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RESEARCH ARTICLE

# De novo assembly and discovery of genes involved in the response of *Solanum sisymbriifolium* to *Verticillium dahlia*

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**Abstract** Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae*, is a devastating disease of eggplant (*Solanum* spp.) and causes substantial losses worldwide. Although some genes or biological processes involved in the interaction between eggplant and *V. dahliae* have been identified in some studies, the underlying molecular mechanism is not yet clear. Here, we monitored the transcriptomic profiles of the roots of resistant *S. sisymbriifolium* plants challenged with *V. dahliae*. Based on the measurements of physiological indexes (T-SOD, POD and SSs), three time points were selected and subsequently divided into two stages (S_12 h vs. S_0 h and S_48 h vs. S_12 h). KEGG enrichment analysis of the DEGs revealed several genes putatively involved in regulating plant-*V. dahliae* interactions, including mitogen-activated protein kinase (MAPK) genes (MEKK1 and MAP2K1), WRKY genes (WRKY22 and WRKY33) and cytochrome P450 (CYP) genes (CYP73A/C4H, CYP98A/C3′H and CYP84A/F5H). In addition, a subset of genes that play an important role in activating *V. dahliae* defence responses, including *Ve* genes as well as genes encoding PR proteins and TFs, were screened and are discussed. These results will help to identify key resistance genes and will contribute to a further understanding of molecular mechanisms of the *S. sisymbriifolium* resistance response to *V. dahliae*.

**Keywords** *Solanum sisymbriifolium* · Transcriptome · *Verticillium dahliae* · Defence-response genes

## Introduction

Eggplant (*Solanum melongena* L.) is the third solanaceous fruit crop of outstanding importance worldwide after potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) (Polignano et al. 2010). The global production of eggplants has increased considerably, especially in Asian and African countries. According to data of the Food and Agriculture Organization of the United Nations (http://faostat.fao.org), eggplant production has increased from 29 to 49 million tons in the last decade. Eggplant verticillium wilt (EVW), which is caused by *Verticillium dahliae*, is a devastating disease of eggplant worldwide and causes substantial yield losses (Collonnier et al. 2001). *Verticillium dahliae* is a destructive plant fungal pathogen that is widely distributed in agricultural soils, and more than 400 plant species, including many economically important crops, are hosts for this pathogen (Bhat and Subbarao 1999); however, no effective fungicides or chemicals are available to cure infected commercial plants. Cultivation methods such as solarization, soil amendments and rotation also have very limited effects on EVW (Yadeta et al. 2014). Thus, genetic resistance still constitutes the most ideal strategy for

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controlling plant pathogenic fungi with respect to economic benefits and environmental protection, but genetic resistance has received little attention due to a lack of genetic resources (Fradin and Thomma 2006).

Some wild relatives of eggplant, such as *Solanum torvum*, *Solanum aculeatissimum*, and *Solanum sisymbrifolium*, are resistant to EVW (Bletsos et al. 2003; Collonnier et al. 2001; Liu et al. 2015; Zhou et al. 2016). However, fertile $F_1$s are rarely obtained, as the above wild *Solanum* species and *S. melongena* are not cross compatible (Borua 1990; Gramazio et al. 2016; Plazas et al. 2016; Tamura et al. 2002; Toppino et al. 2008). Recently, efforts have been made to uncover the mechanisms underlying the molecular interactions between *V. dahliae* and *Arabidopsis* (Pantelides et al. 2010), tomato (Diwan et al. 1999; Fradin et al. 2009; Kawchuk et al. 2001), potato (Yang et al. 2015) and cotton (Sun et al. 2013; Xu et al. 2011; Zhang et al. 2012, 2013) plants. In studies on the responses of eggplant to *V. dahliae*, several resistance-related genes have been isolated and studied (Chai et al. 2003; Fei et al. 2004), and RNA-Seq (RNA sequencing) technologies have been applied to analyze gene expression profiles in eggplant defence responses (Yang et al. 2013, 2014; Zhou et al. 2016). However, these data are insufficient to elucidate the defence mechanism of eggplant challenged with *V. dahliae*. As such, it is necessary and urgent to excavate useful resistance genes for eggplant improvement.

*Solanum sisymbriifolium* is a wild relative of *S. melongena* and is resistant to EVW (Bletsos et al. 2003; Zhou et al. 2016). However, compared with other wild relatives of eggplant, *S. sisymbriifolium* has been very rarely studied. For better utilization of this potential material, more genomic information is demanded. Therefore, the first transcriptome sequencing of *S. sisymbriifolium* with and without *V. dahliae* infection was performed using Illumina next-generation sequencing technology both to provide an overview of the *S. sisymbriifolium* root transcriptome and to identify the genes and pathways involved in the pathogen-plant interaction (PPI) system. The results generated in this study can provide important fundamental information for understanding the control of plant development and disease resistance in eggplant.

## Materials and methods

### Plant material and inoculation

The highly toxic pathogenic *V. dahliae* strain QZ-S was isolated from the roots of a diseased eggplant in the Kunming area of China (Wu et al. 2017). The isolate was cultured on potato dextrose agar (PDA) plates for 15–20 days, after which it was transferred to Czapek's liquid medium in a shaker at 110 rpm under a temperature of 25 °C for 5–7 days. The suspension liquid was then filtered and adjusted to approximately $1 \times 10^7$ spores/mL with sterilized distilled water prior to inoculation.

Seeds of *S. sisymbriifolium* were obtained from the Kunming Institute of Botany, Chinese Academy of Science. After the seeds were sterilized, they were allowed to germinate in a growth chamber at 30 °C. The germinated seeds were subsequently transplanted to sterilized soil and cultivated in the greenhouse at 25/15 °C (day/night). When they had two pairs of leaves, the seedlings were inoculated by dipping their roots into the fungal spore suspension for 30 min. The roots of the *V. dahliae*-treated plants were then harvested after 6, 12, 24, 48 and 72 h. Control plants were not inoculated but were treated with distilled water in the same manner. At each time point, 18 plants were randomly divided into three groups, and the roots of the six plants in each group were pooled (n = 3 groups). All of the samples were rapidly frozen in liquid nitrogen and stored at − 80 °C.

### Measurement of physiological indexes

For enzyme extracts and assays, the roots (0.1 g) were ground in liquid nitrogen, and were then suspended in a 0.9-mL solution containing 10 mM phosphate buffer (pH 7.4). The homogenate was then centrifuged at 2500 rpm at 4 °C for 10 min, after which the resulting supernatant was collected for determining the T-SOD and POD activities and the SS content. The T-SOD activity was measured using a total superoxide dismutase assay kit (hydroxylamine method, A001-1), the POD activity was measured using a POD assay kit (A084-3), and the SS content was assayed using a plant SS content test kit (A145). The commercial assay kits mentioned above were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and a DDS-306 electrical conductivity meter (Fangzhou Company, Chengdu, China) was used for measuring all of the indexes.

### RNA isolation, library construction and sequencing

The total RNA of each sample was extracted using RNAout 1.0 (Tianenze, Beijing, China). RNA degradation and contamination were monitored on 1% agarose gels, and a NanoPhotometer® spectrophotometer (Implen, CA, USA) was used to check the RNA purity. A Qubit® RNA Assay Kit in conjunction with a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA) was used to measure the RNA concentrations, and RNA integrity was assessed using an RNA Nano 6000 Assay Kit together with an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

To construct a cDNA library for each time point, each group of three replicates was sampled at each time point. All cDNA library construction and Illumina sequencing were performed by the Beijing Novogene Bioinformatics Technology Co., Ltd.

Briefly, poly-T oligo-conjugated magnetic beads were used to purify the mRNA from the total RNA. Fragmentation was then carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5 ×). First-strand cDNA synthesis was primed by random hexamer and transcribed by M-MuLV Reverse Transcriptase (RNase H). Second-strand was subsequently synthesized using DNA Polymerase I and RNase H. Second-strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. The remaining overhangs were converted to blunt ends via exonuclease/polymerase activities. After the 3′ ends of the DNA fragments were adenylated, NEBNext adaptors having a hairpin loop structure were ligated in preparation for hybridization. To select cDNA fragments that were preferentially 150–200 bp in length, an AMPure XP system (Beckman Coulter, Beverly, USA) was used to purify the library fragments. Then, 3 µl of USER Enzyme (NEB, USA) was used with size-selected adaptor-ligated cDNA at 37 °C for 15 min followed by 95 °C for 5 min prior to PCR. Phusion High-Fidelity DNA polymerase, universal PCR primers and index (X) primer were then used to perform PCR. The PCR products were purified (via the AMPure XP system) a final time, after which the Agilent Bioanalyzer 2100 system was used to assess the library quality.

Clustering of the index-coded samples was performed on a cBot Cluster Generation System using a TruSeq PE Cluster Kit v3-cBot-HS (Illumina) in accordance with the manufacturer's instructions. After the clusters were generated, the library preparations were sequenced on an Illumina HiSeq 2000 platform, after which paired-end reads were generated.

## De novo transcriptome assembly and gene functional annotation

In-house Perl scripts were used to first process the raw data (raw reads) in fastq format. During this step, clean data (clean reads) were obtained by removing reads containing adaptors, reads containing poly-N sequences and low-quality reads ($Q \leq 20$) from the raw data. At the same time, Q20 and Q30 values, the GC content and the sequence duplication level of the clean data were calculated. All downstream analyses were based on high-quality clean data. Besides, the reads of *V. dahlia* were removed by comparing the clean data with the Nt database. The clean reads were subsequently assembled using Trinity software (version r20140413p1) as described for de novo transcriptome assembly in the absence of a reference genome (Kanehisa et al. 2008). To avoid redundant transcripts, in-house Perl scripts were applied to extract the longest transcripts as unigenes. The duplicate genes were removed by TGICL (TGI Clustering tools) software (Pertea et al. 2003) to attain the final set of unigenes.

