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ORIGINAL PAPER

De Novo Sequencing and Analysis of the Transcriptome of the Wild Eggplant Species *Solanum Aculeatissimum* in Response to *Verticillium dahliae*

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Abstract Eggplant verticillium wilt, caused by *Verticillium dahliae*, is one of the most severe diseases of eggplant worldwide. Transferring of resistance genes from wild relatives would be valuable for the continued improvements of eggplant. *Solanum aculeatissimum*, a wild relative of eggplant possessing resistance to verticillium wilt, is potentially useful for genetically enhancing eggplant. To better understand the defense response to *V. dahliae*, transcriptome analysis of roots from *S. aculeatissimum* in response to *V. dahliae* infection was performed. A total of 69,824 all-unigenes were obtained through Illumina/Solexa sequencing. Functional annotations by aligning all-unigenes with public protein databases including NCBI non-redundant protein (NR), Clusters of Orthologous Groups (COG), Swiss-Prot protein, and Kyoto Encyclopedia of Genes and Genomes (KEGG) were obtained. The gene expression profiles of *S. aculeatissimum* infected with *V. dahliae* were compared to those of non-infected plants, and 11,696 upregulated and 5949 downregulated genes were identified. Gene Ontology (GO) enrichment and metabolic pathway-enrichment analysis were performed. Some *V. dahliae*-resistant related genes, including putative resistance genes (R-genes), phenylpropanoid pathway-related genes, genes encoding pathogenesis-related proteins (PRs) and transcription factors were identified. Taken together, this is the first transcriptome sequencing of *S. aculeatissimum* under *V. dahliae* stress.

Keywords *Solanum aculeatissimum* · Transcriptome · *Verticillium dahliae* · Illumina/Solexa

Introduction

Eggplant (*Solanum melongena* L.) belongs to the Solanaceae family, is an important vegetable crop and is of substantial economic importance in Asia, Africa, and the subtropics (Collonnier et al. 2001; Zhuang, Zhou, and Wang 2012). However, its production is severely threatened by numerous abiotic or biotic stresses. Particularly, verticillium wilt, caused by *Verticillium dahliae*, is one of the most destructive soilborne diseases. It invades the roots, inducing vascular disorders, vessels browning, and subsequent wilting. A severe outbreak of this disease can cause significant losses in yield (Daunay 2008). Besides eggplant, it can also affect some other economically important crops such as tomato, potato, and cotton (Tjamos and Beckman 1989). Since no fungicide can efficiently control verticillium wilt, there is an urgent need to explore the useful resistance genes for eggplant improvement (Tamura, Murata, and Mukaihara 2002; Toppino, Vale, and Rotino 2008; Zhuang et al. 2012).

Resistance to verticillium wilt has been identified in some wild relatives of eggplant, such as *S. linnaeanum*, *S. sisymbrifolium*, *S. torvum*, and *S. aculeatissimum*, which are potentially useful for genetically enhancing *S. melongena* (Daunay 2008). However, fertile F_1 hybrids were only obtained between *S. linnaeanum* and *S. melongena*, due to the cross-incompatibility problem between above wild *solanum* species and *S. melongena*. Resistance to *V. dahliae* from *S. linnaeanum* has been

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transferred to cultivated eggplant through subsequent backcrossing (Acciarri et al. 2001; Sunseri et al. 2003). Nevertheless, *S. linnaeanum* is only considered as moderate resistant to *V. dahliae*, it also shows mild symptoms of verticillium wilt including vessels browning and plant growth reduction (Liu et al. 2015). New sources of resistance are needed for sustainable eggplant production. The molecular mechanisms of resistance to *V. dahliae* remain unclear and the ability to explore functional genes or markers would facilitate the breeding progress to improve tolerance to verticillium wilt of eggplant.

Several *V. dahliae* resistance related genes have been isolated and studied in tomato (Diwan et al. 1999; Kawchuk et al. 2001; Fradin et al. 2009), mint (Vining and Davis 2009), lettuce (Hayes et al. 2011) and cotton (Zhang et al. 2012). Moreover, RNA-seq technologies have been used to study gene expression profiles in different cotton species under *V. dahliae* infection (Xu et al. 2011; Sun et al. 2013). The terpenoid and phenylpropanoid pathways and genes associated with lignin metabolism were reported to play an important role in pathogen response in cotton and tomato (Tan et al. 2000; Gayoso et al. 2010; Xu et al. 2011). However, in eggplant, the research of molecular mechanisms of resistance to *V. dahliae* is rather limited compared to the economic crops such as tomato and cotton.

S. aculeatissimum, a wild relative of cultivated eggplant, is a well-known economically important plant, which is widely cultivated as a medicinal crop, possessing some desirable characters especially the resistance to soil-borne diseases such as verticillium wilt and root-knot nematode (Handique 1986; Borua 1990; Zhuang et al. 2012). The breeding program of incorporation of resistance from *S. aculeatissimum* to cultivated eggplant is undergoing and fortunately interspecific hybrids between *S. aculeatissimum* and *S. melongena* have been obtained through embryo culture (Rattan et al. 2015). To make better use of this potential, more genomic information is required. Therefore, the first transcriptome sequencing of *S. aculeatissimum* under *V. dahliae* infection using a next-generation sequencing technology, Illumina/Solexa was undertaken to provide an overview of *S. aculeatissimum* root transcriptome and novel insights into the molecular mechanisms involved in *V. dahliae* resistance.

