant hormone signal transduction" pathway, paralleling the JA response to *C. fulvum* infection (Fig. 2b). Similarly, the expression level of gene 100,736, 444 (Fig. 8i), which encodes the disease-resistance protein RPM1, was increased at least 27-fold in Cf-16 tomato. In addition, the expression levels of gene 101,259, 487 (Fig. 8m), which encodes a peroxidase, and gene 101,256,817 (Fig. 8b), which encodes the calcium binding protein CML, were increased at least 11-fold.
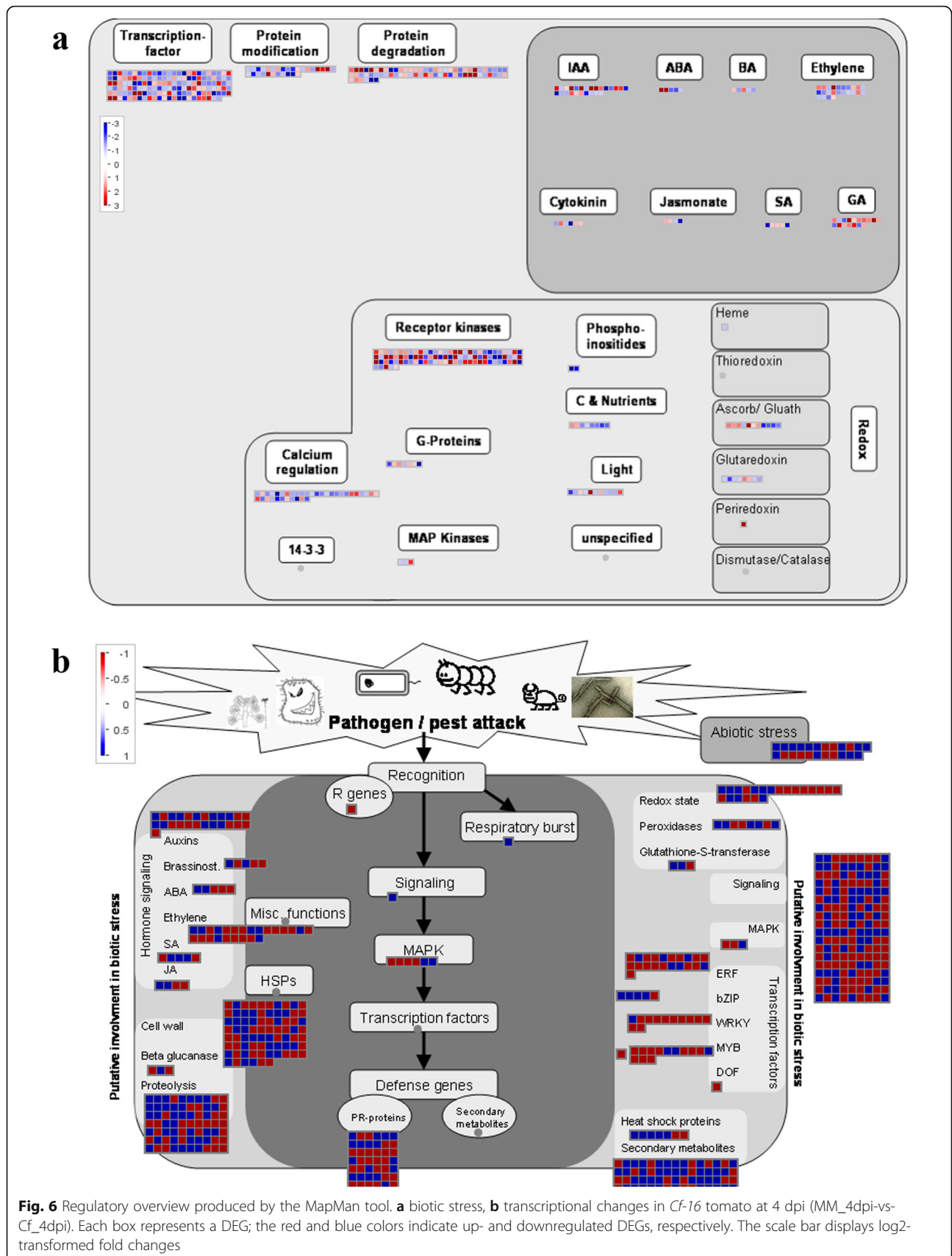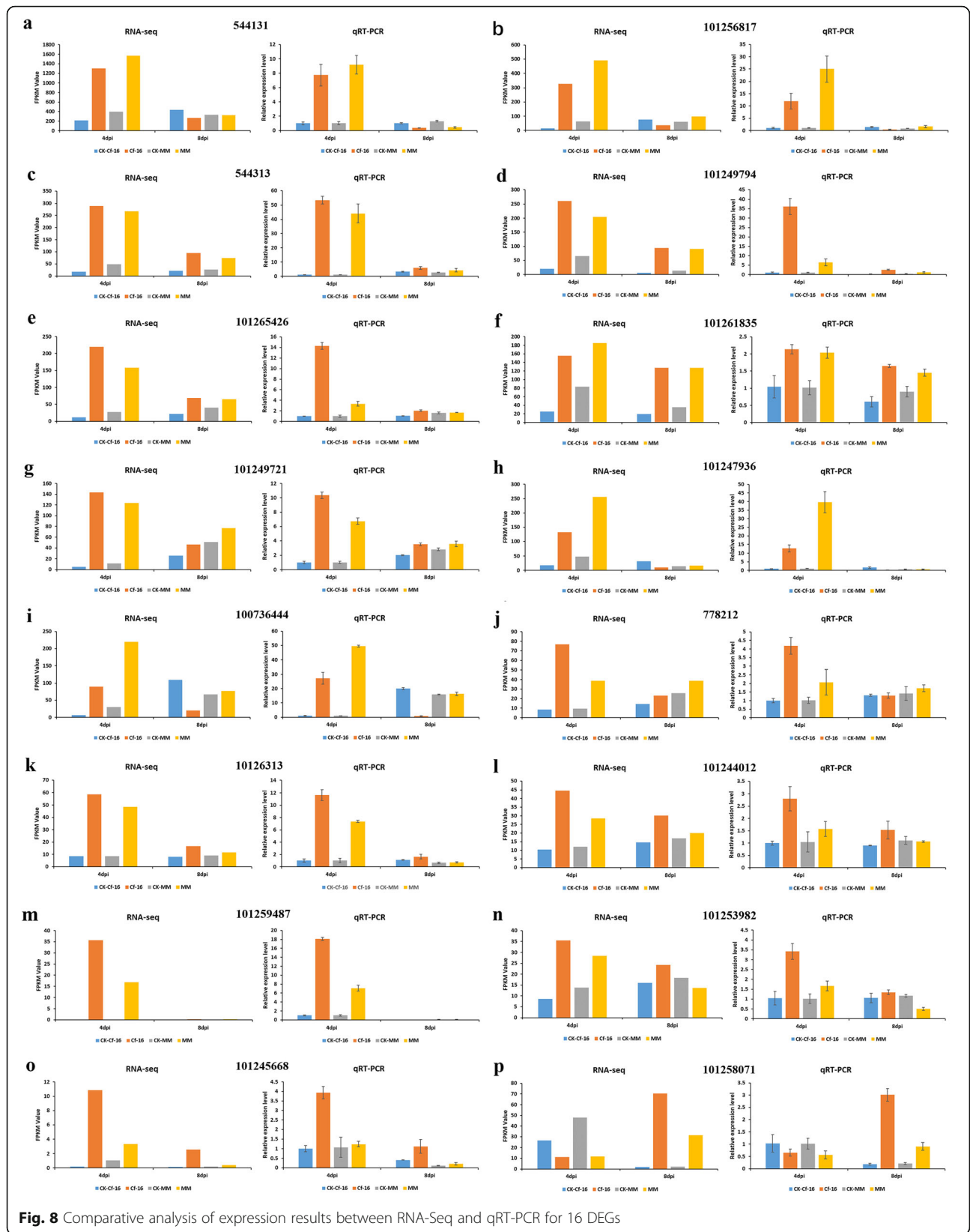
## Discussion