Gene function was annotated based on the following databases: Nr, Nt, Pfam, KOG/COG, Swiss-Prot (a manually annotated and reviewed protein sequence database), KEGG orthologue (KO), and GO.

## Differential expression analysis

The DESeq R package (v1.10.1) was used to analyse the differential expression of both groups. Using a model based on negative binomial distribution, DESeq provides statistical routines to determine differential expression among digital gene expression data. For the samples with biological replicates, the DEGs were filtered for a corrected *P* value threshold of 0.05 (the *P* values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate (FDR)). Functional enrichment analyses including the use of GO and KEGG were performed to identify which GO terms or metabolic pathways were significantly enriched in DEGs. The GO enrichment analysis of the DEGs was implemented through the GOseq R package (v1.10.0); the analysis was based on the Wallenius non-central hypergeometric distribution (Young et al. 2010), and the GO terms with a corrected $P$ value $< 0.05$ were considered significantly enriched. KOBAS (Mao et al. 2005) software (v2.0.12) was used to test the statistical enrichment of the DEGs among the KEGG pathways. Those pathways with an FDR value of $\leq 0.05$ were defined as containing genes with significant differential expression.

## RT-qPCR analysis of DEGs

Eighteen co-expressed DEGs were chosen for validation using RT-qPCR. The total RNA was isolated from the roots of both *V. dahliae*-treated and control plants. First-strand cDNA was synthesized using a Transcript® All-in-one First-Strand cDNA Synthesis SuperMix for qPCR kit (Trans, Beijing). The primers used for RT-qPCR were designed with Primer Premier 5.0 software (Supplementary Table S1). All primer pairs were custom ordered from Tsingke, Kunming. The 18S RNA gene of eggplant served as an internal control. RT-qPCR was performed on an ABI 7500 Real-time PCR system using a SYBR Green-based PCR assay. All RT-qPCRs for each gene were performed for three biological replicates, and each biological replicate included three technical replicates. A reaction containing 10 µl of 2 × TransStart® Tip Green qPCR SuperMix,

0.4 µl of passive reference dye (50 ×), 1 µl of diluted cDNA, and 0.4 µl (10 µM) of each primer in a final volume of 20 µl was performed. The amplification programme consisted of one cycle of 94 °C for 30 s, followed by 40 cycles of 94 °C for 5 s and 60 °C for 30 s. The relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

## Results

### Changes in physiological indexes of *S. sisymbriifolium* infected with *V. dahliae*

To investigate the physiological responses of *S. sisymbrifolium* challenged with *V. dahliae* and to choose the key time points for the transcriptome analysis, the total superoxide dismutase (T-SOD) and peroxidase (POD) activities as well as the soluble sugar (SS) content were measured at 0 (mock control), 6, 12, 24, 48 and 72 h post inoculation (hpi).

After the plants were infected with *V. dahliae*, the T-SOD, POD and SS enzymes within the *S. sisymbrifolium* roots showed greater activity than did those within the control roots (0 hpi). The maximum T-SOD (Fig. 1a) was observed at 12 hpi, and a second, smaller increase was detected at 48 hpi. The POD enzyme activity increased markedly after the plants were inoculated with the pathogen (Fig. 1b) and exhibited two similar peaks at 12 and 48 hpi. The SS content also increased sharply after the plants were inoculated; it peaked at 12 hpi, after which it decreased (Fig. 1c).

Based on the above results, we can conclude that the physiological activity of *S. sisymbriifolium* changed markedly under stress from *V. dahliae*, especially at 12 and 48 hpi. To identify the resistance-related genes, samples at three time points (0, 12 and 48 hpi) of those examined above were selected for RNA-Seq.

### Illumina sequencing and de novo assembly

Nine transcriptome libraries by using an Illumina HiSeq™ 2000 platform for *S. sisymbrifolium*, three time points (0, 12 and 48 hpi) and their biological replicates were constructed and sequenced. A total of 480,282,756 raw reads were generated from the nine libraries. After removing the reads containing ploy-N, reads containing adaptor sequence fragments and low quality reads by stringent quality checks and data cleaning, 454,990,956 clean reads with a mean Q20 of 96.87% were obtained from all of the libraries (Supplementary Table S2). The de novo assembly of these clean reads produced 430,269 transcripts and 221,695 unigenes by using Trinity softwore (r20140413p1)

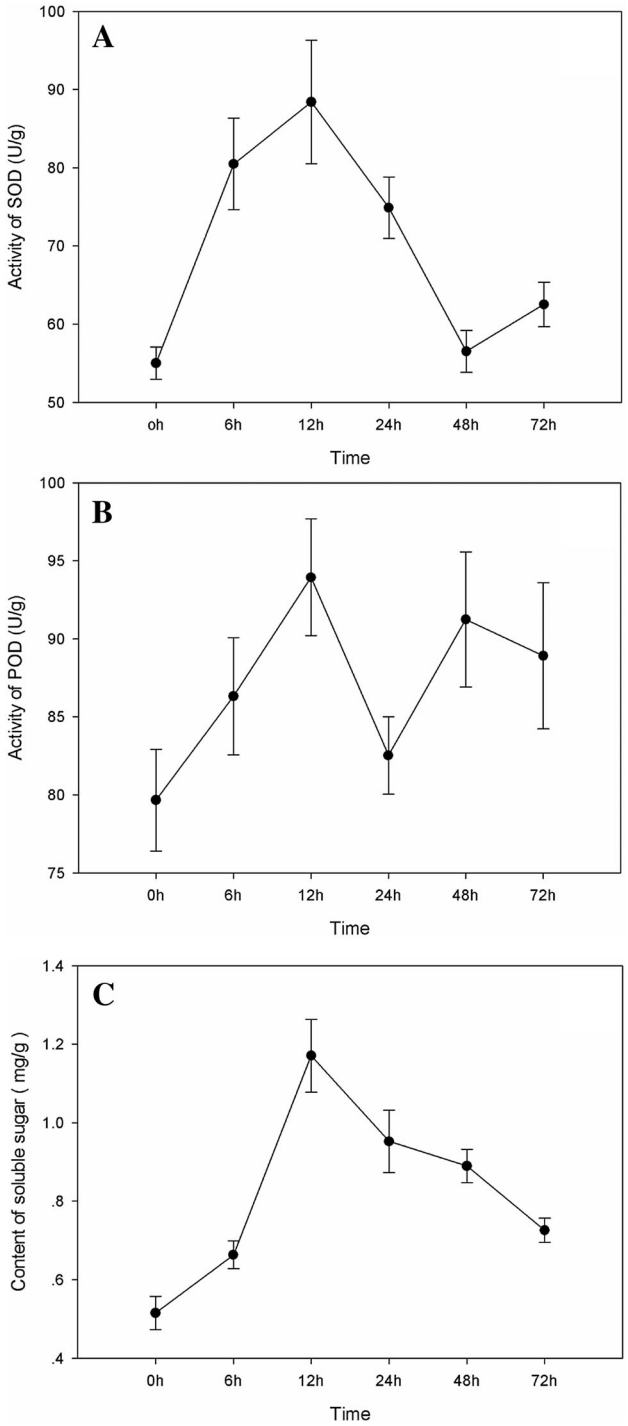


**Fig. 1** Determination of physiological indexes. The activities of T-SOD (**a**) and POD (**b**) as well as the SS content (**c**) in the roots of plants challenged with *V. dahliae* were measured at 0, 6, 12, 24, 48 and 72 hpi

(Grabherr et al. 2011). And the N50 of total unigenes was 1772 bp. The sequence size distribution is presented in Figure S1. RNA-seq read data have been deposited in the NCBI Sequence Read Archive (SRA) database under accession No. PRJNA495012 (https://www.ncbi.nlm.nih.

gov/sra/PRJNA495012). The sequencing and assembly results suggest that the unigene data were highly reliable for further analysis.

### Functional annotation and classification

The all-unigenes were tested via BLAST analysis against seven databases (the NCBI non-redundant protein sequences (Nr) and non-redundant nucleotide sequences (Nt), Swiss-Prot, Protein family (Pfam), Clusters of Orthologous Groups of proteins (KOG/COG), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases) with an *e*-value threshold of $10^{-5}$. The results indicated that the unigenes (221,695) with homologous sequences were distributed among the above databases as follows: 141,213 (63.69%), 170,754 (77.02%), 107,976 (48.70%), 50,281 (22.68%), 111,391 (50.24%), 59,105 (26.66%) and 110,326 (49.76%) unigenes were matched to the Nr, Nt, Pfam, KOG, Swiss-Prot, KEGG and GO databases, respectively. Among these unigenes, 192,578 (86.86%) with homologous sequences were present in at least one database. The Nr database annotation results indicated that the majority of the data could be compared with and mapped to *S. tuberosum* (35.1%) or *S. lycopersicum* (17%).

We used the GO database as an international standardized classification system to classify the possible functions of the unigenes based on the Nr annotation. A total of 110,326 unigenes were classified via sequence homology into three major functional categories (biological process (BP), cellular component (CC) and molecular function (MF)) and 57 subcategories (Supplementary Fig. S2). Within the BP category, the top three categories were 'cellular process' (63,727), 'metabolic process' (61,791) and 'single-organism process' (48,950). Within the CC category, the most frequent categories were 'cell' (36,204), 'cell part' (36,191) and 'organelle' (25,126). Genes in the MF category primarily sorted into 'binding' (59,763), 'catalytic activity' (51,536) and 'transporter activity' (8245) categories.