Materials and Methods

Plant Material and Inoculation

Verticillium dahliae VW003 isolated from eggplant with verticillium wilt in Nanjing area of China (Zhou et al. 2012) was cultured on potato dextrose agar (PDA) plate for 15 days and then was inoculated into Czapek's liquid medium on a shaker at 120 rpm at 25 °C for 4–7 days. The suspension liquid was adjusted to 1×10^7 spores ml^{-1} for inoculation.

S. aculeatissimum seeds were introduced from CGN (Center for Genetic Resources, The Netherlands). After sterilization, seeds were allowed to germinate in 30 °C. The uniform germinated seeds were sown in sterilized soil and cultured in the greenhouse at 25 °C/15 °C, with a photoperiod of 16 h light and 8 h dark. The seedlings were divided into two groups (*V. dahliae* inoculated and control samples), each group contained 15 seedlings. For *V. dahliae* treated group, the seedlings were inoculated by dipping the roots in the suspension of fungal spores for 15 min. Control plants were not inoculated but were treated and sampled with distilled water in the same way. The roots of *V. dahliae* treated and control plants were harvested after 72 h. The collected roots were pooled with three plants for each group and rapidly stored at −80 °C until use.

cDNA Preparation for Illumina Sequencing

Total RNA of each sample was extracted using the RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The total RNA concentration of each samples was estimated using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). The integrity of the RNA samples was assessed with 1.2 % agarose gel electrophoresis. The two samples were sequenced using an Illumina HiSeqTM 2000. Sequencing was performed by the Beijing Genomics Institute (BGI)-Hongkong, China according to the manufacturer's instructions (Illumina, San Diego, CA). The mRNA was first fragmented into small pieces using divalent cations under elevated temperature. Taking these short fragments as templates, random hexamer primer was used to synthesize the first-strand cDNA. The second-strand cDNA was synthesized using buffer, dNTPs, RNase H, DNA polymerase I. Short fragments were purified with a QiaQuick PCR Purification Kit (Qiagen, CA, USA) and resolved with EB buffer for end-repairing and tailing A. After that, the short fragments were connected with sequencing adapters. After resolution by agarose gel electrophoresis, the suitable fragments were selected for the PCR amplification as templates. At last, the two libraries were sequenced using Illumina HiSeq^{TM} 2000.

Raw Sequence Processing and de Novo Assembly

A stringent filtering process of raw sequencing reads was carried out before the transcriptome assembly. The reads with adaptors, ambiguous sequences represented as "N," and those of law quality (<20 % of bases with a quality score $Q \leq 10$) were removed. Q20, the percentage of bases whose quality is larger than 20 in clean reads was used as an index of sequencing error rate. Transcriptome de novo assembly was carried out with short reads assembling program, Trinity (Grabherr

et al. 2011). Trinity first combined reads with certain lengths of overlap to form longer fragments, known as contigs. The reads were then mapped back to contigs; with paired-end reads it was able to detect contigs from the same transcript as well as the distances between these contigs. Finally, Trinity connected the contigs, and get sequences that cannot be extended on either end. Such sequences were defined as unigenes.

Functional Annotation

The unigene sequences were annotated by BLASTx alignment (e value < 0.00001) to protein databases such as the NCBI NR protein database (http://www.ncbi.nlm.nih.gov), the Swiss-Prot protein database (http:// www.expasy.ch/sprot), the KEGG pathway database (http://www.genome.jp/kegg) and the COG database (http://www.ncbi.nlm.nih.gov/COG). The best-aligning results from the four above databases were used to decide the sequence direction of unigenes. If the alignment results from these four databases conflicted with each other, a priority order of NR, Swiss-Prot, KEGG and COG was considered. With regard to the other sequences that failed to be annotated to any one of the above databases, the ESTScan program (http://www.ch.embnet.org/software/ESTScan.html) was used to predict the coding regions (CDS) and their orientations.

For the Nr annotations, the Blast2GO program was used to obtain GO functional annotation, and the GO functional classification for all unigenes were obtained to understand the distribution of gene functions at the macroscopic level using the WEGO software. For COG annotations, the unigene sequences were aligned to the COG database to predict and classify possible functions. For pathway-enrichment analysis, all unigenes were mapped to terms in the KEGG pathway database.

Differential Expression Analysis

FPKM (fragments per kb per million fragments) (Mortazavi et al. 2008) was used to calculate unigene expression levels. The FDR (False Discovery Rate) control method was applied to determine the threshold of p value in multiple tests and analyses (Reiner, Yekutieli, and Benjamini 2003). After the FDR was obtained, the ratio of FPKM was used to calculate the fold-change in the expression of unigenes in two samples simultaneously. An FDR < 0.001 and the absolute value of log2Ratio ≥1 were used as the threshold for the judgment of the significance of the gene expression differences. The different expressed genes were then subjected to GO and KEGG Ontology enrichment analysis.

Identification of Disease Resistance Genes

The identification of plant resistance genes in *S. aculeatissimum* was conducted according to Yang et al. (2014). Amino acid sequences for 112 reference resistance genes were downloaded from the Plant Resistance Genes database (PRGdb: http://prgdb.crg.eu/wiki/Main_Page) (Sanseverino et al. 2013). BlASTP was used to identify and classify putative resistance genes in *S. aculeatissimum* (parameters:*-evalue 1e-5 –outfmt 6 –max_target_seqs 1*). By parsing tabular outputs using in-house PERL scripts, results were filtered with a threshold cutoff of 40 % identity and 50 % coverage, and then homologous sequences were extracted and classified.

