Comparative transcriptome profiling conferring of resistance to *Fusarium oxysporum* infection between resistant and susceptible tomato

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ABASTRCAT

Tomato Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a destructive disease of tomato worldwide which causes severe yield loss of the crops. As exploring gene expression and function approaches constitute an initial point for investigating pathogen– host interaction, we performed a transcriptional analysis to unravel regulated genes in tomato infected by FOL. Differentially expressed genes (DEG) upon inoculation with FOL were presented at twenty-four hours post-inoculation including four treatments: Moneymaker_H_2O, Moneymaker_FOL, Motelle_H_2O and Motelle_FOL. A total of more than 182.6 million high quality clean reads from the four libraries were obtained. A large overlap was found in DEGs between susceptible tomato cultivar Moneymaker and resistant tomato cultivar Motelle. All Gene Ontology terms were mainly classified into catalytic activity, metabolic process and binding. However, Gene Ontology enrichment analysis evidenced specific categories in infected Motelle. Statistics of pathway enrichment of DEGs resulted that the taurine and hypotaurine metabolism, the stibenoid, diarylheptanoid and gingerol biosynthesis, the starch and sucrose metabolism were the top three pathway affected in both groups. Interestingly, plant-pathogen pathway was greatly regulated in Motelle treated with FOL. Combining with qRT-PCR facilitated the identification of regulated pathogenicity associated genes upon infected resistant or susceptible tomato. Our data showed that a coordinated machinery played a critical role in prompting the response, which could help in generating models of mediated resistance responses with assessment of genomic gene expression patterns.

INTRODUCTION

Fusarium oxysporum f. sp. *lycopersici* (hereafter referred to as FOL) is a biotrophic pathogen which is the causal agent of tomato wilt. Accumulating data indicate that

F. oxysporum is a large species complex, with more than 150 host-specific forms causing disease in vegetables, fruit trees, wheat, corn, cotton and ornamental crops (Di Pietro *et al.* 2003; Leslie and Summerell 2006). *F. oxysporum* infects vascular bundles in the plant host, leading to clogged vessels, yellowing of leaves, wilting and finally death of the whole plant. According to their specific pathogenicity to tomato cultivars, three physiological races (Di Pietro *et al.* 2003; Leslie and Summerell 2006; Takken and Rep 2010) of *F. oxysporum* are distinguished (Kawabe *et al.* 2005).

Tomato (*Solanum lycopersicum*) is a worldwide economic crop, and also has been studied as a crucial model plant for studying the genetics and molecular basis of resistance mechanisms. Four plant resistance (*R*) genes have been discovered in cultivated tomato from wild tomato species including the *I* and *I-2* genes from *S. pimpinellifolium*, and the *I-3* and *I-7* gene from *S. pennellii*. Among these four *R* genes, so far, *I-2*, *I-3* and *I-7* have been cloned, encode an NB-LRR protein like most known *R* genes (Ori *et al.* 1997; Simons *et al.* 1998; Kawabe *et al.* 2005; Catanzariti *et al.* 2015; Gonzalez-Cendales *et al.* 2016). Previous works have demonstrated that the *I-2* and *I-3* gene confers resistance to race 2 and race 3 strains of FOL, respectively (Simons *et al.* 1998; Catanzariti *et al.* 2015). The *I-2* locus encodes an *R* protein that recognizes the *avr2* gene product from *F. oxysporum* (race 2) (Houterman *et al.* 2009). The *I-3* encodes an S-receptor-like kinase (SRLK) genes that confers *Avr3*-dependent resistance to FOL (race 3) (Catanzariti *et al.* 2015). Previously, two near-isogenic tomato cultivars susceptible Moneymaker (*i-2*/*i-2*) and resistant Motelle (*I-2*/*I-2*) were recruited to study the interaction between tomato and FOL (Ouyang *et al.* 2014). The genotypes of these two tomato cultivars are for *I-2* and respond to FOL infection (Di Pietro and Roncero 1998; De Ilarduya *et al.* 2001; Yu and Zou 2008). We unrevealed the microRNA diversifications responding to FOL infection in tomato by high-throughput RNA sequencing (RNA-seq) approach (Ouyang *et al.* 2014). Basically, transcriptome analysis is a very important tool to discover the molecular basis of plant-pathogen interaction globally, allowing dissection of the pattern of pathogen activities and molecular repertoires available for defense responses in host plant. By taking advantage of RNA-seq technology, a few of transcriptome profiling studies of plants following inoculation with *Fusarium* fungus have been reported, including studies in banana (Guo *et al.* 2014), cabbage (Xing *et al.* 2016), watermelon (Liu *et al.* 2015), mango (Liu *et al.* 2016), and *Arabidopsis* (Chen et al. 2014; Gupta *et al.* 2014). Upon to pathogens infection, plants activate a few of defense responses to resistant diseases caused by according pathogens. Resistance response may associated with hypersensitive reaction (HR), structural alterations, reactive oxygen species (ROS) accumulation, synthesis of secondary metabolites and defense molecules (Park *et al.* 2003; Shah 2003; Ros *et al.* 2004).