KOG is a database for protein orthology classification. The alignment of the all-unigenes to the KOG database resulted in the identification of 50,281 unigenes assigned to 26 function clusters (Supplementary Fig. S3). Among these categories, the largest group was 'posttranslational modification, protein turnover, and chaperones' (6928 unigenes, 13.78%), then the following groups were 'translation, ribosomal structure and biogenesis' (6221 unigenes, 12.37%), 'general function prediction only' (5980 unigenes, 11.89%), 'energy production and conversion' (3889 unigenes, 7.73%) and 'signal transduction mechanisms' (3595 unigenes, 7.15%).

The KEGG database was used for the functional classification and pathway assignment of the all-unigenes. A total of 59,105 unigenes were assigned to 130 KEGG pathways. The first 19 pathways are shown in Supplementary Fig. S4. The main pathways were 'translation' (7034, 11.90%), 'carbohydrate metabolism' (6070, 10.27%), 'overview' (5101, 8.63%), 'folding, sorting and degradation' (4484, 7.59%) and 'energy metabolism' (3445, 5.83%). The KEGG pathway analysis also revealed that the 'plant-pathogen interaction' (1479, 2.50%), 'plant hormone signal transduction' (1080, 1.83%) and 'phenylpropanoid biosynthesis' (835, 1.41%) clusters were the largest subcategories that were involved in the 'environmental adaptation', 'signal transduction' and 'biosynthesis of secondary metabolites' pathways, respectively.

### Differential expression analysis

A comparison of the three time points identified 6218 (2958 up-regulated, 3260 down-regulated), 7059 (4137 up-regulated, 2922 down-regulated) and 10,402 (5831 up-regulated, 4571 down-regulated) differentially expressed genes (DEGs) in the S_12 h versus S_0 h, S_48 h versus S_0 h and S_48 h versus S_12 h pairs, respectively (Fig. 2).

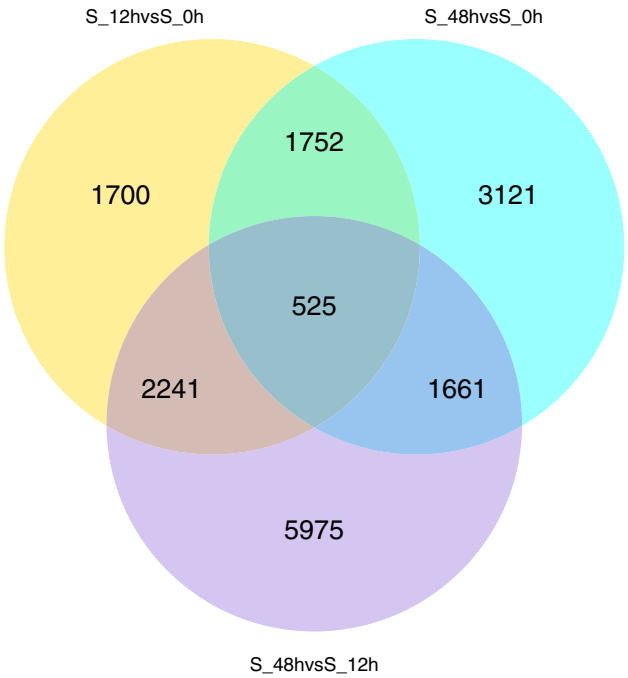


**Fig. 2** Venn diagram showing DEGs from the comparison of three time points. Yellow, blue and purple represent the DEGs of S_12 h versus S_0 h, S_48 h versus S_0 h and the S_48 h versus S_12 h, respectively. The overlapping values correspond to the number of DEGs in two/three comparisons (color figure online)

To analyse the gene expression trends between the different time points, we divided the three time points into stage 1 (S_12 h vs. S_0 h) and stage 2 (S_48 h vs. S_12 h). A comparative analysis between these two stages revealed 13,854 DEGs, including 2766 unigenes, that were significantly regulated during both periods, with 3452 and 7636 unigenes differentially regulated in stage 1 and stage 2, respectively.

The DEGs were hierarchically clustered according to the fragments per kilobase of transcript per million mapped reads (FPKM) values of the samples; this clustering allowed us to observe the overall gene expression pattern. The results (Fig. 3) indicated that the expression patterns of the DEGs varied greatly at the different time points and that the gene expression trends at 0 hpi and 48 hpi were more similar than those at 12 hpi. At the same time, after the plants were inoculated with *V. dahliae*, the majority of the DEGs were expressed at only one or two time points, and a small subset of DEGs were co-expressed at all three time points. The majority of the DEGs exhibited similar expression patterns at 0 hpi and 48 hpi, but these patterns were opposite from those at 12 hpi.

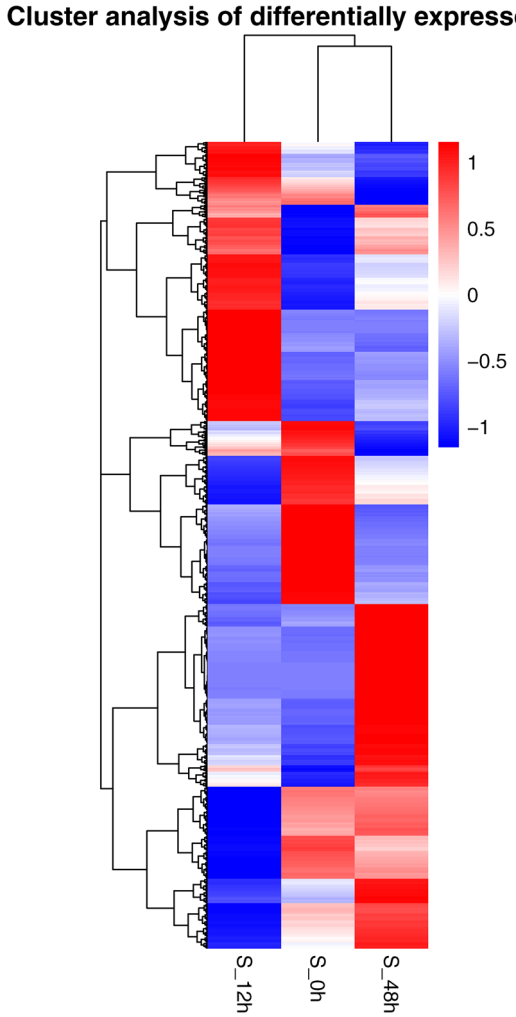


**Fig. 3** Expression profiles of DEGs at both stages. The graph indicates the similarity in the tendencies for changes in gene expression. The shorter the line is, the more similar the genes. Red colour indicates a high expression level, while blue colour indicates a low expression level (color figure online)

GO assignments were used to classify the functions of the predicted *S. sisymbriifolium* genes. The enriched GO terms ($P < 0.05$) are listed in Supplementary Fig. S5. During stage 1, in the BP category, 'oxidation–reduction process', 'phosphorylation' and 'single-organism carbohydrate metabolic process' were significantly overrepresented. In the CC category, 'transcription factor complex', 'extracellular matrix' and 'cytosolic part' were the top three significantly enriched terms. In the MF category, many genes were involved in 'cation binding', 'metal ion binding' and 'oxidoreductase activity'. During stage 2, 'single-organism process', 'single-organism metabolic process' and 'oxidation–reduction process' were the most enriched in the BP category, and 'chloroplast part', 'plastid part' and 'thylakoid membrane' were the top three enriched terms in the CC category. For the MF category, 'catalytic activity', 'ion binding' and 'cation binding' were the most enriched terms.

We performed a KEGG analysis of the DEGs to explore their involvement in biological pathways. The pathways that were significantly enriched in both stages are listed in Supplementary Table S3; the top 20 are shown in Fig. 4. The KEGG pathway enrichment analysis indicated that the major pathways for both stages involved the metabolism and biosynthesis of secondary metabolites. For stage 1 (Fig. 4a), 'oxidation–reduction process', 'phosphorylation' and 'single-organism carbohydrate metabolic process' were the three most enriched terms. For stage 2 (Fig. 4b), 'single-organism process', 'single-organism metabolic process' and 'oxidation–reduction process' were the most enriched. These results are consistent with those of the GO enrichment analysis. In particular, some pathways that play important roles in plant resistance to fungal pathogens, such as 'plant hormone signal transduction', 'plant-pathogen interaction' and 'phenylpropanoid biosynthesis', contained many genes and were significantly enriched in both stages. In contrast, several pathways were enriched in only one stage. For example, 'arginine and proline metabolism', 'cutin, suberin and wax biosynthesis', 'flavonoid biosynthesis' and 'stilbenoid, diarylheptanoid and gingerol biosynthesis' were enriched specifically in stage 1, whereas 'diterpenoid biosynthesis', 'photosynthesis', 'regulation of autophagy' and 'zeatin biosynthesis' were enriched only in stage 2.