In this study, we characterized the interaction between *C. fulvum* and Cf-16 tomato or Moneymaker through microscopic observations. HR was observed in Cf-16 tomato at 8 dpi, whereas in Moneymaker, hyphae emerged through the stomata and continued to increase in number and grow at 8 dpi (Fig. 1). Systemic defense response mediated by resistance genes was activated during the early stage of *C. fulvum* infection. This finding was consistent with previous studies of other Cf genes [37–39]. Based on comparative transcriptome analysis, we demonstrated the resistance response to *C. fulvum* in Cf-16 tomato. In response to *C. fulvum* infection, drastic transcriptional changes occurred at 4 dpi in both tomato cultivars, although a number of DEGs were also detected between the respective control groups (Table 1). Because these results suggested that many of the DEGs in each comparison may not be linked to resistance, we screened resistance genes in Cf-16 tomato more stringently by comparing the transcriptomes of the control groups. Significant GO terms primarily included "biological regulation", "cellular process", "metabolic process" and

"response to stimulus" in the biological process category, and these terms are associated with disease resistance. Furthermore, KEGG enrichment analysis indicated that most of the DEGs were classified into "Plant hormone signal transduction" and "Plant-pathogen interaction". In this preliminary comparison, more up-regulated DEGs were detected in Cf-16 compared to Cf-10 and Cf-12 tomatoes during the early stage of *C. fulvum* infection [38, 39]. A comprehensive comparative analysis of Cf-19, Cf-12, Cf-10 and Cf-16 will provide important information for further exploration of the mechanism of Cf-gene-mediated resistance response to *C. fulvum* infection.

Plants have a series of defense mechanisms to respond to pathogen attack. PRRs are the first line of defense [44, 45]; these receptors recognize *C. fulvum* and activate a resistance response [46]. In our study, chitin elicitor receptor kinase 1 (CERK1; BGI_novel_G000519), a pattern recognition protein, was significantly up-regulated in *Cf-16* tomato at 4 dpi (Additional file 6: Table S6). This result was consistent with the study by Xue et al. (2017) on Cf-12. We will investigate whether increased expression of CERK1 is associated with the activation of chitin signaling and determine whether this increase affects the tomato-*C. fulvum* interaction process.