Quantitative Real-Time PCR Analysis

The selected unigenes were then validated by quantitative real-time PCR. First-strand cDNA was generated from the total RNA isolated from roots of both *V. dahliae* treated and control plants by using the PrimeScript™ II 1st Strand cDNA synthesis kit (Takara, Japan). Primers for qRT-PCR were designed using Primer 3 (version 4.0) software (Rozen and Skaletsky 2000). All primer pairs were custom ordered from Invitrogen, Shanghai. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene of eggplant was used as internal control gene (Zhou, Liu, and Zhuang 2014). qRT-PCR was performed on the ABI 7500 Real-time PCR system using a SYBR Green-based PCR assay. Three independent biological replicates of each sample and three technical replicates of each biological replicate were used for qRT-PCR analysis. The reaction containing 10 μl of Fastsmart Universal SYBR Green Master (Rox) (Roche), 5 μl of diluted cDNAs, and 0.5 μl (10 pmol) of each primer to a final volume of 20 μl was performed. The PCR conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001). The sequences of the primers used were listed in Supplementary file 1.

Results

Illumina Sequencing and De Novo Assembly

The *S. aculeatissimum* seedlings were randomly divided into two groups (*V. dahliae* inoculated and Control samples). Total RNA of the roots of three plants from both the *V. dahliae* treated and control groups were isolated. The cDNA samples representing the two groups were prepared and sequenced using Illumina HiSeqTM 2000, labeled as VW and CK, respectively.

A total of 28,831,882 raw reads were generated for the *V. dahliae* inoculated (VW) sample, and 28,426,946 raw reads for the control sample (CK). After discarding the low-quality reads, a total of 26,525,328 (total clean nucleotides 2,387,279,520 nt) and 26,578,026 (total clean nucleotides 2,392,022,340 nt) clean reads were obtained from VW and CK samples, respectively. The Q20 percentage was over 97 % for both samples (Table 1).

The short reads were assembled into 126,093 (VW) and 106,026 (CK) contigs. The average contig size was over 300 nt for both samples, with the N50 of 467 (VW) and 523 (CK), respectively. The contigs of each sample were further assembled into unigenes, generating 71,291 unigenes with a mean length of 535 bp for VW sample, and 64,413 unigenes with a mean length of 597 bp for CK sample. A total of 69,824 all-unigenes were obtained after long-sequence clustering between the two samples. The total length of all unigenes was 46,403,534 nt, with a mean length of 665 nt. The size distribution of all unigenes indicated that 40,136 (58.91 %) unigenes were 300 to 500 nt, and 3340 (4.78) unigenes were longer than 2000 nt.

Functional Annotation and Classification

All of the unigenes were tested with BLAST analysis against protein databases with a cutoff *e* value of 10^{-5}. The public protein databases included the NCBI non-redundant protein (Nr) database, the Clusters of Orthologous Groups (COG) databases, the Swiss-Prot protein database, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A total of 54,113 (77.5 %) unigenes with homologous sequences were found in at least one of the above databases. Among them, 46,835, 29,626, 27,568, and 18,630 unigenes were found in NR, Swiss-Prot, KEGG and COG, respectively.

The GO classification system was used to classify the possible functions of the unigenes based on Nr annotations. A total of 34,814 unigenes were classified into three main categories, including biological process, cellular component and molecular function, which were then categorized into 58 functional groups. For biological process category, the top five largest categories were: "cellular process" (23,294), "metabolic process" (22,450), "response to stimulus" (12,160), "biological regulation" (9814), and "regulation of biological process" (9035). For cellular components, the top three categories were: "cell" (23,150), "binding" (17,630), and "membrane" (10,884). For molecular function, the top three categories were: "catalytic activity" (18,182), "transporter activity" (2387), and "structural molecule activity" (1095). Only a few unigenes (<100) were clustered in the following categories: "viral reproduction," "locomotion," "nitrogen utilization," "carbon utilization," "sulfur utilization," "cell killing," "extracellular region part," "extracellular matrix," "extracellular matrix part," "protein binding transcription factor," "nutrient reservoir activity," "metallochaperone activity," "protein tag," and "translation regulator activity" (Fig. 1).

The all-unigenes were aligned to the COG database in which orthologous gene products were classified. A total of 18,630 unigenes were identified and assigned into 25 functional clusters (Fig. 2). Among these COG categories, the cluster "general function prediction only" was the largest group (5751 of 18,630 unigenes, 30.9 %), followed by "transcription" (3312 unigenes, 17.8 %), "translation, ribosomal structure and biogenesis" (2742, 14.7 %), "replication, recombination and repair" (2692, 14.4 %), "posttranslational modification, protein turnover, chaperones" (2576, 13.8 %), "Signal transduction and metabolism" (2382, 12.8 %), "carbohydrate transport and metabolism" (2189, 11.7 %). There were also 1828 unigenes annotated as "function unknown."

The BLAST analysis of all-unigenes was also performed against the KEGG database to further demonstrate functional classification and pathway assignment. In total, 27,568 unigenes were involved in 128 KEGG pathways. The major pathways were "metabolic pathways," with 6768 of 27,568 unigenes (24.55 %) related to it, followed by "biosynthesis of secondary metabolites" with 3264 unigenes (11.84 %) involved, "plant-pathogen interaction" with 1535 unigenes (5.57 %) involved, "plant hormone signal transduction" with 1353 unigenes (4.91 %) involved, and "RNA transport" with 1322 unigenes (4.8 %) involved.