To provide basic information for further study of the interaction between *S. sisymbriifolium* and *V. dahliae*, several sets of genes involved in important pathways, such

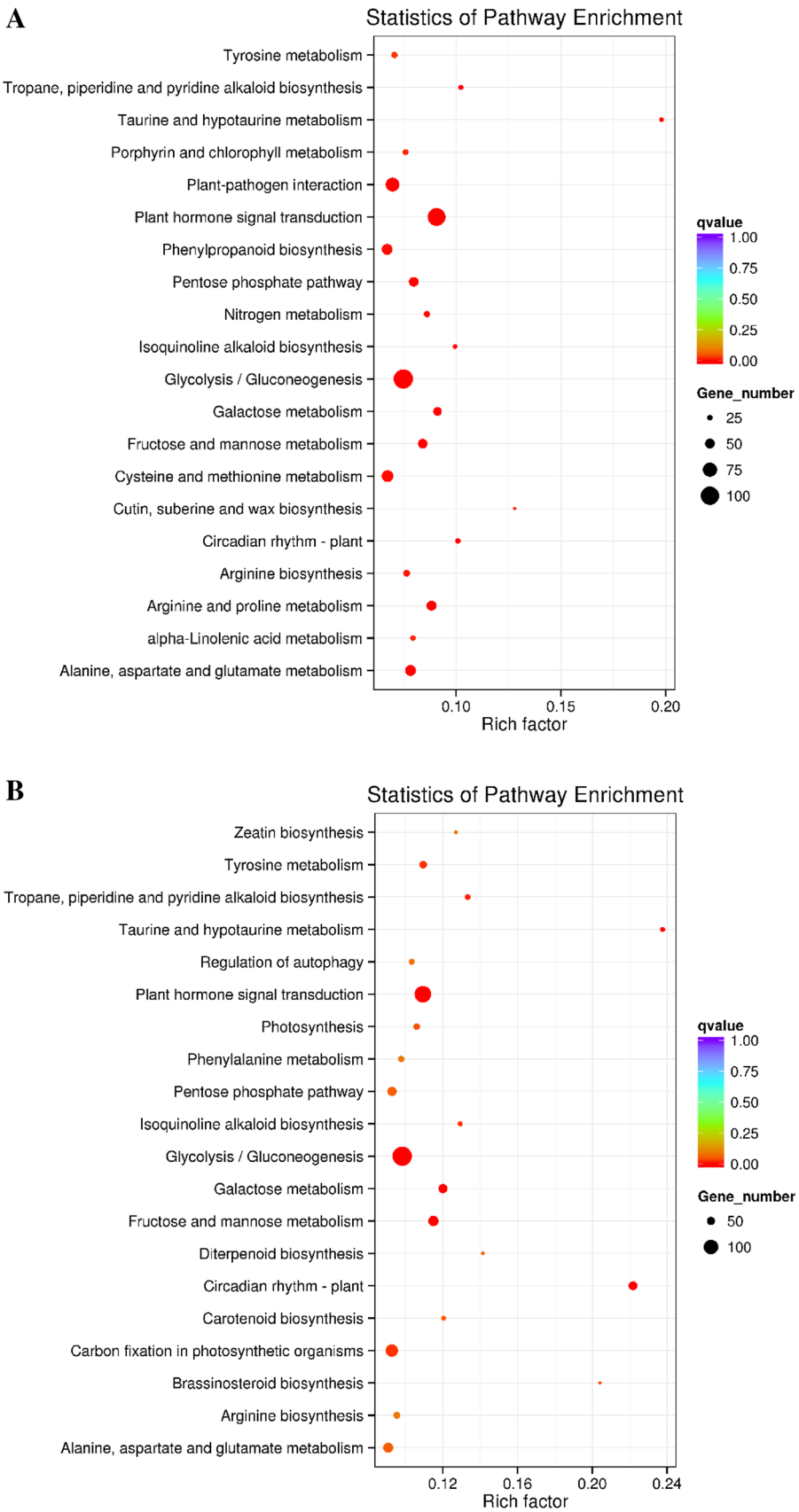


**Fig. 4** KEGG pathway enrichment analysis of stage 1 (A) and stage 2 (B). The top 20 enriched KEGG pathways are listed. The X-axis indicates the enrichment factor of the KEGG term, and the Y-axis indicates the name of the KEGG term. The size of the points indicates the number of genes within the pathway term. The colour of each point indicates the degree of enrichment (color figure online)

as the 'plant hormone signal transduction', 'plant-pathogen interaction' and 'phenylpropanoid biosynthetic' pathways, were selected. Additionally, a subset of the genes that played important roles in activating *V. dahliae* defence responses, including *Ve* genes as well as those encoding pathogenesis-related (PR) proteins and transcription factors (TFs), were screened in this study.

## Selection of the major genes related to resistance to *V. dahliae*

### *DEGs involved in the plant hormone signal transduction pathway*

Many regulated genes that participate in the plant hormone signal transduction (PHST) pathway were identified based on their KEGG pathway assignment (Table 1, Supplementary Fig. S6). The results show that most of the PHST-related genes are involved in abscisic acid (ABA), auxin (AUX), ethylene (ET), cytokinin (CTK) and salicylic acid (SA) biosynthesis during both time periods; however, stage 2 involved more genes than did stage 1.

Additionally, most of the enzymes or TFs emerged in both time courses but with contrasting expression trends. For example, the expression of AUX1, A-ARR, PYL, EBF1_2 and ERF1 was up-regulated in stage 1 but down-regulated in stage 2, and the expression of ARF, B-ARR, BKI1 and JAR1 was down-regulated in stage 1 but up-regulated in stage 2. Some enzymes or TFs were detected only in one time course. All of the genes related to the PIT4 and MYC2 TFs were down-regulated and were detected specifically in stage 1. For the genes that emerged only in stage 2, the expression of AHP, GID1, EIN2, EIN3, BSK and PR1 increased, but the expression of TIR1 and BIN2 decreased.

### *DEGs involved in the PPI pathway*

In the PPI pathway, 70 and 83 DEGs were identified in stage 1 and stage 2, respectively, and the majority of those genes were down-regulated in both stages (Table 2, Supplementary Fig. S7). In stage 1, the expression of RBOH, NOA1, Pti4, Pti1, RPM1, EIX1_2, and CERY1 increased, but that of CDPK, WRKY33, FLS2, MEKK1, NHO1, and RPS2 decreased. In stage 2, the MEKK1, MAP2K1, NHO1, and PR1 genes were up-regulated, but the NOA1, WRKY33, WRKY22, Pti4, Pti5, Pti1, RIN4, PBS1, and EIX1_2 genes were down-regulated.

Furthermore, in the PPI pathway, more genes participated in stage 2 than in stage 1, such as MAP2K1, WRKY22, Pti5, PR1, PBS1, RIN4 and SGT1. The majority of the enzymes and proteins encoded by the unigenes were identified in both stages, but their gene expression patterns were very different. For example, the expression of EIX1_2, NOA1, Pti1 and Pti4 increased in stage 1 but decreased in stage 2, and, the expression of NHO1 and MEKK1 increased in stage 1 but decreased in stage 2.

### *DEGs involved in phenylpropanoid biosynthetic (PB) pathway-related genes*

Several sets of genes assigned to the PB pathway were also detected in this study (Table 3, Supplementary Fig. S8). In stage 1, several genes encoding enzyme transcripts, including COMT, C3′H, C4H, and CCoAOMT, were up-regulated; however, the genes encoding F5H and CALDH were down-regulated. In stage 2, the expression of BGLU, PAL, CCoAOMT, 4CL, C4H, HCT, and F5H increased, but the expression of C3′H and CALDH decreased.

More genes in the PB pathway were identified in stage 2 than in stage 1, and most of those genes were up-regulated. Regarding the enzymes that were present in both stages, the expression of C4H and CCoAOMT increased continuously during the two stages, but the expression of F5H and C3′H showed the opposite pattern. In addition, one CALDH gene was down-regulated and differentially regulated only in stage 1, whereas two up-regulated PAL genes were detected only in stage 2.

### *Ve genes and PR protein-encoding genes*

*Ve* genes (Fradin et al. 2009; Kawchuk et al. 2001) and PR protein-encoding genes (Zhang et al. 2013, 2016a, b; Zhou et al. 2016) play important roles in defence reactions against verticillium wilt.

In this study, the Nr annotation described two non-DEGs (Cluster-33922.112117 and Cluster-33922.87359) as *Ve* genes (Table 4), and NCBI queries revealed that those genes shared high identity with the *S. torvum* genes *StVe1* (93.0%, DQ020574.1) and *StVe2* (95.6%, KT946796.1), respectively.

Seven unigenes that were described as or predicted to be PR proteins of *Solanum* species on the basis of their Nr description and that were expressed differentially after the plants were inoculated with *V. dahliae* were screened (Table 4). Among those unigenes, three were annotated as PR10 and were up-regulated in both stages, and two were annotated as PR5 and were down-regulated in stage 1 but up-regulated in stage 2. In addition, three PR1 genes that are involved in both the PHST (Table 1) and PPI pathways (Table 2) exhibited different expression trends.

### *TFs*

TFs have been known to play significant roles in plant responses to biotic stresses as important regulatory