The objects of this study were to determine the transcript profile between susceptible Moneymaker and resistant Motelle tomato plants in response to FOL infection and to reveal genes underlying the innate immune response against the fungal pathogen. To achieve these goals, we performed transcriptome analysis using RNA-seq approach. In addition to genes known to response to pathogen infection, our results also uncovered a bunch of novel fungal pathogen-responsive genes for further functional characterization, and provided a broader view of the dynamics of tomato defense transcriptome triggered by FOL infection.

MATERIALS and METHODS

Tomato materials and fungal culture

Two tomato near-isogenic cultivars (cv.) Motelle (*I-2*/*I-2*) and Moneymaker (*i-2*/*i-2*) that exhibit different susceptibilities to the root pathogen FOL were used for plant infection and libraries construction. Profiling experiments were performed on two-week-old tomato seedlings grown at 25°C with a 16/8-h light/dark cycle. The wild-type *Fusarium oxysporum* f. sp *lycopersici* strain used for all experiments is FGSC 9935 (also referred to as FOL 4287 or NRRL 34936). Two-week-old tomato seedlings were removed from soil and roots incubated in a solution of FOL conidia at a concentration of $1x10^8$/ml for 30 min. Control tomato plants were treated with water. Plants were then replanted in soil and maintained in a growth chamber at 25°C for 24 h with constant light. Plants were removed from soil, and roots were rinsed and excised, then immediately frozen in liquid nitrogen and stored at -80°C.

RNA extraction, library preparation, and sequencing

Total RNA was isolated from roots using TRIzol® Reagent (#15596026, Life Technologies, CA, USA) according to the manufacturer's recommendations. After the total RNA extraction and DNase I treatment, magnetic beads with Oligo (dT) were used to isolate mRNA. Mixed with the fragmentation buffer, the mRNA was sheared into short fragments. Then cDNA was synthesized using the mRNA fragments as templates. cDNAs were purified and resolved with EB buffer for end reparation and single nucleotide A (adenine) addition followed by adding adapters to cDNAs. After agarose gel electrophoresis, the suitable cDNAs were selected for the PCR amplification as templates. During the quality control (QC) steps, Agilent 2100 Bioanaylzer and ABI StepOnePlus Real-Time PCR System were used in quantification and qualification of the sample library. The libraries were sequenced using Illumina HiSeqTM 2000.

RNA-seq analysis, normalization of sequence reads and identification of differentially expressed genes (DEGs)

Primary sequencing data that produced by Illumina HiSeqTM 2000, called as raw reads, were subjected to QC. After QC, raw reads were filtered into clean reads which were aligned to the reference sequences as described by previous report. (Trapnell *et al.* 2012). All sequence reads were trimmed to remove the low-quality sequences. The trimmed reads were then aligned to the tomato reference genome downloaded from the Sol Genomics Network using Bowtie v0.12.5 (Langmead *et al.* 2009) and TopHat v2.0.0 (Trapnell *et al.* 2009; Trapnell *et al.* 2012) with default settings. Cufflinksv0.9.3 (Trapnell *et al.* 2010) was used to calculate transcript abundance based on fragments per kilo base of transcript permillion fragments mapped (FPKM) using all parameters on default settings. The transcript was considered as expressed when the FPKM value was greater than 0.1 and the lower boundary for FPKM value was greater than zero at 95% confidence interval. Once the transcript abundance was calculated for individual sample files using Cufflinks, the output files were further merged pairwise for each comparison (in vitro comparison between two populations, in planta comparison between two populations and in planta versus *in vitro* for each population) using Cufflinks utility program-Cuffmerge (Trapnell *et al.* 2012). The pairwise comparisons of gene expression profiles between the two populations were done using the Cuffdiff program of the Cufflinks version 1.3.0 (Trapnell *et al.* 2010). The genes were considered significantly differentially expressed if Log_2 FPKM (fold change) was ≥1.0 and false discovery rate (FDR, the adjusted P value) was <0.01. The q-value which was a positive FDR analogue of the p-valuewas set to <0.01 (Storey and Tibshirani 2003).