Protein-Coding Region Prediction

A total of 46,823 coding sequences (CDS) from unigene sequences were extracted and translated into peptide sequences after subjecting all-unigene sequences to BLASTx (*e* value < 0.00001) against protein databases (Nr, Swiss-Prot, KEGG and COG). ESTScan was used to predict 6096 coding sequences and translated them into peptide sequences for those unigenes with no BLAST hits.

Differential Expression Analysis

The FPKM method (fragments per KB per million fragments) was used to identify the genes with different expression levels. The results showed that a total of 17,645 genes were significantly changed ($|(\log2\text{Ratio})| \geq 1$) with p values < 0.05 and FDR ≤ 0.001, and were labeled as differentially expressed genes (DEGs). Among these genes, 11,696 genes were upregulated and 5949 genes were downregulated upon inoculation of *S. aculeatissimum* roots with *V. dahliae* (Fig. 3).

The differentially expressed genes in VW and Control samples were subjected into GO enrichment analysis to characterize the major biological functions. The most common terms were "cellular process," "cell"/"cell part," and "catalytic activity" in each of the three main categories (biological process, cellular component, and molecular function),

Table 1 Summary for the *S. aculeatissimum* transcriptome

	Control	VW inoculated	Total
Total Raw Reads	28426946	28831882	
Total Clean Reads	26578026	26525328	
Total Clean Nucleotides (nt)	2392022340	2387279520	
Q20 percentage	97.85 %	97.74 %	
N percentage	0.00 %	0.00 %	
GC percentage	43.73 %	44.89 %	
Contig			
Total Number	106026	126093	
Total Length (nt)	34897735	38936799	
Mean Length (nt)	329	309	
N50	523	467	
Unigene			
Total Number	64413	71291	69824
Total Length (nt)	38463937	38143007	46403534
Mean Length (nt)	597	535	665
N50	957	793	1005
Total Consensus Sequences	64413	71291	69824
Distinct Clusters	17201	16683	19726
Distinct Singletons	47212	54608	50098

respectively. It was noticed that "cell proliferation" was the most significantly enriched GO-term of "Biological process," "cytosolic ribosome" was the most significantly enriched GO-term of "Cellular component," and "structural molecule activity" was the most significantly enriched GO-term of "Molecular Function" (Supplementary file 2).

The pathway analysis of the differentially expressed genes was also performed to identify the primary biochemical pathways and signal transduction pathways in which gene expression changes associated with VW treatment. A total of 128 pathways were obtained. The major pathways were metabolic pathways and biosynthesis of secondary metabolites. The most important metabolite pathways, e.g. plant-pathogen interactions, plant hormone signal transduction and phenylpropanoid biosynthesis were well covered.

Identification of Disease Resistance Genes Involved in *V. dahliae* Stress

In our study, the Plant Resistance Genes database (PRGdb, http://prgdb.org) was used to analysis the resistance genes in *S. aculeatissimum*. PRGdb is a web accessible open-source database that represents bioinformatic resource providing a comprehensive overview of resistance genes (R-genes) in plants (Sanseverino et al. 2010; Sanseverino et al. 2013). In this database, a set of 112 known R-genes were manually curated to

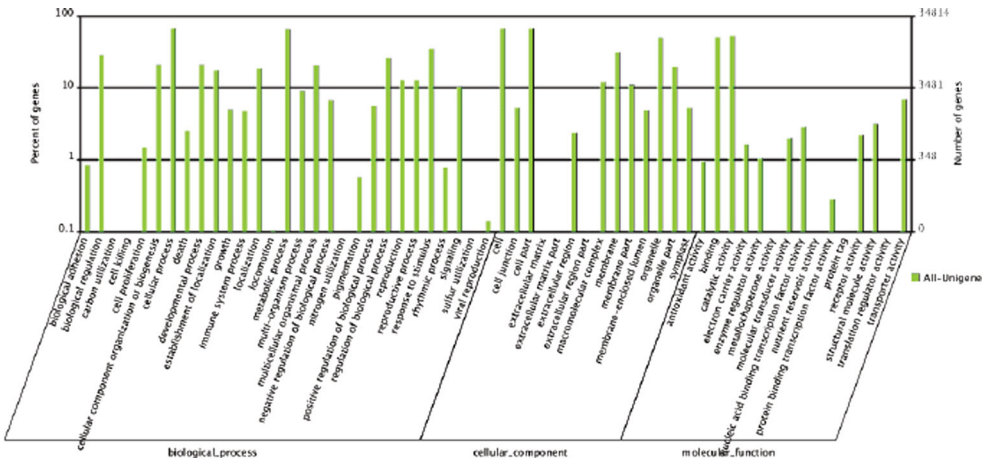

Fig. 1 Histogram presentation of gene ontology classification. A total of 34,814 unigenes were assigned to at least one GO-term and were grouped into three main categories. The *left-hand Y-axis* indicates the percentage of genes in a category, and the *right-hand* indicates the number of genes