**Table 1** DEGs involved in the PHST pathway

| Terms | | | Stage 1 (S_12 h vs. S_0 h) | Stage 2 (S_48 h vs. S_12 h) |
|---|---|---|---|---|
| Hormone category | Gene name | Gene definition | Unigenes annotated | Unigenes annotated |
| Auxin | | | 22 (9 up-, 13 down-regulated) | 24 (16 up-, 8 down-regulated) |
| | AUX1 | auxin influx carrier (AUX1 LAX family) | 1 (up-regulated) | 2 (down-regulated) |
| | ARF | auxin response factor | 2 (down-regulated) | 4 (up-regulated) |
| | IAA | auxin-responsive protein IAA | 6 (5 up-, 1 down-regulated) | 5 (1 up-, 4 down-regulated) |
| | GH3 | auxin responsive GH3 gene family | 5 (1 up-, 4 down-regulated) | 6 (up-regulated) |
| | SAUR | SAUR family protein | 8 (2 up-, 6 down-regulated) | 6 (5 up-, 1 down-regulated) |
| | TIR1 | transport inhibitor response 1 | 0 | 1 (down-regulated) |
| Cytokinin | | | 11 (5 up-, 6 down-regulated) | 11 (8 up-, 3 down-regulated) |
| | A-ARR | two-component response regulator ARR-A family | 5 (up-regulated) | 2 (down-regulated) |
| | CRE1 | arabidopsis histidine kinase 2/3/4 (cytokinin receptor)[EC:2.7.13.3] | 4 (down-regulated) | 5 (4 up-, 1 down-regulated) |
| | B-ARR | two-component response regulator ARR-B family | 2 (down-regulated) | 2 (up-regulated) |
| | AHP | histidine-containing phosphotransfer peotein | 0 | 2 (up-regulated) |
| Gibberellin | | | 1 (down-regulated) | 2 (up-regulated) |
| | PIT4 | phytochrome-interacting factor 4 | 1 (down-regulated) | 0 |
| | GID1 | gibberellin receptor GID1[EC:3.-.-.-] | 0 | 2 (up-regulated) |
| Abscisic acid | | | 33 (9 up-, 24 down-regulated) | 32 (21 up-, 11 down-regulated) |
| | PYL | abscisic acid receptor PYR/PYL family | 7 (up-regulated) | 10 (down-regulated) |
| | PP2C | protein phosphatase 2C [EC:3.1.3.16] | 16 (down-regulated) | 9 (8 up-, 1 down-regulated) |
| | ABF | ABA responsive element binding factor | 3 (down-regulated) | 5 (up-regulated) |
| | SnRK2 | serine/threonine-protein kinase SRK2 [EC:2.7.11.1] | 7 (2 up-, 5 down-regulated) | 8 (up-regulated) |
| Ethylene | | | 18 (18 up-regulated) | 18 (6 up-, 12 down-regulated) |
| | ETR | ethylene receptor [EC:2.7.13.-] | 6 (up-regulated) | 6 (1 up-, 5 down-regulated) |
| | CTR1 | serine/threonine-protein kinase CTR1 [EC:2.7.11.1] | 3 (up-regulated) | 2 (1 up-, 1 down-regulated) |
| | EBF1_2 | EIN3-binding F-box protein | 7 (up-regulated) | 5 (down-regulated) |
| | ERF1 | ethylene-responsive transcription factor 1 | 2 (up-regulated) | 1 (down-regulated) |
| | EIN2 | ethylene-insensitive protein 2 | 0 | 1 (up-regulated) |
| | EIN3 | ethylene-insensitive protein 3 | 0 | 3 (up-regulated) |
| Brassinosteroid | | | 2 (2 down-regulated) | 6 (5 up-, 1 down-regulated) |
| | BKI1 | BRI1 kinase inhibitor 1 | 2 (down-regulated) | 3 (up-regulated) |
| | BSK | BR-signalling kinase [EC:2.7.11.1] | 0 | 2 (up-regulated) |
| | BIN2 | protein brassinosteroid insensitive 2 [EC:2.7.11.1] | 0 | 1 (down-regulated) |
| Jasmonic acid | | | 3 (down-regulated) | 3 (up-regulated) |
| | JAR1 | jasmonic acid-amino synthetase | 1 (down-regulated) | 3 (up-regulated) |
| | MYC2 | transcription factor MYC2 | 2 (down-regulated) | 0 |
| Salicylic acid | | | 7 (2 up-, 5 down-regulated) | 21 (17 up-, 4 down-regulated) |
| | NPR1 | regulatory protein NPR1 | 3 (1 up-, 2 down-regulated) | 5 (4 up-, 1 down-regulated) |
| | TGA | transcription factor TGA | 4 (1 up-, 3 down-regulated) | 13 (10 up-, 3 down-regulated) |

**Table 1** continued

| Terms | | | Stage 1 (S_12 h vs. S_0 h) | Stage 2 (S_48 h vs. S_12 h) |
|---|---|---|---|---|
| Hormone category | Gene name | Gene definition | Unigenes annotated | Unigenes annotated |
| | PR1 | pathogenesis-related protein 1 | 0 | 3 (up-regulated) |
| Total | | | 97 (43 up-, 54 down-regulated) | 117 (78 up-, 39 down-regulated) |

**Table 2** DEGs involved in the PPI pathway

| Terms | | Stage 1 (S_12 h vs. S_0 h) | Stage 2 (S_48 h vs. S_12 h) |
|---|---|---|---|
| Gene name | Gene description | Unigenes annotated | Unigenes annotated |
| Rboh | respiratory burst oxidase [EC:1.6.3.- 1.11.1.-] | 7 (up-regulated) | 5 (4 up-, 1 down-regulated) |
| NHO1 | glycerol kinase [EC:2.7.1.30] | 2 (down-regulated) | 2 (up-regulated) |
| CaM | calmodulin | 20 (5 up-, 15 down-regulated) | 21 (6 up-, 15 down-regulated) |
| MAP2K1 | mitogen-activated protein kinase kinase 1 [EC:2.7.12.2] | 0 | 2 (up-regulated) |
| CNGC | cyclic nucleotide gated channel, plant | 12 (8 up-, 4 down-regulated) | 5 (2 up-, 3 down-regulated) |
| HSP90 | heat shock protein 90 kDa beta | 2 (1 up-, 1 down-regulated) | 4 (1 up-, 3 down-regulated) |
| SGT1 | suppressor of G2 allele of *SKP1* | 0 | 2 (1 up-, 1 down-regulated) |
| CDPK | calcium-dependent protein kinase | 8 (down-regulated) | 8 (6 up-, 2 down-regulated) |
| MEKK1 | mitogen-activated protein kinase kinase kinase 1 [EC:2.7.11.25] | 1 (down-regulated) | 1 (up-regulated) |
| FLS2 | LRR receptor-like serine/threonine-protein kinase *FLS2* [EC:2.7.11.1] | 2 (down-regulated) | 2 (1 up-, 1 down-regulated) |
| WRKY33 | WRKY transcription factor 33 | 5 (down-regulated) | 1 (down-regulated) |
| WRKY22 | WRKY transcription factor 22 | 0 | 1 (down-regulated) |
| NOA1 | NOA1,nitric-oxide synthase, plant [EC:1.14.13.39] | 1 (up-regulated) | 1 (down-regulated) |
| CERK1 | chitin elicitor receptor kinase 1 | 2 (up-regulated) | 10 (1 up-, 9 down-regulated) |
| PBS1 | disease resistance protein *RPS2* | 0 | 1 (down-regulated) |
| Pti4 | pathogenesis-related genes transcriptional activator *PTI4* | 1 (up-regulated) | 1 (down-regulated) |
| Pti5 | pathogenesis-related genes transcriptional activator *PTI5* | 0 | 2 (down-regulated) |
| Pti1 | pto-interacting protein 1 [EC:2.7.11.1] | 1 (up-regulated) | 3 (down-regulated) |
| PR1 | pathogenesis-related protein 1 | 0 | 3 (up-regulated) |
| RIN4 | *RPM1*-interacting protein 4 | 0 | 2 (down-regulated) |
| RPM1 | disease resistance protein *RPM1* | 3 (up-regulated) | 2 (1 up-, 1 down-regulated) |
| RPS2 | disease resistance protein *RPS2* | 2 (down-regulated) | 2 (1 up-, 1 down-regulated) |
| EIX1_2 | EIX receptor 1/2 | 1 (up-regulated) | 1 (down-regulated) |
| **Total** | | **70 (30 up-, 40 down-regulated)** | **83 (32 up-, 50 down-regulated)** |

proteins. In the present study, querying the unique transcripts of the DEGs resulted in the identification of 499 and 663 TFs in stage 1 and stage 2, respectively, and their classification into 57 and 63 families. The top 20 groups of TFs are listed in Fig. 5. Analysis of the TFs revealed that the groups and numbers of TFs were similar between the two stages, and the MYB, AP2-EREBP, Orphan, WRKY, bHLH and bZIP groups were the six largest groups in both stages.

## Validation of DEGs by real-time quantitative PCR (RT-qPCR)

To confirm the assembled sequences and the expression profiles of RNA-Seq, RT-qPCR was performed on a subset of 18 select DEGs (Fig. 6, Supplementary Table S4). These genes were differentially expressed in both stages and consisted of nine PHST genes, seven PPI genes and two phenylpropanoid biosynthesis genes (Supplementary

**Table 3** DEGs involved in the PB pathway

| Terms | | Stage 1 (S_12 h vs. S_0 h) | Stage 2 (S_48 h vs. S_12 h) |
|---|---|---|---|
| Gene name | Gene description | Unigenes annotated | Unigenes annotated |
| CYP73A/C4H | trans-cinnamate 4-monooxygenase [EC:1.14.13.11] | 2 (up-regulated) | 2 (up-regulated) |
| COMT | caffeic acid 3-O-methyltransferase [EC:2.1.1.68] | 3 (up-regulated) | 5 (3 up-, 2 down-regulated) |
| CYP98A/C3′H | coumaroylquinate(coumaroylshikimate) 3′-monooxygenase [EC:1.14.13.36] | 3 (up-regulated) | 2 (down-regulated) |
| CCoAOMT | caffeoyl-CoA O-methyltransferase [EC:2.1.1.104] | 1 (up-regulated) | 2 (up-regulated) |
| CYP84A/F5H | ferulate-5-hydroxylase [EC:1.14.-.-] | 1 (down-regulated) | 1 (up-regulated) |
| CALDH | coniferyl-aldehyde dehydrogenase [EC:1.2.1.68] | 1 (down-regulated) | 0 |
| 4CL | 4-coumarate–CoA ligase [EC:6.2.1.12] | 4 (1 up-, 3 down-regulated) | 2 (up-regulated) |
| HCT | shikimate O-hydroxycinnamoyltransferase [EC:2.3.1.133] | 4 (1 up-, 3 down-regulated) | 1 (up-regulated) |
| CAD | cinnamyl-alcohol dehydrogenase [EC:1.1.1.195] | 7 (3 up-, 4 down-regulated) | 13 (8 up-, 5 down-regulated) |
| BGLU | beta-glucosidase [EC:3.2.1.21] | 3 (2 up-, 1 down-regulated) | 6 (up-regulated) |
| POD | peroxidase [EC:1.11.1.7] | 27 (18 up-, 9 down-regulated) | 29 (23 up-, 6 down-regulated) |
| PAL | phenylalanine ammonia-lyase [EC:4.3.1.24] | 0 | 2 (up-regulated) |
| UGT72E | coniferyl-alcohol glucosyltransferase [EC:2.4.1.111] | 0 | 1 (down-regulated) |
| CCR | cinnamoyl-CoA reductase [EC:1.2.1.44] | 0 | 4 (1 up-, 3 down-regulated) |
| Total | | 56 (34 up-, 22 down-regulated) | 70 (51 up-, 19 down-regulated) |