After recognizing the infection, Cf-16 tomato quickly activated a complex series of defense-associated signaling pathways. Ca$^{2+}$ influx is considered to play a key role in the early downstream response of numerous PAMP sensing processes, resulting in local and systemic acquired resistance [47, 48]. Ca$^{2+}$ activates calcium-dependent protein kinases (CDPKs), which play important roles in plant responses to both abiotic stress and pathogens [49, 50]. In our study, CDPKs (101,249,495, 101,055,527 and 101,255,379) were expressed at high levels in Cf-16 tomato during the early stage of infection (Additional file 2: Table S2). This result was consistent with those of previous studies, suggesting that these genes play crucial roles in the Cf-16-mediated resistance response to *C. fulvum* infection [51]. In addition, the binding of Ca$^{2+}$ to CML results in the production of nitric oxide (NO), which further promotes plant HR or
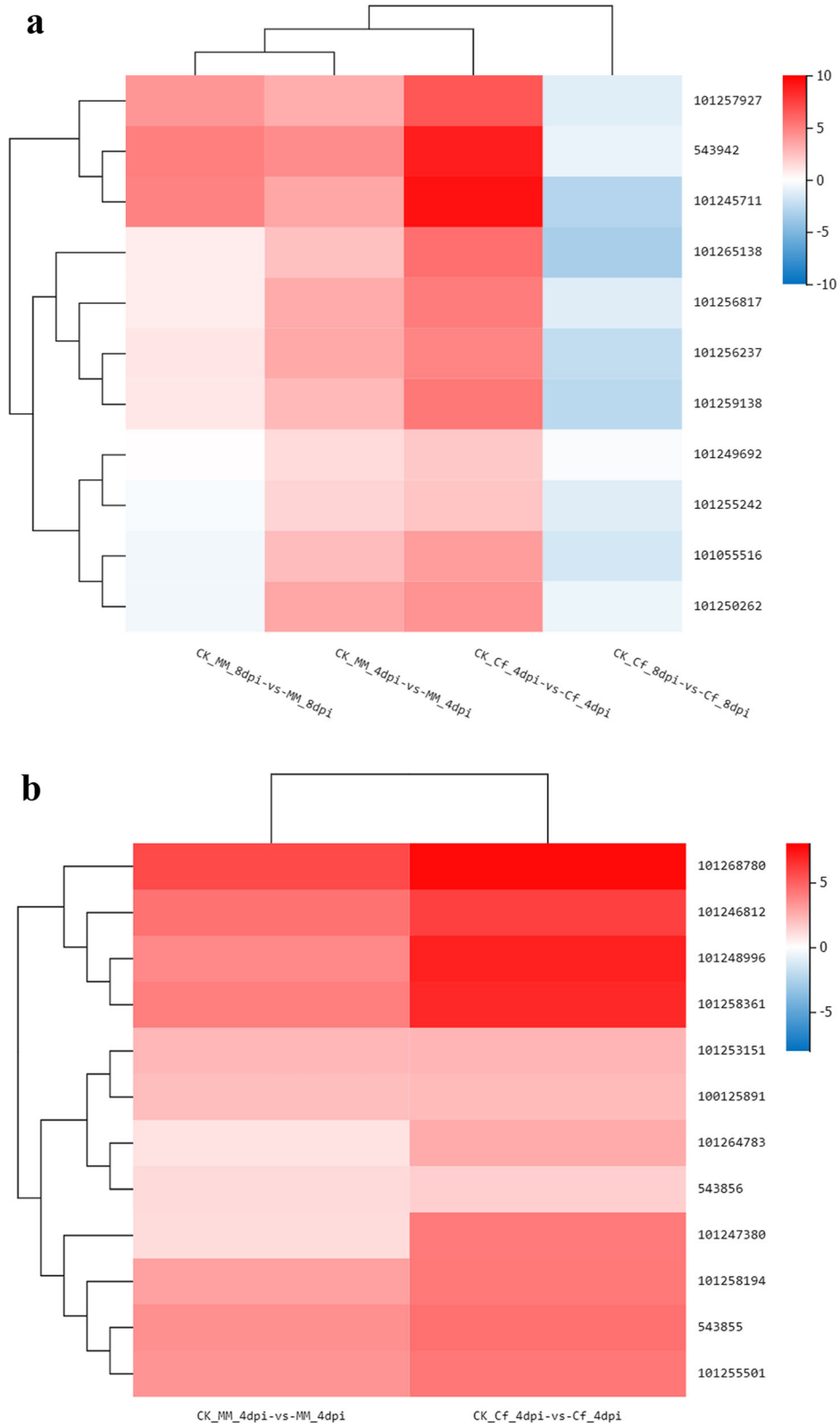
**Fig. 6** Regulatory overview produced by the MapMan tool. **a** biotic stress, **b** transcriptional changes in *Cf-16* tomato at 4 dpi (MM_4dpi-vs-Cf_4dpi). Each box represents a DEG; the red and blue colors indicate up- and downregulated DEGs, respectively. The scale bar displays log2-transformed fold changes

**Fig. 7** Gene co-expression network analysis by WGCNA. **a** Gene dendrogram colored according to correlations between gene expression levels. Different colors represent different gene modules and indicate coefficients of dissimilarity between genes. **b** Module-sample association. The abscissa represents the samples; the ordinate represents the modules. The numbers in each cell are the correlation coefficient (top) and *P*-value (bottom). The variation from blue (low) to orange (high) indicates the ranges of the DEGs

autoimmune reactions [52]. Interestingly, the results of our study showed that 11 CML genes were significantly expressed at 4 dpi in Cf-*16* tomato compared with Moneymaker (Fig. 9a). In particular, the genes with IDs 543,942 and 101,245,711 were up-regulated approximately eight-fold in Cf-*16* tomato compared with Moneymaker. Based on these results, we propose that these CML genes are involved in the Cf-*16* tomato defense response against *C. fulvum*. Similarly, Ranty (2016) demonstrated that CML genes play crucial roles in plant responses to both abiotic stress and pathogens [53]. FLS2 recognizes flg22 and subsequently activates downstream signaling pathways that involve WRKY TFs to promote defense responses against bacterial and

fungal pathogens and nematodes [54, 55]. In our study, 12 WRKY genes were specifically up-regulated at 4 dpi, as shown by the hierarchical clustering of DEGs in both tomato cultivars (Fig. 9b). Among them, the genes with IDs 101,268,780, 101,258,361, 101,248,996 and 101,246,812 were up-regulated more than six-fold in Cf-*16* tomato compared with Moneymaker. These results suggest that these WRKY genes may activate a series of downstream PR genes and thus play pivotal roles in the resistance response of Cf-*16* tomato to *C. fulvum*. Our results also showed that PR-1 genes (IDs 544,123 and 100,191,111) were significantly up-regulated in Cf-*16* tomato after inoculation (Additional file 6: Table S6). Overall, our results suggest that PRRs activate and promote the expression of downstream CDPKs, CMLs and WRKY

**Fig. 8** Comparative analysis of expression results between RNA-Seq and qRT-PCR for 16 DEGs

**Fig. 9 a** Differentially expressed CML genes in *Cf-16* tomato and Moneymaker. **b** Differentially expressed WRKY genes in *Cf-16* tomato and Moneymaker at 4 dpi

TFs; induce the accumulation of reactive oxygen species; and cause the deposition of cystatin in the cell wall, thereby inducing PTI in Cf-*16* tomato.