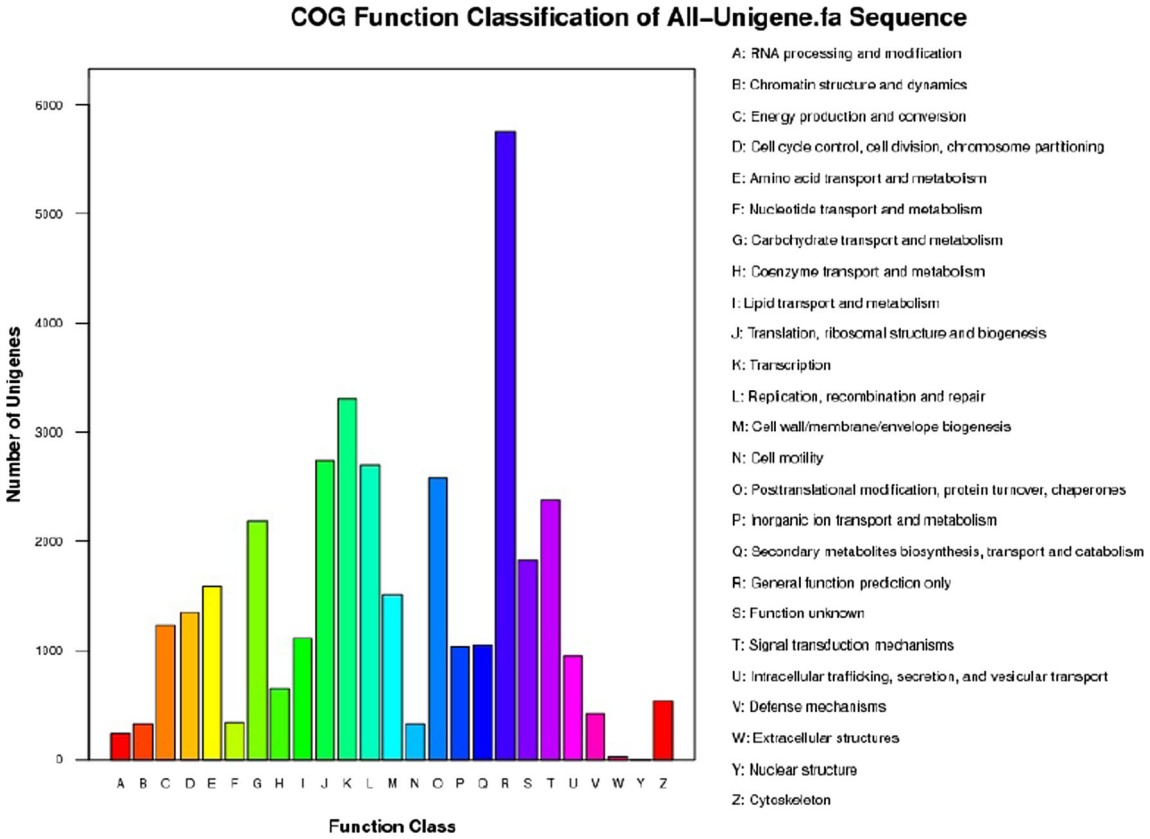

Fig. 2 COG classification. A total of 18,630 unigenes were assigned to 25 classifications. *Capital letters* on the *X-axis* indicate the COG categories as listed on the right of the histogram, and the Y-axis indicates the number of transcripts

confirm that they were described in the literature to confer resistance to pathogens and they are grouped into seven distinct classes based on the presence of specific domains or partial domains, including N-terminal coiled coil-nucleotide-binding site-leucine-rich repeat (CNL), Toll interleukin1 receptor-nucleotide-binding site-leucine-rich repeat (TNL), receptor-like protein (RLP), receptor-like kinase (RLK), three truncated classes (Kinase, NL and TN) and "other" which has no typical resistance related domains (van Ooijen et al. 2007; Sanseverino et al. 2010; Sanseverino et al. 2013; Yang et al. 2014).

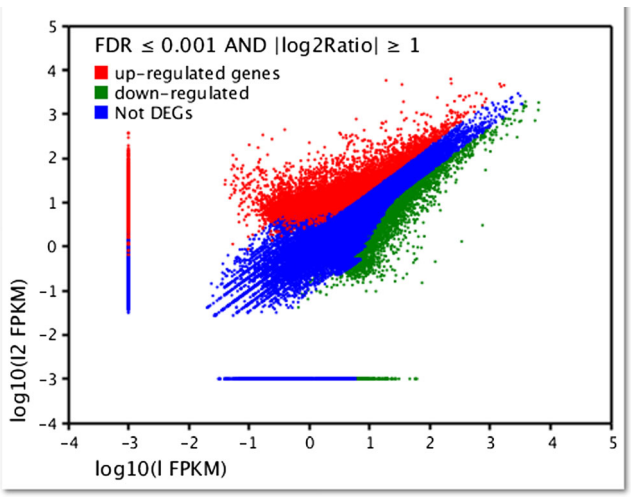

Fig. 3 Gene expression levels of VW-inoculated and control sample. The differentially expressed genes are shown in *red* and *green*. Genes without expression changes are shown in *blue*

Amino acid sequences of the 112 reference resistance genes were downloaded from the PRGdb and used to identify and classify putative R-genes in *S. aculeatissimum*. A total of 198 differential expressed resistance genes were revealed, including 47 genes of CNL class, 16 genes of TNL class, 58 genes of RLK class, 30 genes of RLP class, 4 genes of NL class, 9 genes of Kinase class, and 34 genes of "other" class, and no gene was found in TN class. Next, we focused our analysis on KEGG pathways to identify resistance genes in response to *V. dahliae*. The major pathway in which the 198 differential expressed resistance genes involved was plant-pathogen interactions, with 81 resistance genes (40.9 %) involved, including 17 genes of CNL class, 5 genes of TNL class, 19 genes of RLP class, 28 genes of RLK class, 2 genes of NL class, 3 genes of Kinase class, and 7 genes of "other" class (Table 2). In the plant hormone signal transduction pathway, only 10 genes of RLK class, 9 genes of RLP class and 5 genes of "other" class were involved.