**Table 4** Expression profiles of several known unigenes in response to infection by *V. dahlia*

| Gene name | Unigene ID | Gene length | Description | FPKM | | |
|---|---|---|---|---|---|---|
| | | | | 0 hpi | 12 hpi | 48 hpi |
| Ve2 | Cluster-33922.87359 | 3756 | verticillium wilt disease resistance protein precursor *[Solanum torvum*] | 29.09 | 10.12 | 13.85 |
| Ve1 | Cluster-33922.112117 | 3615 | verticillium wilt disease resistance protein [*Solanum torvum*] | 1.28 | 0.63 | 1.04 |
| PR10 | Cluster-33922.86915 | 1578 | pathogenesis-related protein 10 [*Solanum virginianum*] | 0.87 | 11.07 | 36.84 |
| PR10 | Cluster-33922.88265 | 1767 | pathogenesis-related protein 10 [*Solanum virginianum*] | 2.77 | 17.23 | 21.99 |
| PR5 | Cluster-33922.53642 | 1413 | PREDICTED: pathogenesis-related protein 5 [*Solanum lycopersicum*] | 4.45 | 1.47 | 7.36 |
| PR5 | Cluster-33922.53641 | 1423 | PREDICTED: pathogenesis-related protein 5 [*Solanum lycopersicum*] | 6.55 | 2.79 | 14.21 |
| PR10 | Cluster-33922.86911 | 896 | pathogenesis-related protein 10 [*Solanum virginianum*] | 382.66 | 641.74 | 1909.39 |
| PR | Cluster-33922.69006 | 1717 | PREDICTED: pathogen-related protein-like [*Solanum tuberosum*] | 0.13 | 0.46 | 0.00 |
| PR1 | Cluster-33922.28369 | 716 | pathogenesis related protein 1 | 2.64 | 1.40 | 48.66 |
| PR1 | Cluster-20063.0 | 874 | pathogenesis-related protein 1 | 0.06 | 0.00 | 2.18 |
| PR1 | Cluster-33922.147658 | 1145 | pathogenesis-related protein 1 | 0.02 | 0.19 | 2.37 |

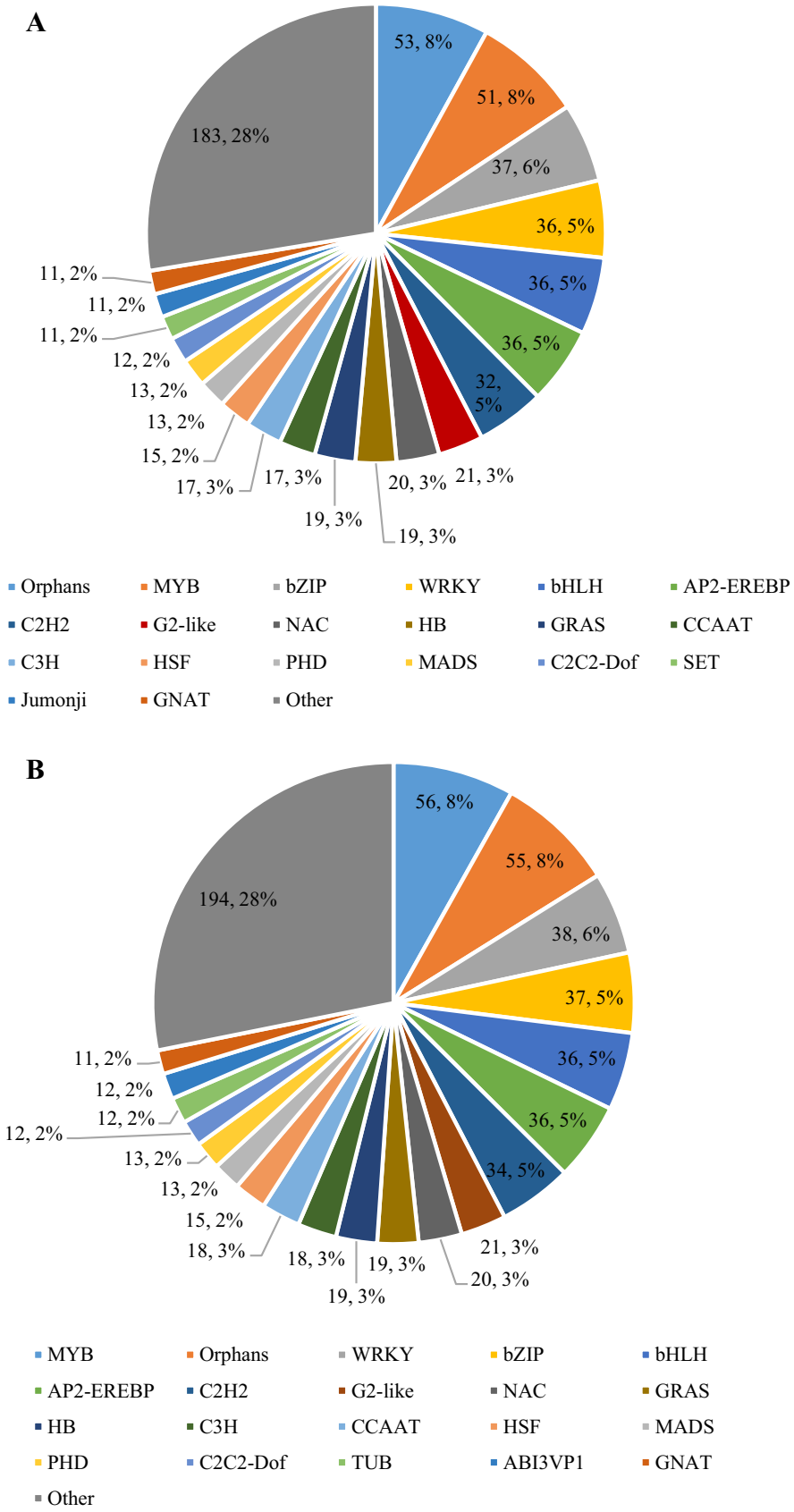


**Fig. 5** Classification of TFs in stage 1 (A) and stage 2 (B). The top 20 groups of TFs are listed. Different colours represent different TF types. Because the different stage has different TF groups, the same TF type may have different colour in graph A and B (color figure online)

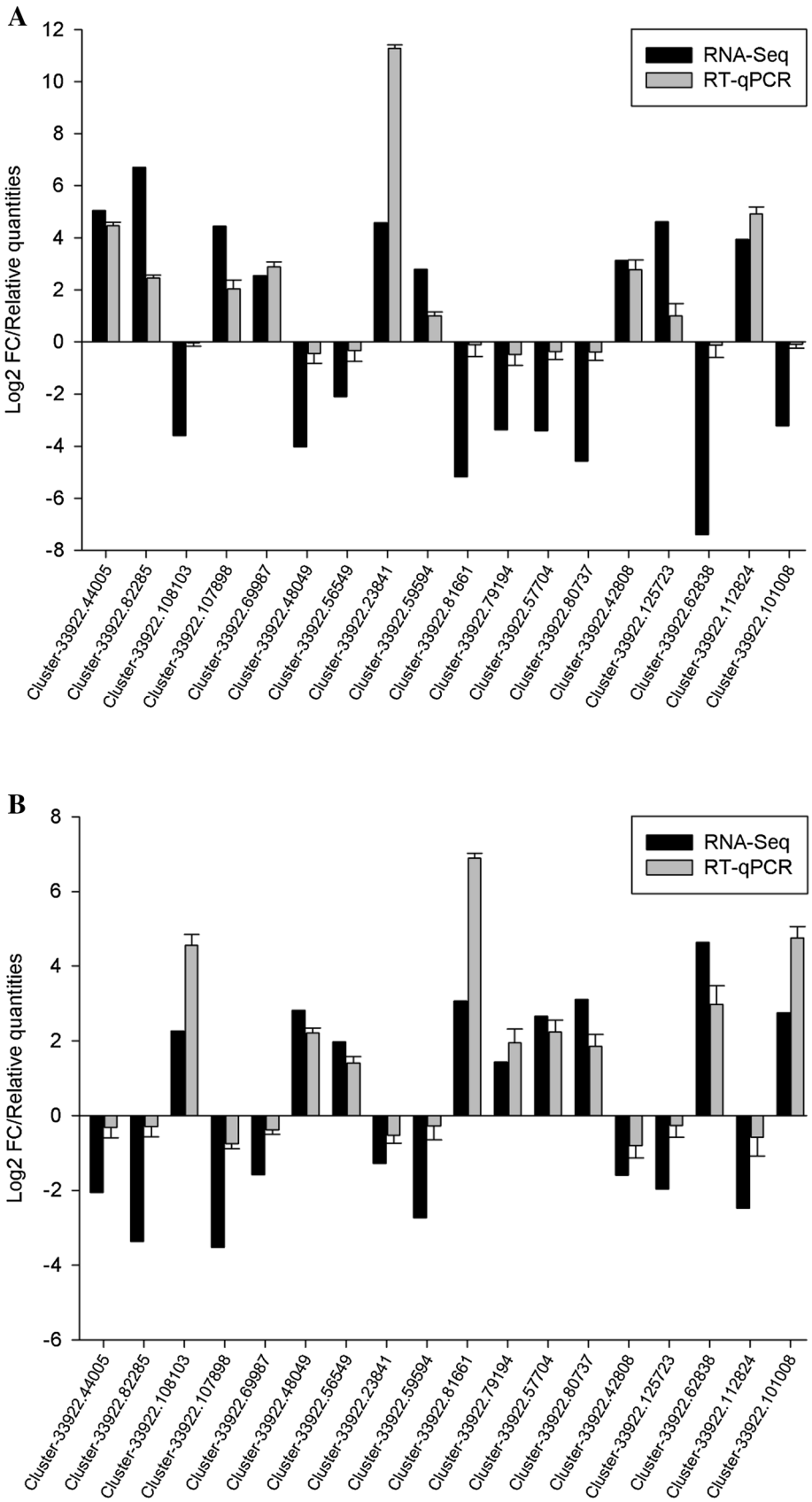


**Fig. 6** RT-qPCR validation of stage 1 (A) and stage 2 (B). Eighteen co-expressed DEGs were selected from the PHST, PPIand PB pathways. The Y-axis indicates the normalized expression patterns based on RNA-Seq and RT-qPCR, which are transformed by either the $\log_2$ FC and the mean relative quantities (RQ), respectively. Error bars indicate SD. The calculation of RQ were controlled by 0 hpi in stage 1 (S_12 h vs. S_0 h) and by 12 hpi in stage 2 (S_48 h vs. S_12 h)