Functional categorization of DEGs.

DEGs were functionally categorized online for all pairwise comparisons according to the Munich Information Center for Protein Sequences (MIPS) functional catalogue (Ruepp *et al.* 2004). The functional categories and subcategories were regarded as enriched in the genome if an enrichment P- and FDR- value was below <0.05. The Kyoto Encyclopediaof Genes and Genomes (KEGG) pathway analyses were performed using interface on blast2GO (Blast2GO v2.6.0, http://www.blast2go.com/b2ghome) for all DEGs to identify gene enrichment on a specific pathway.

Gene Ontology (GO) and pathway enrichment analysis

Gene Ontology (GO) and pathway enrichment were performed using DAVID software (Smyth 2005). Graphs of the top 20 enriched GO terms for each library were generated using the Cytoscape Enrichment Map plugin (Smoot *et al.* 2011; Merico *et al.* 2010).

Quantitative real time-PCR (qRT-PCR) analysis.

qRT-PCR analysis was performed according to our previous protocol (Ouyang *et al.* 2014). The reverse transcription reaction was done on 1 μg of total RNA using the SMART MMLV Reverse Transcriptase (Takara, Mountain View, CA). cDNA was diluted two times and used as template for quantitative RT-PCR, which was performed with the CFX96 real-time PCR system (Bio-Rad, Hercules, California, USA). Primers used for qRT-PCR were designed from 3-UTR for individual gene. For each cDNA sample, three replications were performed. Each reaction mixture (20 μL) contained 1 μL of cDNA template, 10 μL of SYBR1 Green PCR Master Mix (Applied Biosystems, Foster, CA) and 1 μL of each primer (10 μM). Relative expression levels of genes were normalized using the 18S rRNA as internal control, and were calculated as the fold change by comparison between in water treated and in FOL treated samples.

Statistical analyses

All data in this study were subjected to ANOVA analysis or Student's t-test analysis using SPSS 11.5 (SPSS Company, Chicago, IL).

RESULTS

General features of Moneymaker and Motelle transcriptomes

We investigated transcriptomes in roots of tomato during infection with the tomato wilt disease fungus FOL through construction of transcript libraries and RNA-seq. By taking advantage of two near-isogenic cultivars that show differential interaction with FOL – Moneymaker (susceptible) and Motelle (resistant), we generated four libraries including: Moneymaker treated with water (MM_H_2O), Moneymaker treated with FOL (MM_Foxy), Motelle treated with water (Mot_H_2O) and Motelle treated with FOL (Mot_Foxy). Using Illumina sequencing, we obtained a total of more than 182.6 million high quality clean reads from the four libraries. Of these, 45,616,330 from MM_H_2O, 45,635,428 from MM_Foxy, 45,680,034 from Mot_H_2O, and 45,661,734 from Mot_Foxy. The number of expressed transcripts were 22,796 and 22,639 from MM_H_2O and MM_Foxy library respectively, and 22,825 and 22,725 from Mot _H_2O and Mot _Foxy library respectively (Table 1). Sequence reads presented reasonable correlation between related two populations (t >0.95, p = 0.29) (Figure 1). A number of 21,808 and 21,753 genes were co-expressed between MM_H_2O and MM_Foxy library and Mot _H_2O and Mot _Foxy library, respectively. The co-expressed genes increased slightly to 21,887 between MM_Foxy and Mot _Foxy library (Figure 2). The scatter of all expressed genes of each pair were presented in figure 3.

Of the sequence reads from MM_H_2O and MM_Foxy library, 75.49% and 67.89% were mapped to the reference genome of tomato, respectively. For Mot _H2O and Mot _Foxy library, 75.87% and 70.46% were aligned to the reference genome of

Table 1 Summary of sequence reads (in millions) from four libraries.