Among these 198 differential expressed resistance genes, there were 112 downregulated genes and 86 upregulated genes, including 41 downregulated genes and 6 upregulated

Table 2 The KEGG pathways in which the differential expressed resistance genes involved

Pathway	CNL	TNL	RLK	RLP	TN	NL	Kinase	Other	Total
Metabolic pathways	5	0	0	0	0	4	0	2	11
Purine metabolism	5	0	0	0	0	4	0	0	9
Pyrimidine metabolism	5	0	0	0	0	4	0	0	9
ABC transporters	0	0	4	0	0	0	4	10	18
Spliceosome	0	0	0	0	0	0	0	8	8
Regulation of autophagy	0	0	1	0	0	0	0	0	1
RNA polymerase	5	0	0	0	0	4	0	0	9
Plant-pathogen interaction	17	5	28	19	0	2	3	7	81
Plant hormone signal transduction	0	0	10	9	0	0	0	5	24
Ubiquitin mediated proteolysis	0	0	0	1	0	0	0	0	1

CNL N-terminal coiled coil-nucleotide-binding site-leucine-rich repeat, *TNL* Toll interleukin 1 receptor-nucleotide-binding site-leucine-rich repeat, *RLK* receptor-like kinase, *RLP* receptor-like protein

genes of CNL class, 13 downregulated genes and 3 upregulated genes of TNL class, 23 downregulated genes and 35 upregulated genes of RLK class, 7 downregulated genes and 23 genes of RLP class, 16 downregulated genes 18 upregulated genes of "other" class. All 4 genes of NL class were downregulated, and genes of Kinase class were downregulated except unigene CL2230. Contig2. Notablely, one unigene CL5609. Contig2 of RLP class which showed 50 % identity of tomato *Ve1*(88 % coverage, *e* value = 0), was downregulated in *S. aculeatissimum*.

Identification of Major Genes Involved in *V. dahliae* Stress

Several sets of genes were also identified based on the KEGG pathway analysis, including phenylpropanoid pathway-related genes, genes encoding pathogenesis-related proteins (PRs) and transcription factors (Supplementary file 3).

A variety of regulated genes participating in the phenylpropanoid biosynthetic pathway were identified. Among these genes, most of the genes encoding enzyme transcripts were found to be upregulated, including phenylalanine ammonia-lyase (PAL) family genes, cinnamyl-alcohol dehydrogenase, caffeic acid 3-0-methyltransferase, catalase-peroxidase. Some of the genes were both up- and downregulated, including coniferyl-alcohol glucosyltransferase, 4-coumarate-CoA ligase, ferulate-5-hydroxylase, suggesting these pathway genes might play important roles in *S. aculeatissimum* in response to *V. dahliae*.

Genes encode pathogenesis-related proteins (PRs) were found to be differentially expressed upon the *V. dahliae* infection. PR-1 involved in the plant-pathogen interaction pathway was found to be upregulated. The genes annotated as thaumatine-like protein (PR-5) and bet_v1-related protein (PR-10) were also upregulated. Besides, several genes encoding WRKY transcription factors involved in the plant-pathogen interaction pathway were both up- and down- regulated. In addition, genes involved in the plant hormone signal transduction were also affected by *V. dahliae*. The transcription level of AUX1/LAX family (LAX2, LAX3, LAX4), ethylene receptor, ethylene-responsive transcription factor 1 were increased, while the transcription level of transport inhibitor response 1 (TIR1), jasmonic acid-amido synthetase were decreased.

Validation of Differentially Expressed Genes from RNA-seq

Quantitative real-time PCR was performed on a subset of 18 randomly selected differentially expressed genes to validate the expression data from the Illumina RNA-seq analysis. These genes were involved in plant-pathogen interaction, plant hormone signal transduction, or phenylpropanoid biosynthesis pathways, and also included upregulated and downregulated unigenes (Table 3). The expression patterns for all the 18 genes were in agreement with the RNA-seq data except for unigene43290_All. The Pearson correlation coefficient was 0.82 (correlation is significant at the 0.01 level), which showed a good correlation between RNA-seq expression and qRT-PCR analysis.