Table S5). For all of the detected genes, the trends observed from the RT-qPCR expression were in agreement with those from the RNA-Seq data.

## Discussion

Physiological and biochemical studies on EVW have been conducted in several plants (Chen et al. 2015; Derksen et al. 2013; Faino et al. 2012), and some verticillium wilt resistance genes have been cloned (Chai et al. 2003; Diwan et al. 1999; Fradin et al. 2009). Nevertheless, the molecular mechanism of wild eggplant resistance to *V. dahliae* is still unclear; the disease resistance mechanism is complex, and there are several resistance genes involved. Moreover, previous studies on the eggplant defence response have not provided enough information to determine the complex transcriptomic dynamics. In recent years, the accessibility of high-throughput sequencing technologies has provided a good opportunity to thoroughly analyse the large-scale expression profile of the defence mechanism, despite uncharacterized genome sequences (Grabherr et al. 2011). In this study, we chose *S. sisymbriifolium*, which has proven to be a valuable resistance resource against verticillium wilt, as the subject of a transcriptome analysis, both to investigate its response process under stress from *V. dahliae* and to explore new resistance genes and resistance mechanisms for overcoming EVW. Considering the large differences between the cultivated and wild species of eggplant, the genomic sequence of eggplant was not used in this study despite being published.

### Selection of time points

Signal perception, transduction and exchange in plant defense responses usually occur during the early periods of pathogen infection (Jones and Dangl 2006; Kunkel and Brooks 2002), especially within the first 12 hpi, which often corresponds to fungal spore germination and fungal penetration of plant epidermal cells (Fradin and Thomma 2006). Additionally, 48 hpi with *V. dahliae* has also been used as a key time point for RNA-Seq analysis in previous studies (Gayoso et al. 2010; Sun et al. 2013; Tan et al. 2015).

In the present study, measurements of physiological activity of the *S. sisymbriifolium* roots following infection with *V. dahliae* (Fig. 1) revealed that the maximum T-SOD and POD activities and SS content occurred at 12 hpi and that a second increase in both T-SOD and POD activities occurred at 48 hpi. GO enrichment analysis of the DEGs revealed that all six SOD-related unigenes were up-regulated in stage 1 but down-regulated in stage 2. Regarding POD-related genes, there were 25 up-regulated and 14 down-regulated unigenes in stage 1 but 48 up-regulated and 7 down-regulated unigenes in stage 2. The same trend was observed for unigenes related to glucose (54 up-regulated and 9 down-regulated unigenes in stage 1; 28 up-regulated and 56 down-regulated unigenes in stage 2) and fructose (35 up-regulated and 3 down-regulated unigenes in stage 1; 22 up-regulated and 36 down-regulated unigenes in stage 2), which are the main classes of SSs. This result coincided with the physiological measurement results of SOD, POD and the SSs. Those results indicated that 12 hpi and 48 hpi were the key inflection points during the interaction between *S. sisymbriifolium* and *V. dahliae*, reflecting the reliability and coherence of the physiological measurements and transcriptomic data.

Next, three time points (0, 12, and 48 hpi) were chosen and divided into two stages (S_12 h vs. S_0 h; S_48 h vs. S_12 h) that coincided with the crucial stages of fungal infection and the analysis of the basal gene expression patterns between the different time periods, according to the measurements of the physiological indexes. This setup enabled us to avoid any confounding factors, focus on the key time points, and accurately identify the critical genes from among the enormous amounts of RNA-Seq information.

### Identification of DEGs during the *S. sisymbriifolium* response to *V. dahliae*

Comparison of S_12 h versus S_0 h (stage 1) revealed 6218 DEGs, including 2958 up-regulated unigenes whose mean $\log_2$ fold change (FC) was 4.79 and 3260 down-regulated unigenes whose mean $\log_2$ FC was − 4.24. For S_48 h versus S_12 h (stage 2), 5831 up-regulated unigenes whose mean $\log_2$ FC was 2.88 and 4571 down-regulated unigenes whose mean $\log_2$ FC was − 2.42 were revealed in this study. The results indicated that stage 2 has more DEGs than does in stage 1, especially up-regulated DEGs, but the change in amplitude of gene expression was larger in stage 1 than in stage 2. In addition, the hierarchical clustering of the DEGs showed that the expression patterns of the DEGs at 0 hpi and 48 hpi were more similar than those at 12 hpi (Fig. 3). These results indicate a major change at 12 hpi and the crucial role of that time point following inoculation of *S. sisymbriifolium* with *V. dahliae*; these results were consistent with those of the physiological experiments (Fig. 1). The results also indicated that negative regulation of the genes occurred promptly in the early stage but that positive regulation was pronounced in the later stage of the *S. sisymbriifolium* response to *V. dahliae*. Here, we predicted the important roles of the down-regulated genes during plant resistance to *V. dahliae*, and our predictions were consistent with those of *Guo* et al. (2017) in sunflower.

## DEGs involved in the main pathways

In this study, both the GO and KEGG enrichment analyses of the DEGs in both stages indicated that many genes are involved in the three most important pathways (the PHST, PPI and PB pathways), which indicates that these processes play an important role in the response of *S. sisymbriifolium* to *V. dahliae*. These pathways have previously been studied with respect to the interaction between *V. dahliae* and cotton (Sun et al. 2013; Zhang et al. 2017), tomato (Tan et al. 2015) and sunflower (Guo et al. 2017), but this is the first time a comprehensive and detailed investigation has been conducted in eggplant.

### *DEGs involved in the PHST pathway*

As signaling molecules, plant hormones are considered to play an important role in modulating signaling networks involved in plant responses to various biotic stresses (Bari 2009). For example, SA, which suppresses JA-mediated defences against necrotrophic signals, is activated in cotton upon *V. dahliae* infection (Gao et al. 2013; Pieterse et al. 2009, 2012). ET has complex functions in the response to verticillium wilt; for example, ET triggers induced systemic resistance (ISR) in plants (Pieterse et al. 1996; Wang et al. 2011), and the release of ET induces cotton susceptibility to verticillium wilt and can cause leaves to wilt (Beyer 1979; Veronese et al. 2003). AUX is involved in general transcriptional induction during the response of cultivated eggplant to *V. dahliae* infection (Yang et al. 2013). Several recent papers also have reported that ABA plays important roles in plant defence responses (Adie et al. 2007; de Wit 2007; Mohr and Cahill 2003).

In this study (Table 1), the largest group of ABA-related genes and the pathway 'CRE1 → AHP → B-ARR → A-ARR → Stomatal closure' were detected in both stages, suggesting an important role of ABA during the response of *S. sisymbriifolium* to *V. dahliae*. In contrast, two other important pathways, 'SA → NPR1 → TGA → PR-1 → Disease resistance' and 'AUX1 → TIR1 → AUX/IAA → ARF → Cell enlargement/Plant growth', of PHST were found in stage 2. These pathways were also reported in the transcriptome profiling of the defence responses of both *S. aculeatissimum* (Zhou et al. 2016) and cotton (Zhang et al. 2013) against *V. dahliae*, and the presence of these pathways might indicate that SA and AUX play important functions in resistance to *V. dahliae*.

Regarding the SA-mediated signal transduction pathway, NPR1 plays a key role in the induction of defence genes by activating the transcription factor TGA (Zhou et al. 2000; Johnson et al. 2003). In the present study, the NPR1 and TGA genes also showed a similar expression trend of down-regulation in stage 1 followed by up-regulation in stage 2, a pattern that was especially pronounced for the TGA genes. In addition, all of the ET genes were up-regulated in stage 1, but most of them were down-regulated in stage 2. In addition, only one JAR1 gene and two genes for MYC2 transcription factors in the JA-mediated signal transduction pathway were identified, all of which were down-regulated in stage 1 and up-regulated in stage 2. These results indicated that, after the plants were infected with the hemibiotrophic pathogen *V. dahliae*, SA-mediated defence was induced in *S. sisymbriifolium*, but the JA/ET signalling pathways were suppressed. SA-dependent responses are involved in responses to biotrophic or hemibiotrophic pathogens, whereas the JA/ET pathways are activated during resistance to necrotrophic pathogens (Glazebrook 2005; Pieterse et al. 2009).

In general, the majority of the PHST genes were involved in the biosynthesis of ABA, AUX, ET, CTK and SA, but their expression patterns varied. This variation indicated important roles for these hormones and their differential regulation during the interaction between *S. sisymbriifolium* and *V. dahliae*. Nevertheless, the mechanisms underlying the protective effects of these hormones remain largely unknown; the role of these hormones appears to be complex, and additional studies are still needed.