Sample name	Clean reads	Genome map rate	Gene map rate	Expressed gene	Novel gene	Alternative splicing	SNP	Indel
MM_H_2O	45,616,330	75.49%	76.87%	22,796	775	32,482	14,046	1,232
MM_Foxy	45,635,428	67.89%	68.47%	22,639	761	32,689	13,371	1,228
Mot_H_2O	45,680,034	75.87%	75.92%	22,825	795	33,706	17,286	1,506
Mot_Foxy	45,661,734	70.46%	71.26%	22,725	705	32,965	16,409	1,360

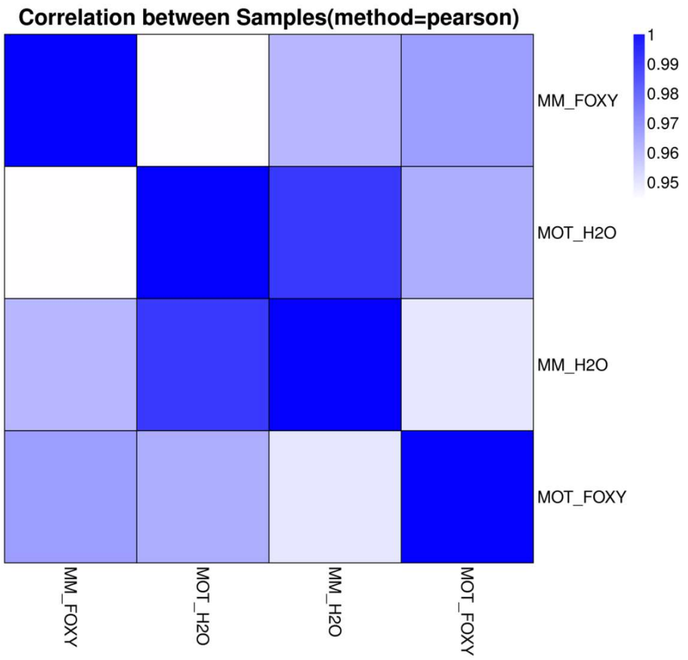

Figure 1 Correlations value between each two libraries.

tomato, respectively. Among the reads mapped to the tomato genome, perfect match reads were 63.00% and 55.52% for MM_H_2O and MM_Foxy library respectively, and 62.61% and 57.41% for Mot _H_2O and Mot _Foxy library respectively (Table 2).

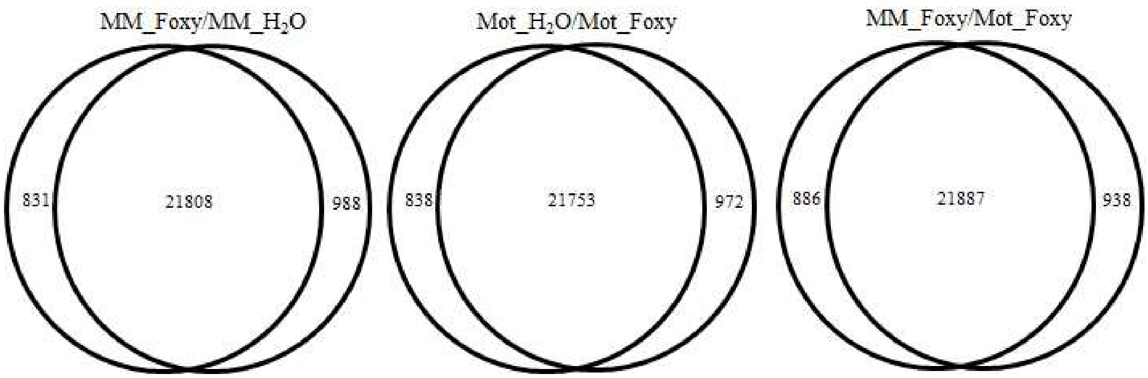

Figure 2 Venn Chart of Co-expressed Genes between MM_H_2O and MM_Foxy, Mot _H_2O and Mot _Foxy, and MM_Foxy and MM_Foxy.