During long-term evolutionary interactions with plants, several pathogens successfully cause ETS by producing a number of effectors. Simultaneously, plants have evolved R genes that recognize these effectors and function through highly specific interactions between effectors and their corresponding NB-LRR class receptors. The rice CC-NB-LRR protein Pi-to can directly interact with Avr factors, which the LRR domain is able to directly recognize the effector Avrpita of *Magnaporthe oryzae* and induce ETI [56]. It has also been demonstrated that the NBS-LRR protein from *Arabidopsis thaliana* RPM1 confers resistance to *Pseudomonas syringae*. RPM1 is also involved in the onset of HR [16, 57]. Consistent with previous studies, our results showed that genes encoding RPM1 (101,261, 141, 100,736,444, 109,120,689, 101,253,178 and BGI_novel_G001591) were significantly up-regulated in Cf-*16* tomato at 4 dpi (Additional file 3: Table S3a). These genes may play a key role in the response of Cf-*16* tomato to *C. fulvum* infection. More importantly, the genes with IDs 109,120,689, 101,253,178 and BGI_novel_G001591 were identified as DEGs that overlapped between Cf-*16* tomato and Moneymaker only at 4 dpi (Table 2). These genes may be the most promising candidate genes involved in leaf mold resistance.

Phytohormones are known to be important in the regulation of defense responses in plants [58–60]. SA, a crucial regulator of plant-pathogen interactions, induces HR and systemic acquired resistance [61]. In our study, 32 DEGs were identified in the significantly enriched KEGG pathway "Plant hormone signal transduction" (Additional file 7: Table S7). Interestingly, TGA (104,645,854, 101,250,172 and 101,253,982) and PR-1 (544123), which are involved in the SA signaling pathway, were significantly up-regulated in Cf-*16* tomato after *C. fulvum* inoculation. Importantly, the expression of PR-1 (544123) was significantly higher in Cf-*16* tomato than in Moneymaker at 4 and 8 dpi, suggesting that PR-1 may have a significant function in the response of Cf-*16* tomato to *C. fulvum* infection. This result is consistent with previous studies [62]. We also showed that JAZ (jasmonate-zim-domain) genes (101,247,936 and 100,134,911), which encode major proteins in JA signaling, were up-regulated in Cf-*16* tomato at 4 dpi. This finding is consistent with the previously measured change in JA content after infection. Additionally, SAUR family proteins (BGI_novel_G000650, BGI_novel_G001679, 101,255,313, 101,257,321, 104,648, 957 and 101,264,326) and PP2Cs (101,249,794 and 101, 261,835) in the KEGG pathway "Plant hormone signal transduction" (Additional file 7: Table S7) were identified in the present study, suggesting that SAUR family proteins and PP2Cs also play roles in the resistance of Cf-*16*

tomato to *C. fulvum*. In particular, the SAUR family protein with ID 101264326 was identified among the DEGs that overlapped between Cf-*16* tomato and Moneymaker only at 8 dpi (Table 3). Elucidation of the function of this protein in the response of Cf-*16* tomato to *C. fulvum* is needed. Overall, discrepancies among different studies suggest that the specific hormones involved may vary and behave differently in different tomato-*C. fulvum* interactions under different conditions and at different time points. Therefore, it is important to explore crosstalk between SA and JA signaling in the activation of Cf-*16*-mediated defense systems against *C. fulvum* attack and close interactions with other Cf genes.

## Conclusions

This study analyzed the first comprehensive transcriptome of the Cf-*16* resistant tomato cv. Ontario7816 and susceptible tomato cv. Moneymaker and explored interactions between Cf-*16* tomato and *C. fulvum*. Some DEGs related to disease resistance were identified and predicted to be associated with the plant innate immune response, $Ca^{2+}$ channels and plant hormone signal transduction pathways. These results contribute to our understanding of the potential mechanism by which Cf-*16* tomato combats *C. fulvum* infection and will facilitate the fine mapping and cloning of the Cf-*16* gene in the future.

## Methods

### Plant materials and *C. fulvum* inoculation

Two tomato cultivars, the resistant cultivar Ontario7816, including the Cf-*16* gene (kindly provided by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Science) [23], and the susceptible cultivar Moneymaker, which lacks *C. fulvum*-resistance genes (kindly provided by the Tomato Genetic Resource Center, LA2706), were used in this study. Tomato seeds were sown in pots filled with soil and grown under controlled conditions (16 h light, 25 °C and 95% ambient humidity) in a greenhouse at the Horticultural Station of Northeast Agricultural University (Harbin, China). At the four- to six-leaf stage, the abaxial leaf surfaces of 40 plants per line were sprayed with a suspension of *C. fulvum* (race 1.2.3.4) at $1 \times 10^7$ sporangia per milliliter [63]. Mock-treated plants of each line were sprayed with sterilized water under the same conditions. All plants were maintained at 25 °C with 95% relative humidity.

### Microscopic observation of *C. fulvum* in Cf-*16* tomato

To assess the process of Cf-*16*-mediated HR and the key time points involved in the resistance mechanism, the lactophenol trypan blue staining method was performed according to Franco's approach [64]. Leaf samples from the resistant and susceptible lines were harvested at 0–21 dpi, immediately stained, clarified overnight in chloral

hydrate solution (2.5 mg/ml) [65], and examined using an Olympus SZX10 dissecting microscope (Olympus, Japan).

### Endogenous JA and SA levels
Leaf samples from the inoculation and control groups of the resistant and susceptible cultivars were harvested at 0, 2, 3, 4, 8, 12, 16 and 21 dpi. Endogenous SA and JA were extracted from the leaves using the modified method of Llugany et al. (2013) [66]; levels were measured via HPLC-MS/MS using an AB SCIEX Triple TOF5600$^+$ mass spectrometer (SCIEX, USA) [67].