Discussion

This study presents the first use of Illumina sequencing technology to investigate the transcriptome difference of a wild species of eggplant *S. aculeatissimum* in response to *V. dahliae* infection. The verticillium wilt of eggplant causes significant economic losses, and the cultivated eggplant gene pool is lack of resistance. Thus, transferring of resistant genes from wild relatives of eggplant will be valuable for the continued improvements of eggplant (Liu et al. 2015). Although verticillium wilt resistance in *S. linnaeanum* has been transferred to cultivated eggplant through subsequent backcrossing (Acciarri et al. 2001; Sunseri et al. 2003), it is also important

Table 3 Quantitative real-time PCR analysis of selected differentially expressed genes

Unigene	Annotation (BLASTX)	qRT-PCR fold	Illumina/ Solexa fold
Unigene37540_All	PREDICTED: probable receptor-like protein kinase [*Solanum tuberosum*]	2.76 ± 0.08	3.33
Unigene34938_All	PREDICTED: PTI1-like tyrosine-protein kinase 3-like [*Solanum tuberosum*]	2.38 ± 0.26	3.00
Unigene43104_All	PREDICTED: beta-glucosidase 13-like [*Solanum tuberosum*]	2.01 ± 0.07	4.33
Unigene9328_All	PREDICTED: cationic peroxidase 1-like [*Solanum tuberosum*]	29.45 ± 5.60	4.83
Unigene20339_All	PREDICTED: lignin-forming anionic peroxidase -like [*Solanum tuberosum*]	3.22 ± 0.17	4.72
Unigene16512_All	PREDICTED: probable inactive receptor kinase [*Solanum tuberosum*]	1.9 ± 0.38	3.63
Unigene43321_All	glucan endo-1,3-beta-glucosidase-like protein 3-like [*Solanum tuberosum*]	2.89 ± 0.04	3.21
Unigene21521_All	PREDICTED: probable WRKY transcription factor 71 [*Solanum lycopersicum*]	2.13 ± 0.04	1.51
Unigene29445_All	PREDICTED: phenylalanine ammonia-lyase [*Solanum lycopersicum*]	2.09 ± 0.11	2.75
Unigene757_All	PREDICTED: G-type lectin S-receptor-like serine/ threonine-protein kinase [*Solanum pennellii*]	−4.89 ± 0.55	−1.97
Unigene22870_All	PREDICTED: flavonoid 3′-monooxygenase-like [*Solanum tuberosum*]	−10.9 ± 1.33	−1.15
Unigene19886_All	PREDICTED: 7-deoxyloganetic acid glucosyltransferase-like [*Solanum pennellii*]	−17.43 ± 2.69	−1.05
Unigene7232_All	PREDICTED: serine carboxypeptidase-like 11 [*Solanum lycopersicum*]	−27.73 ± 5.39	−1.87
Unigene11753_All	PREDICTED: probable WRKY transcription factor 40 [*Solanum tuberosum*]	−27.03 ± 5.05	−1.62
Unigene20920_All	double WRKY type transfactor [*Solanum tuberosum*]	−15.31 ± 2.01	−1.70
Unigene2470_All	ABA responsive element-binding protein [*Solanum torvum*]	−43.92 ± 3.03	−2.95
Unigene11975_All	PREDICTED: *Solanum tuberosum* probable disease resistance protein	−7.4 ± 0.14	−2.07
Unigene43290_All	PREDICTED: probable LRR receptor-like serine/ threonine-protein kinase [*Solanum pennellii*]	−2.63 ± 0.12	3.15

to explore new resistant sources for verticillium wilt. *S. aculeatissimum* has proven to be a valuable resource because of its desirable traits for resistance to verticillium wilt, and root-knot nematode. Moreover, the interspecific hybridization has been successfully made between *S. aculeatissimum* and cultivated eggplant, and the cloning of verticillium wilt and root-knot nematode resistant genes are undergoing. Therefore, we chose this specific wild relative of eggplant for transcriptome analysis.

A comprehensive transcriptome analysis could obtain a deeper understanding of the molecular mechanisms of *S. aculeatissimum* responses to VW resistance. Despite that the genome sequence of eggplant has been published, considering the big differences between cultivated eggplant and wild species, the genome sequence of cultivated eggplant was not used in this bioinformation analysis. In the present study, a total of 57,258,828 raw reads were obtained, corresponding to about 4.5 Gb data and were further assembled into unigenes. About 22.5 % of the predicted all-unigenes could not be assigned annotation in any of the NR, NT, Swiss-Prot, KEGG, COG, and GO databases, which may be due to the limited information about the genomes or transcriptomes of *S. aculeatissimum*. Among the genes with KEGG pathway annotations, a total of 1535 all-unigenes were involved in the group "plant-pathogen interaction," which represented the third largest group in our results, indicating the efficiency and feasibility of Illumina sequencing to identify genes related to VW resistance in *S. aculeatissimum*. The quality of the different expressed genes was also validated by qRT-PCR analysis, and the results showed a good correlation between the two analysis techniques.

The fundamental strategy for controlling *Verticillium* disease in eggplant is the isolation of resistance genes from their resistant relatives to be used in conventional breeding, genetic engineering and biotechnological approaches (Collonnier et al. 2001; Yang et al. 2014; Liu et al. 2015). The *Ve1* of tomato is the first Verticillium wilt resistant gene to be reported (Kawchuk et al. 2001). Some *Ve* homologous genes such as

StVe1 from *Solanum torvum* (Fei et al. 2004), *GbVe1* and *Gbvdr5* from cotton (Zhang et al. 2012; Yang et al. 2015) have been verified. All these genes encode leucine-rich-repeat receptor-like proteins (eLRR-RLPs). The RLP genes frequently occur in clusters of related genes previously observed for the distribution of NBS-LRR and RLK-type resistance genes (Kruijt, Brandwagt, and de Wit 2004; Yang et al. 2015). In our study, different classes of putative resistance genes involved in the plant-pathogen interaction pathway were identified. Unigene CL5609. Contig2_ALL of RLP class was found to have high identity with *Ve* gene of tomato. Further investigation of these genes may provide valuable candidates for genetic improvement of eggplant.