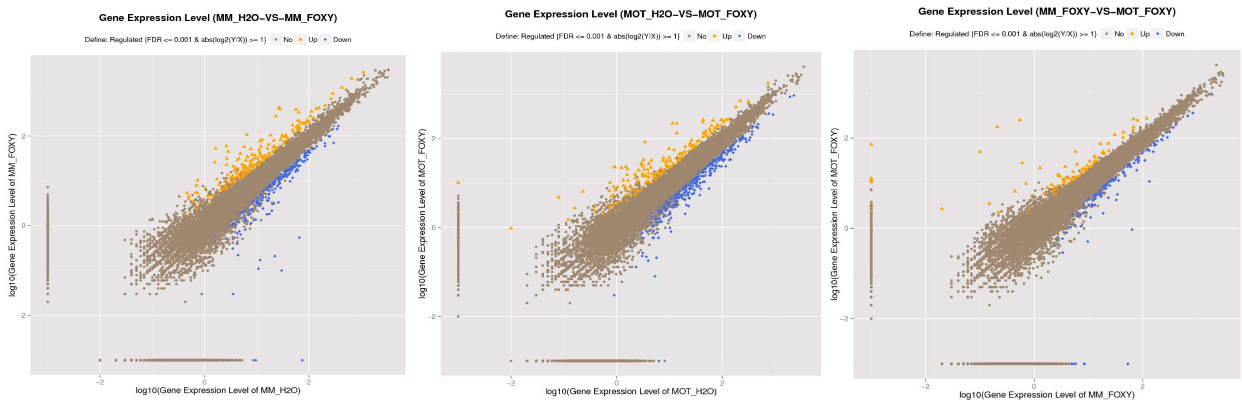

Figure 3 Scatter chart of all expressed genes of each pair between MM_H_2O and MM_Foxy, Mot _H_2O and Mot _Foxy, and MM_Foxy and MM_Foxy.

Analysis of differentially expressed genes (DEGs) and functional classification of DEGs by gene ontology (GO) enrichment analysis

After expression levels Fragments Per Kilobase of exon model per Million mapped reads (FPKM) for each gene were calculated. Differentially expressed genes (DEGs) were defined as genes with fold-change > 2 fold and P_{adjust} value < 0.05. A total number of 3,942 and 4,168 genes showed significantly differential expression in MM_H_2O vs. MM_Foxy library and Mot _H_2O vs. Mot _Foxy library, respectively.

Table 2 Mapping statistics of four libraries.

		MM_H_2O	MM_Foxy	Mot_H_2O	Mot_Foxy
Total mapped reads	Map to genome	75.49%	67.89%	75.87%	70.46%
	Map to gene	76.87%	68.47%	75.92%	71.26%
Perfect match	Map to genome	63.00%	55.52%	62.61%	57.41%
	Map to gene	66.21%	57.68%	64.46%	59.64%
Mismatch	Map to genome	12.49%	12.38%	13.26%	13.05%
	Map to gene	10.66%	10.79%	11.46%	11.63%
Unique match	Map to genome	74.32%	66.85%	74.79%	69.46%
	Map to gene	69.47%	61.75	68.83%	64.43%
Multi-position match	Map to genome	1.17%	1.04%	1.07%	0.99%
	Map to gene	7.40%	6.72%	7.09%	6.83%
Total Unmapped reads	Map to genome	24.51%	32.11%	24.13%	29.54%
	Map to gene	23.13%	31.53%	24.08%	28.74%

Among these DEGs, 221/219 genes were down-regulated, and 261/415 genes were up-regulated (MM_H_2O vs. MM_Foxy/ Mot _H_2O vs. Mot _Foxy) (Figure 4). A majority of these DEGs were overlapped in both water and FOL treated two tomato cultivars.

To explore the distribution of DEGs, gene ontology (GO) enrichment analyses were conducted based on these DEGs. A total of 530 and 769 GO terms were discovered in MM_H_2O vs. MM_Foxy and Mot _H_2O vs. Mot _Foxy library, respectively. For both libraries, all GO terms were assigned to three groups including the biological process, the cellular component and the molecular. All GO terms were mainly classified into catalytic activity (104 out of 530 in MM_H_2O vs. MM_Foxy library, and 141 out of 769 in Mot _H_2O vs. Mot _Foxy library, (the same define in