### RNA extraction, cDNA library construction and sequencing
Total RNA was obtained from each group at 4 and 8 dpi, for a total of 24 samples, and used for RNA-Seq and qRT-PCR analyses. Total RNA was extracted from three biological replicates for each group with three plants using the RNAprep Pure Plant Kit (ThermoFisher, USA) and then used for qRT-PCR [68, 69]; quantified RNA samples were used for cDNA library construction. Library preparation and sequencing were conducted by BGI Tech (Shenzhen, China). The libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina R (NEB, USA) and sequenced using a BGISEQ-500, with 150-bp paired-end reads generated. The raw sequencing data were deposited in NCBI Sequence Read Archive under the accession number GSE133678 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133678).

### Sequencing read mapping and identification of DEGs
Raw reads in FASTQ format were generated by base calling, statistically analyzed using SOAPnuke v1.4.0 and filtered using Trimmomatic v0.36 [70]. Clean reads were obtained by removing reads with adapters, reads containing more than 5% poly-N (where N represents unknown bases), and low-quality reads (with a mass value less than 10 and proportion of total number of bases in the reads greater than 20%).

The clean reads were aligned to the *S. lycopersicum* reference genome sequence (NCBI_GCF_000188115.3_SL2.50) using HISATv2.1.0 [71]. Gene expression levels were quantified with the FPKM method using RSEMv1.2.8 [40]. DEGs were detected using DEGseq methods based on the Poisson distribution [72]. Genes with an adjusted *P*-value of ≤0.001 and a log$_2$ fold change of ≥2 were defined as differentially expressed [73].

### Functional annotation and enrichment pathway analyses of DEGs
GO and KEGG pathway enrichment analyses of DEGs were performed using the Phyper function in R software;

GO terms and KEGG pathways with an adjusted P-value of ≤0.01 were regarded as significantly enriched. For a graphical overview, DEGs were mapped to various metabolic and regulatory pathways (bins) using the MapMan tool. The colored boxes in each bin represent the log$_2$-transformed fold change values of the DEGs.

### Gene co-expression network analysis
Gene co-expression network analysis was performed using the WGCNA package v1.48. Gene dendrograms were constructed with colors based on the correlations between the expression levels of genes and used to build clustering trees and to divide modules. In addition, the correlation between modules and samples was analyzed using WGCNA.

### qRT-PCR analysis
Sixteen DEGs were validated using qRT-PCR to verify the expression profiles obtained by RNA-Seq. qRT-PCR was performed using AceQ® qPCR SYBR® Green Master Mix (Vazyme, USA) and a qTOWER$^3$G Detection System (Analytik Jena, Germany). Each sample was replicated three times, and data analysis was performed using the $2^{-\triangle\triangle CT}$ method [74]. The gene *EFα1* was used as a reference control for normalization (R: 5′-CCACCAATCTTGTACACATCC-3′, S: 5′-AGACCACCAAGTACTACTGCAC-3′) (Additional file 5: Table S5).

## Supplementary information
**Supplementary information** accompanies this paper at https://doi.org/10.1186/s12870-020-2245-5.

---

**Additional file 1: Table S1.** Summary of RNA-Seq data.

**Additional file 2: Table S2.** All FPKM values for every gene.

**Additional file 3: Table S3.** All fold changes and *P*-values for DEGs between the different groups.

**Additional file 4: Table S4.** DEGs between Moneymaker and Cf-*16* tomato at 4 dpi, based on MapMan analysis.

**Additional file 5: Table S5.** Primers used for qPCR.

**Additional file 6: Table S6.** Up-regulated DEGs in the significantly enriched KEGG pathway "Plant-pathogen interaction" in Cf-*16* tomato and Moneymaker at 4 dpi.

**Additional file 7: Table S7.** Up-regulated DEGs in the significantly enriched KEGG pathway "Plant hormone signal transduction" in Cf-*16* tomato and Moneymaker at 4 dpi.

**Additional file 8: Table S8.** Common DEGs in the significantly enriched KEGG pathway "Plant-pathogen interaction" between the MM_4dpi-vs-Cf_4dpi and CK_Cf_4dpi-vs-Cf_4dpi comparisons.

**Additional file 9: Figure S1.** Classification of raw reads from different samples.

**Additional file 10: Figure S2.** Scatter plot of KEGG pathway enrichment of the three modules from WGCNA.

---

### Abbreviations
ABA: Abscisic acid; cDNA-AFLP: cDNA-amplified fragment length polymorphism; CDPK: Calcium-dependent protein kinase; CERK1: Chitin elicitor receptor kinase 1; CML: Calcium binding protein; CNCG: Plant cyclic nucleotide-gated ion channel; DEG: Differentially expressed gene; dpi: Day

Zhang *et al. BMC Plant Biology*        (2020) 20:33

Page 14 of 16

post inoculation; ETI: Effector-triggered immunity; ETS: Effector-triggered susceptibility response; FPKM: Fragments per kilobase of exon per million fragments mapped; GO: Gene Ontology; HPLC-MS/MS: High-performance liquid chromatography-tandem mass spectrometry; HR: Hypersensitive response; IAA: Indole-3-acetic acid; JA: Jasmonic acid; JAZ: Jasmonate-zim-domain gene; KEGG: Kyoto Encyclopedia of Genes and Genomes; NB-LRR: Nucleotide-binding site and leucine-rich repeat; PAMP: Pathogen-associated molecular pattern; PRR: Pattern recognition receptor; PTI: PAMP-triggered immunity; qRT-PCR: Real-time quantitative polymerase chain reaction; SA: Salicylic acid; TF: Transcription factor; WGCNA: Weighted gene coexpression network analysis

## Authors' contributions
XX conceived of and designed the project. DZ performed the experiments, analyzed the RNA-Seq data and wrote the manuscript. HY revised the manuscript. YB and YS participated in the preliminary preparation of the experiments. YB, YS, TZ, HL, CD, JJ, JL and LX participated in the data discussion. All authors have read and approved the final manuscript.

## Availability of data and materials
All the data pertaining to the present study have been included in the tables and figures of the manuscript. The raw sequencing data were deposited in NCBI Sequence Read Archive under the accession number GSE133678 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE133678).