Progress has been made in investigations of defense mechanisms by cotton against *V. dahliae* infection. The phenylpropanoid pathway has been reported to play a critical role during the plant defense response to *V. dahliae* (Smit and Dubery 1997; Pomar et al. 2004; Xu et al. 2011; Sun et al. 2013; Zhang et al. 2013). In particular, the lignin pathway was also reported to play an important role in plant responses to *V. dahliae*. Inoculation of tomato with *V. dahliae* induced an increase in the total amount of lignin in roots of both a susceptible line and a resistant line (Gayoso et al. 2010). Inoculation of pepper with *V. dahliae* increased the total stem lignin content (Pomar et al. 2004). A similar result was also observed in the cotton defense response to *V. dahliae* (Xu et al. 2011). In this study, we also detected some DEGs involved in the phenylpropanoid pathway. The enzymes that function in the core phenylpropanoid pathway, including PAL, catalase-peroxidase, caffeicacid 3-O-methyltransferase, coniferyl-aldehyde dehydrogenase, caffeoyl-CoA O-methyltransferase, and cinnamyl-alcohol dehydrogenase exhibited increased expression in the *S. aculeatissimum* defense response to *V. dahliae*. Most of the identified genes involved in the specific branch pathways for the formation of lignins were upregulated after pathogen inoculation. Thus, the overall results indicated the activation of the phenylpropanoid pathway in the *S. aculeatissimum* defense response to *V. dahliae*.

Phytohormones such as ABA, JA, ET, SA, auxin, GA, and cytokinin are important regulators in the complex signaling cascades and involved in the defense responses (Bari and Jones 2009; Xu et al. 2011). SA induces systemically acquired resistance and is implicated in plant tolerance to biotrophic pathogens (Spoel and Dong 2008; Leon-Reyes et al. 2009; Zhang et al. 2013). Based on our studies, an important branch in the plant hormone signal transduction pathway: "SA → NPR1 → TGA → PR-1 → Disease resistance" was found. This finding was also reported in the transcriptome profiling of cotton defense response to *V. dahliae*. The presence of this branch might suggest its role in the resistance response (Zhang et al. 2013). Another important branch in the plant hormone signal transduction pathway from our study was "AUX1 → TIR1 → AUX/IAA → ARF → Cell enlargement/Plant growth." The AUX1, TIR1, and AUX/IAA were differently expressed in *S. aculeatissimum* in response to *V. dahliae* infection, indicating auxin pathway may play important roles in resistance to *V. dahliae*. Yang et al. (2013) also reported the changes in miR393 expression in cultivated eggplant point to a general transcriptional induction of the auxin response to *V. dahliae* infection. SA was also reported to inhibit pathogen growth in plants through repression of auxin signaling pathway (Wang et al. 2007). However, the exact role of these hormones and the cross-talk between them during the defense response to *V. dahliae* of *S. aculeatissimum* still remains to be discovered.

Several genes encode pathogenesis-related proteins such as pathogenesis-related protein 1 (PR1), thaumatine-like proteins (PR-5), and bet_v1-related protein (PR-10) were differentially expressed upon the inoculation of *V. dahliae*. To date, seventeen families of PR proteins have been identified. PR-1 proteins are often used as markers of the enhanced defensive state conferred by pathogen-induced systemic acquired resistance (SAR) (Van Loon and Van Strien 1999; Van Loon, Rep, and Pieterse 2006). The PR-5 family belongs to the thaumatin-like proteins with homology to permatins that permeabilize fungal membranes (Vigers, Roberts, and Selitrennikof 1991). Some members of this family have been shown to possess antifungal activity (Van Loon and Van Strien 1999). The PR-10 family is structurally related to ribonucleases (Moiseyev et al. 1997) and has a broad spectrum of roles significantly in biotic and abiotic stresses (Agarwal and Agarwal 2014). The induction of PR-10 expression has been reported in hot pepper in response to tobacco mosaic virus (Park et al. 2004). KEGG analysis revealed that several WRKY transcription factors were involved in the plant-pathogen interaction pathway, and the expression patterns of unigene 21521_ALL, unigene11753_All, and unigene20920_ALL, which were annotated as WRKY transcription factors have been confirmed by qRT-PCR, indicating WRKY transcription factors were involved in *S. aculeatissimum* in response against *V. dahliae* infection. The family of WRKY transcription factors as principal regulators of gene expression are reported to play important functions in plant innate immunity (Zhang and Wang 2005). The WRKY transcription factors obtained in this study are presented for the first time to be associated with *S. aculeatissimum* against *V. dahliae* infection. Further detailed analysis on their functions will deepen our understanding of their regulatory roles in *S. aculeatissimum* in response to *V. dahliae*.

In conclusion, the first use of Illumina sequencing technology to investigate the transcriptome difference of a wild species of eggplant *S. aculeatissimum* in response to *V. dahliae* infection was performed in the present study. The genetic resource and putative signaling pathways related to *S. aculeatissimum* defense response against *V. dahliae* provide valuable information for molecular studies of *S. aculeatissimum*.

The findings of this study will hopefully promote research on resistance in *S. aculeatissimum* to *V. dahliae* and the genetic improvement of eggplant.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Standard This article does not contain any studies with human participants or animals performed by any of the authors.

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