### *DEGs involved in the PPI pathway*

The PPI pathway is one of the most important pathways during the plant defence response to pathogens, including *V. dahliae*. During the PPI process, PAMPs (or MAMPs) are recognized by plants through pattern recognition receptors (PRRs), resulting in PAMP-triggered immunity (PTI) that can halt further colonization as the first layer of the response (Brutus and He 2010). A second layer, effector-triggered immunity (ETI), specifically recognizes the effector; this recognition occurs via NB-LRR proteins (Nürnberger et al. 2004; Zhang and Zhou 2010). ETI is an accelerated response, while PTI is amplified, resulting in disease resistance and usually a hypersensitive response (HR) at the infection site (Dodds and Rathjen 2010; Jones and Dangl 2006).

In the present study, the majority of genes previously reported to be involved in the PPI pathway during plant and *V. dahliae* interactions (Ekengren et al. 2003; Guo et al. 2017; Zhang et al. 2017) were obtained in our library (Table 2). These genes include the PRR proteins EIX receptor 1/2 (EIX1_2), chitin elicitor receptor kinase 1 (CERK1), and LRR receptor-like serine/threonine-protein kinase (FLS2)(*Eulgem and Somssich,* 2007), and plant cyclic nucleotide-gated channels (CNGCs), as well as subfamilies of $Ca^{2+}$ sensors (CaM, CML and CDPK) (Boudsocq et al. 2010). RIN4 and serine/threonine-protein

PBS1, which contribute to *V. dahliae* resistance in sunflower (Guo et al. 2017) and cotton (Zhang et al. 2016a, b), were also detected in this study. In addition, the HR during the interaction between *S. sisymbriifolium* and *V. dahliae* was activated via Pti1, SGT1 and HSP90B and was mediated by RBOH and NOS (Supplementary Fig. S5), which is consistent with studies on sunflower (Guo et al. 2017) and cotton (Zhang et al. 2013, 2017). Moreover, the two disease-resistance proteins RPM1 and RPS2 were only detected in stage 2, during which they were down-regulated. Three pathogenesis-related genes, the transcriptional activators Pti1, Pti4 and Pti5, were also identified and showed the same expression trend during the interaction between *S. sisymbriifolium* and *V. dahliae.*

In addition, MAPK cascades are associated with resistance to *V. dahliae* (Asai et al. 2002; Tena et al. 2001), and mitogen-activated protein kinase kinase kinase 1 (MEKK1) in both stages and mitogen-activated protein kinase kinase 1 (MAP2K1) in stage 2 were identified in the present study.

Furthermore, WRKY22 and WRKY33, which are linked to MAPK cascades, activated the expression of defence-related genes in cotton (Zhang et al. 2016a, b; 2017) and they were also shown to be involved in the PPI pathway in the present study. Among those TFs, WRKY22 expression was specifically detected and increased in stage 2. In contrast, three WRKY33-related genes were down-regulated in both stages, indicating negative regulation of those genes during interactions between *S. sisymbriifolium* and *V. dahliae*.

### *DEGs involved in the PB pathway*

Phenylpropanoids function as pre-formed and inducible antimicrobial compounds during plant–microbe interactions (Naoumkina et al. 2010). The phenylpropanoid pathway plays a critical role in the plant defence response to *V. dahliae* (Gayoso et al. 2010; Sun et al. 2013; Xu et al. 2011; Zhang et al. 2013). Important enzymes of the PB pathway, such as PAL, POD, CAD, 4CL, COMT, HCT and CCoAOMT, have been characterized in plants following infection with *V. dahliae* (Gayoso et al. 2010; Pomar et al. 2004; Sun et al. 2013; Xu et al. 2011).

In the present study (Table 3), all of the abovementioned genes were detected, and the majority of them were up-regulated after the plants were inoculated with *V. dahliae*. It also has been reported that hundreds of cytochrome P450 (CYP) genes (Sun et al. 2014) and CCoAOMT enzymes (Schilmiller et al. 2009) are involved in the PB pathway. The present study identified CYP73A (C4H), CYP98A (C3′H) and CYP84A (F5H), which are members of the CYP gene family, as differentially regulated, and the genes encoding CYP73A and CCoAOMT were up-regulated in both stages, indicating their important functions during interactions between *S. sisymbriifolium* and *V. dahliae*.

As reported previously, HCT and 4CL can affect lignification in cotton (Sun et al. 2013) and alfalfa (Shadle et al. 2007), and lignin plays a central role in the cotton defence response (Xu et al. 2011). In this study, all of the HCT and 4CL genes demonstrated increased expression in stage 2, so the same function may exist in eggplant. PAL, which functions as a catalyst for phenylpropanoid metabolism, is the first enzyme in the PB pathway. Several studies have reported increased PAL expression and activity in response to *V. dahliae* (Gayoso et al. 2010; Xu et al. 2011). In the present study, two PAL genes were detected, and their expression increased in stage 2, indicating their positive regulation during the *S. sisymbriifolium* response to *V. dahliae*. Peroxidases are also involved in the polymerization of monolignols into lignin and in the reinforcement of plant cell wall after pathogen infection or wounding (Marjamaa et al. 2009). In the present study, PODs were the largest group in the PB pathway, and the expression of POD-related genes changed markedly, especially in stage 1. These results coincided with those of the physiological index measurements, indicating their important role in the response of eggplant to *V. dahliae*.

### *Ve* genes and PR protein-encoding genes

The only locus responsible for effective resistance to race 1 of *V. dahliae*, *Ve 1*, has been isolated and cloned from tomato (*S. lycopersicum*) (Fradin et al. 2009; Kawchuk et al. 2001). The *Ve* locus comprises two genes, *Ve1* and *Ve2*, both of which encode cell surface receptors that are members of the extracellular leucine-rich repeat class of receptor-like proteins (eLRR-RLPs) (Kawchuk et al. 2001). In the present study (Table 4), two unigenes (Cluster-33922.87359 and Cluster-33922.112117) shared high identity with the *S. torvum* genes *StVe1* and *StVe2*. This result indicated that these unigenes were *Ve* homologues. Although both *Ve1* and *Ve2* provide resistance when expressed in close relatives of potato (Gayoso et al. 2010; Kawchuk et al. 2001), only *Ve1* provides resistance in tomato (Fradin et al. 2009). Whether a single *Ve* gene (and which one) or both dominate the resistance of eggplant to *V. dahliae* remains unclear.

PR proteins also play important roles in the defence reaction against verticillium wilt (Wang et al. 2011; Zhang et al. 2016a, b). Several PR proteins, such as PR1, PR2, PR5 and PR10, have a wide range of roles, specifically in biotic and abiotic stresses (Agarwal and Agarwal 2014; van Ooijen et al. 2007; van der Hoorn and Kamoun 2008). In the present study (Table 4), PR1-, PR5- and PR10-related genes were detected and expressed in different patterns, which is consistent with the results of a study on *S.*

*aculeatissimum* (Zhou et al. 2016). These findings probably indicate that these PR proteins are critical during interactions between eggplant and *V. dahliae*.

### TFs

TFs act as master switches. By slight changes in their expression, TFs control the expression of a set of genes to regulate different aspects of plant development or responses to biotic and abiotic stresses (Singh et al. 2002). It is reported that both of basal disease resistance and gene-for-gene resistance are mediated by several classes of TFs, such as AP2-EREBPE (Gutterson and Reuber 2004) and bZIP (Scholz et al. 2018). Many WRKY family TFs have also been reported to play important roles in mediating plant responses to stress factors (Yang et al. 2012; Zhang et al. 2017). Moreover, bHLH family TFs often interact with MYB family proteins to form complexes that then regulate the downstream expression of target genes (Zhang et al. 2013).

In the present study, in both stages (Fig. 6a, b), the six largest groups of TFs were the MYB, AP2-EREBP, Orphan, WRKY, bHLH and bZIP groups, indicating the significance of their roles during the response of *S. sisymbriifolium* to *V. dahliae*. The results of the transcriptome analysis revealed that the majority of TFs were suppressed after the plants were inoculated with the pathogen. This finding hinted that the negative regulation of some TFs to modulate the plant immune signal network might be required to set up the eggplant resistance to *V. dahliae*, as has been previously reported in cotton (Xu et al. 2011). Further study of the regulatory pattern of TFs during the response of *S. sisymbriifolium* to *V. dahliae* is needed.

## Conclusions

This study provided profiles of the transcripts associated with the interaction between the resistant *S. sisymbriifolium* genotype and the highly toxic pathogen *V. dahliae*. The results revealed putative genes involved in the PHST pathway, the PPI pathway, and PB pathway and several crucial genes, including *Ve* genes, PR genes and WRKY genes, that regulate crucial points in the defence-related pathway and that may contribute to *V. dahliae* resistance in eggplant.

These findings could help us get a deeper understanding of the molecular interactions between eggplant and *V. dahlia*, lay foundations for solanaceae resistance breeding, and provide new insight into the interactions between plants and pathogens. Nevertheless, although some defence-related genes from *S. sisymbriifolium* were perceived, it is probably that extra biosynthetic pathways and more complicated signalling mechanisms in the roots are included in this defence system. Additional studies should be implemented to confirm the functions of the selected candidate DEGs involved in the present study.

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**Author contributions** L.Y.W. and G.H.D. performed the experiments, analysed the data, and drafted the manuscript. R.B. planted and managed the materials in the field. Z.B.L. inoculated the seedlings and helped with sampling. F.Y.L. and Y.J.G. conceived the study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

**Compliance with ethical standards**

**Conflict of interest** The authors declare no competing interests, including financial and non-financial interests.

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