## Ethics approval and consent to participate
Not applicable.

## Consent for publication
Not applicable.

## Competing interests
The authors declare that they have no competing interests.

## Author details
[1]Laboratory of Genetic Breeding in Tomato, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Northeast Region), Ministry of Agriculture and Rural Affairs, College of Horticulture and Landscape Architecture, Northeast Agricultural University, Harbin 150030, China. [2]Horticultural Sub-Academy, Heilongjiang Academy of Agricultural Sciences, Harbin 150069, China.

## References
1. Bhattarai K, Louws FJ, Williamson JD, et al. Diversity analysis of tomato genotypes based on morphological traits with commercial breeding significance for fresh market production in eastern USA. Aust J Crop Sci. 2016;10(8):1098. https://doi.org/10.21475/ajcs.2016.10.08.p7391.
2. Tandon G, Singh S, Kaur S. Comparative analysis of Cf-4 and Cf-19 in tomato (Solanum lycopersicum)-a bioinformatics study. Indian J Agric Sci. 2017; 87(10):1365–70.
3. Cooke MC. New american fungi. Grevillea. 1883;12:32.
4. Butler EJ, Jones SG. Tomato leaf mould, Cladosporium fulvum Cooke [J]. Plant Pathol. 1949:672–8.
5. Jones JB, Jones JP, Stall RE, et al. Compendium of tomato diseases [M]. Am Phytopathol Soc. 1991. https://doi.org/10.2307/3760420.
6. Thomma BPHJ, Van Esse HP, Crous PW, et al. Cladosporium fulvum (syn. Passalora fulva), a highly specialized plant pathogen as a model for functional studies on plant pathogenic Mycosphaerellaceae. Mol Plant Pathol. 2005;6(4):379–93. https://doi.org/10.1111/j.1364-3703.2005.00292.x.
7. Ausubel FM. Are innate immune signaling pathways in plants and animals conserved? Nat Immunol. 2005;6(10):973–9. https://doi.org/10.1038/ni1253.
8. Bigeard J, Colcombet J, Hirt H. Signaling mechanisms in pattern-triggered immunity (PTI). Mol Plant. 2015;8(4):521–39. https://doi.org/10.1016/j.molp.2014.12.022.
9. Dodds PN, Rathjen JP. Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet. 2010;11(8):539. https://doi.org/10.1038/nrg2812.
10. Thomma BPHJ, Nurnberger T, Joosten MHAJ. Of PAMPs and effectors: the blurred PTI-ETI dichotomy [J]. Plant Cell. 2011;23(1):4–15. https://doi.org/10.1105/tpc.110.082602.
11. Jones JD, Dangl JL. The plant immune system. Nature, 2006;444(7117):323–9. https://doi.org/10.1038/nature05286.
12. Wang C, Cai X, Zheng Z. High humidity represses Cf-4/Avr4-and Cf-9/Avr9-dependent hypersensitive cell death and defense gene expression. Planta. 2005;222(6):947–56. https://doi.org/10.1007/s00425-005-0036-8.
13. Nekrasov V, Ludwig AA, Jones JDG. CITRX thioredoxin is a putative adaptor protein connecting Cf-9 and the ACIK1 protein kinase during the Cf-9/Avr9-induced defence response. FEBS Lett. 2006;580(17):4236–41. https://doi.org/10.1016/j.febslet.200 6.06.077.
14. Hammond-Kosack KE, Jones JD. Resistance gene-dependent plant defense responses. Plant Cell. 1996;8(10):1773. https://doi.org/10.1105/tpc.8.10.1773.
15. Soylu EM, Soylu S, Mansfield JW. Ultrastructural characterisation of pathogen development and host responses during compatible and incompatible interactions between Arabidopsis thaliana and Peronospora parasitica. Physiol Mol Plant Pathol. 2004;65(2):67–78. https://doi.org/10.1016/j.pmpp.2004.12.002.
16. Soylu S, Brown I, Mansfield JW. Cellular reactions in Arabidopsis following challenge by strains of Pseudomonas syringae: from basal resistance to compatibility. Physiol Mol Plant Pathol. 2005;66(6):232–43. https://doi.org/10.1016/j.pmpp.2005.08.005.
17. Pei C, Wang H, Zhang J, et al. Fine mapping and analysis of a candidate gene in tomato accession PI128216 conferring hypersensitive resistance to bacterial spot race T3. Theor Appl Genet. 2012;124(3):533–42. https://doi.org/10.1007/s00122-011-1726-1.
18. Mesarich CH, Griffiths SA, van der Burgt A, et al. Transcriptome sequencing uncovers the Avr5 avirulence gene of the tomato leaf mold pathogen Cladosporium fulvum. Mol Plant-Microbe Interact. 2014;27(8):846–57. https://doi.org/10.1094/MPMI-02-14-0050-R.
19. Mesarich CH, Ökmen B, Rovenich H, et al. Specific hypersensitive response–associated recognition of new apoplastic effectors from Cladosporium fulvum in wild tomato. Mol Plant-Microbe Interact. 2017;31(1):145–62. https://doi.org/10.1094/MPMI-05-17-0114-FI.
20. Langford AN. The parasitism of Cladosporium fulvum Cooke and the genetics of resistance to it. Can J Res. 1937;15(3):108–28. https://doi.org/10.1139/cjr37c-008.
21. Kanwar JS, Kerr EA, Harney PM. Linkage of Cf-1 to Cf-11 genes for resistance to leaf mold Cladosporium fulvum. Tomato Genet Coop. 1980;30:20–1.
22. Kerr EA, Bailey DL. Resistance to Cladosporium fulvum Cke. Obtained from wild species of tomato. Can J Bot. 1964;42(11):1541–54. https://doi.org/10.1139/b64-153.
23. Kanwar JS, Kerr EA, Harney PM. Linkage of the Cf-12 to Cf-24 genes for resistance to tomato leaf mold Cladosporium fulvum Cke. Rep Tomato Genet Coop. 1980;30:22–3.
24. Stevens MA, Rick CM. Genetics and breeding [M]//the tomato crop. Dordrecht: Springer; 1986. p. 35–109. https://doi.org/10.1007/978-94-009-3137-4_2.
25. Dickinson MJ, Jones DA, Jones JD. Close linkage between the Cf-2/Cf-5 and Mi resistance loci in tomato. Mol Plant Microbe Interact. 1993;6(3):341–7. https://doi.org/10.1094/MPMI-6-341.
26. Jones DA, Dickinson MJ, Balint-Kurti PJ, et al. Two complex resistance loci revealed in tomato by classical and RFLP mapping of the Cf-2, Cf-4, Cf-5, and Cf-9 genes for resistance to Cladosporium fulvum. Mol Plant Microbe Interact. 1993;6:348. https://doi.org/10.1094/MPMI-6-348.
27. Joosten MHAJ, De Wit PJGM. The tomato–Cladosporium Fulvum interaction: a versatile experimental system to study plant-pathogen interactions. Annu

Zhang *et al. BMC Plant Biology*        (2020) 20:33

Page 15 of 16

Rev Phytopathol. 1999;37(1):335–67. https://doi.org/10.1146/annurev.phyto.37.1.335.

28. Haanstra JPW, Thomas CM, Jones JDG, et al. Dispersion of the Cf-4 disease resistance gene in Lycopersicon germplasm. Heredity. 2000;85(3):266. https://doi.org/10.1046/j.1365-2540.2000.00754.x.

29. Zhao T, Jiang J, Liu G, et al. Mapping and candidate gene screening of tomato Cladosporium fulvum-resistant gene Cf-19, based on high-throughput sequencing technology. BMC Plant Biol. 2016;16(1):51. https://doi.org/10.1186/s12870-016-0737-0.

30. Liu G, Zhao T, You X, et al. Molecular mapping of the Cf-10 gene by combining SNP/InDel-index and linkage analysis in tomato (Solanum lycopersicum). BMC Plant Biol. 2019;19(1):15. https://doi.org/10.1186/s12870-018-1616-7.

31. Phule AS, Barbadikar KM, Maganti SM, et al. RNA-seq reveals the involvement of key genes for aerobic adaptation in rice. Sci Rep. 2019;9(1):5235. https://doi.org/10.1038/s41598-019-41703-2.

32. Zenda T, Liu S, Wang X, et al. Key maize drought-responsive genes and pathways revealed by comparative Transcriptome and physiological analyses of contrasting inbred lines. Int J Mol Sci. 2019;20(6):1268. https://doi.org/10.3390/ijm s20061268.

33. Sui X, Nie J, Li X, et al. Transcriptomic and functional analysis of cucumber (*Cucumis sativus* L.) fruit phloem during early development. Plant J. 2018;96(5):982–96. https://doi.org/10.1111/tpj.14084.

34. Unel NM, Cetin F, Karaca Y, et al. Comparative identification, characterization, and expression analysis of bZIP gene family members in watermelon and melon genomes. Plant Growth Regul. 2019;87(2):227–43. https://doi.org/10.1007/s10725-018-0465-6.

35. Hu P, Li G, Zhao X, et al. Transcriptome profiling by RNA-Seq reveals differentially expressed genes related to fruit development and ripening characteristics in strawberries (Fragaria× ananassa). Peer J. 2018;6:e4976. https://doi.org/10.7717/peerj.4976.

36. Zhu JW, Xu YP, Zhang ZX, et al. Transcript profiling for Avr4/Cf-4-and Avr9/Cf-9-dependent defence gene expression. European J Plant Pathol. 2008;122(2):307–14. https://doi.org/10.1007/s10658-008-9294-1.

37. Zhao T, Liu G, Li S, et al. Differentially expressed gene transcripts related to the Cf-19-mediated resistance response to Cladosporium fulvum infection in tomato. Physiol Mol Plant Pathol. 2015;89:8–15. https://doi.org/10.1016/j.pmpp.2014.11.003.

38. Xue DQ, Chen XL, Zhang H, et al. Transcriptome analysis of the Cf-12-mediated resistance response to Cladosporium fulvum in tomato. Front Plant Sci. 2017;7:2012. https://doi.org/10.3389/fpls.2016.02012.

39. Liu G, Liu J, Zhang C, et al. Physiological and RNA-seq analyses provide insights into the response mechanism of the Cf-10-mediated resistance to Cladosporium fulvum infection in tomato. Plant Mol Biol. 2018;96(4–5):403–16. https://doi.org/10.1007/s11103-018-0706-0.

40. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC bioinformatics. 2011;12(1):323. https://doi.org/10.1186/1471-2105-12-323.

41. Cantu D, Vicente AR, Labavitch JM, et al. Strangers in the matrix: plant cell walls and pathogen susceptibility. Trends Plant Sci. 2008;13(11):610–7. https://doi.org/10.1016/j.tplants.2008.09.002.

42. Claverie J, Balacey S, Lemaître-Guillier C, et al. The cell wall-derived xyloglucan is a new DAMP triggering plant immunity in Vitis vinifera and Arabidopsis thaliana. Front Plant Sci. 2018;9:1725. https://doi.org/10.3389/fpls.2018.01725.

43. Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol. 2005;4(1). https://doi.org/10.2202/1544-6115.1128.

44. Beck M, Heard W, Mbengue M, et al. The INs and OUTs of pattern recognition receptors at the cell surface. Current opinion in plant biology. 2012;15(4):367–74. https://doi.org/10.1016/j.pbi.2012.05.004.

45. Shiu SH, Bleecker AB. Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci. 2001;98(19):10763–8. https://doi.org/10.1073/pnas.181141598.

46. Gimenez-Ibanez S, Hann DR, Ntoukakis V, et al. AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. Curr Biol. 2009;19(5):423–9. https://doi.org/10.1016/j.cub.2009.01.054.

47. Lecourieux D, Lamotte O, Bourque S, et al. Proteinaceous and oligosaccharidic elicitors induce different calcium signatures in the nucleus of tobacco cells. Cell Calcium. 2005;38(6):527–38. https://doi.org/10.1016/j.ceca.2005.06.036.

48. Boudsocq M, Willmann MR, McCormack M, et al. Differential innate immune signalling via Ca 2+ sensor protein kinases. Nature. 2010;464(7287):–418. https://doi.org/10.1038/nature08794.

49. Kobayashi M, Ohura I, Kawakita K, et al. Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell. 2007;19(3):1065–80. https://doi.org/10.1105/tpc.106.048884.

50. Dubiella U, Seybold H, Durian G, et al. Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation [J]. Proc Natl Acad Sci. 2013;110(21):8744–9. https://doi.org/10.1073/pnas.122 1294110.

51. Hu Z, Lv X, Xia X, et al. Genome-wide identification and expression analysis of calcium-dependent protein kinase in tomato. Front Plant Sci, 469. 2016;7. https://doi.org/10.3389/fpls.2016.00469.

52. Ma W, Smigel A, Tsai YC, et al. Innate immunity signaling: cytosolic Ca2+ elevation is linked to downstream nitric oxide generation through the action of calmodulin or a calmodulin-like protein. Plant Physiol. 2008;148(2):818–28. https://doi.org/10.1104/pp.108.125104.

53. Ranty B, Aldon D, Cotelle V, et al. Calcium sensors as key hubs in plant responses to biotic and abiotic stresses. Front Plant Sci. 2016;7:327. https://doi.org/10.3389/fpls.2016.00327.

54. Asai T, Tena G, Plotnikova J, et al. MAP kinase signalling cascade in Arabidopsis innate immunity. Nature. 2002;415(6875):977. https://doi.org/10.1038/415977a.

55. Tena G, Asai T, Chiu WL, et al. Plant mitogen-activated protein kinase signaling cascades. Curr Opinion Plant Biol. 2001;4(5):392–400. https://doi.org/10.1016/S1369-5266(0 0)00191-6.

56. Jia Y, McAdams SA, Bryan GT, et al. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J. 2000;19(1):4004–14. https://doi.org/10.1093/emboj/19.15.4004.

57. Mackey D, Holt BF III, Wiig A, et al. RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell. 2002;108(6):743–54. https://doi.org/10.1016/S0092-8674(02)00661-X.

58. Mao YB, Liu YQ, Chen DY, et al. Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance. Nat Commun. 2017;8:13925. https://doi.org/10.1038/ncomms13925.

59. Betsuyaku S, Katou S, Takebayashi Y, et al. Salicylic acid and jasmonic acid pathways are activated in spatially different domains around the infection site during effector-triggered immunity in *Arabidopsis thaliana*. Plant and Cell Physiol. 2017;59(1):8–16. https://doi.org/10.1093/pcp/pcx181.

60. Berens ML, Berry HM, Mine A, et al. Evolution of hormone signaling networks in plant defense. Ann Rev Phytopathol. 2017;55:401–25. https://doi.org/10.1146/an nurev-phyto-080516-035544.

61. Klessig DF, Choi HW, Dempsey DMA. Systemic acquired resistance and salicylic acid: past, present, and future. Mol Plant Microbe Interact. 2018;31(9):871–88. https://doi.org/10.1094/MPMI-03-18-0067-CR.

62. Aoki K, Yano K, Suzuki A, et al. Large-scale analysis of full-length cDNAs from the tomato (Solanum lycopersicum) cultivar micro-tom, a reference system for the Solanaceae genomics. BMC Genomics. 2010;11(1):210. https://doi.org/10.1186/1471-2164-11-210.

63. Wang A, Meng F, Xu X, et al. Development of molecular markers linked to Cladosporium fulvum resistant gene Cf-6 in tomato by RAPD and SSR methods. HortScience. 2007;42(1):11–5. https://doi.org/10.21273/HORTSCI.42.1.11.

64. Faoro F, Maffi D, Cantu D, et al. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. Biocontrol. 2008;53(2):387–401. https://doi.org/10.1007/s10526-007-9091-3.

65. Keogh RC, Deverall BJ, McLeod S. Comparison of histological and physiological responses to Phakopsora pachyrhizi in resistant and susceptible soybean. Trans Br Mycol Soc. 1980;74(2):329–33. https://doi.org/10.1016/S0007-1536(80)80163-X.

66. Llugany M, Martin SR, Barceló J, et al. Endogenous jasmonic and salicylic acids levels in the Cd-hyperaccumulator Noccaea (Thlaspi) praecox exposed to fungal infection and/or mechanical stress. Plant cell Rep. 2013;32(8):1243–9. https://doi.org/10.1007/s00299-013-1427-0.

67. Pan X, Welti R, Wang X. Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry. Nat Protoc. 2010;5(6):986. https://doi.org/10.1038/nprot.2010.37.

68. Schroeder A, Mueller O, Stocker S, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol. 2006; 7(1):3. https://doi.org/10.1186/1471-2199-7-3.

69. Fang SM, Hu BL, Zhou QZ, et al. Comparative analysis of the silk gland transcriptomes between the domestic and wild silkworms. BMC Genomics. 2015;16(1):60. https://doi.org/10.1186/s12864-015-1287-9.

70. Cock PJA, Fields CJ, Goto N, et al. The sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. Nucleic Acids Res. 2009;38(6):1767–71. https://doi.org/10.1093/nar/gkp1137.

71. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357. https://doi.org/10.1038/nmeth.3317.

72. Wang L, Feng Z, Wang X, et al. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics. 2009;26(1): 136–8. https://doi.org/10.1093/bioinformatics/btp612.

73. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol. 1995;57(1):289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

74. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− $\Delta\Delta$CT method. Methods. 2001;25(4): 402–8. https://doi.org/10.1006/meth.2001.1262.

## Publisher's Note