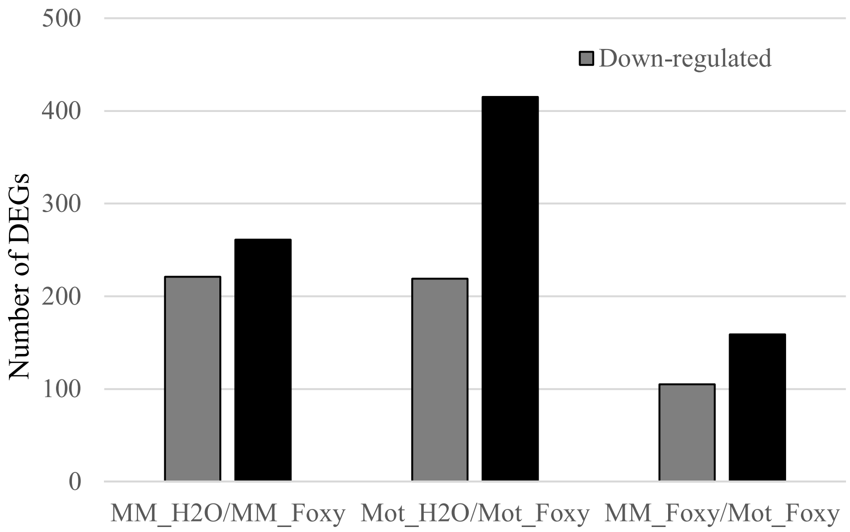

Figure 4 Statistics of Differentially Expressed Genes (DEGs).

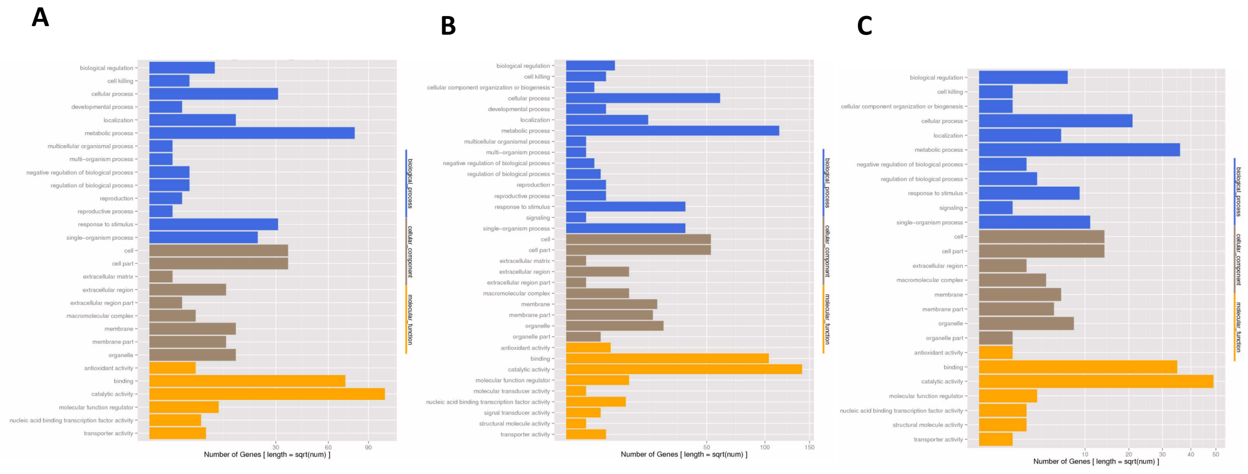

Figure 5 Gene Ontology Analysis of DEGs. The results were basically summarized into three main categories: biological processes, cellular components, and molecular functions. All statistically significant genes from four libraries were assigned to GO terms. A MM_H2O *vs* MM_Foxy. B Mot_H2O *vs* Mot_Foxy. C MM_Foxy *vs* Mot_Foxy.

the following text), metabolic process (81 out of 530, and 118 out of 769), and binding (72 out of 530, and 104 out of 769). For the class of response to stimulus, however, no significant change was presented between these two libraries (31 out of 530, and 36 out of 769) (Figure 5).

To further understand the biological functions, top 20 statistics of pathway enrichment of DEGs were performed to discover the affection of FOL to host plant. Total of 356 and 469 DEGs from MM_H$_2$O *vs*. MM_Foxy library and Mot _H$_2$O *vs*. Mot _Foxy library respective were annotated for pathway enrichment. The taurine and hypotaurine metabolism, the stibenoid, diarylheptanoid and gingerol biosynthesis, the starch and sucrose metabolism were the top three pathway affected in both groups, but in different ranking. The metabolic pathway was the most abundant DEGs in both groups with 122 out of 356 and 148 out of 469 DEGs in MM_H$_2$O vs. MM_Foxy library and Mot _H$_2$O vs. Mot _Foxy library, respectively (Figure 6). Be worth mentioning, plant-pathogen pathway was ranked in the 24th (24 out of 356 DEGs) in MM_H$_2$O vs. MM_Foxy library, however, it was presented in the 8th (40 out of 469 DEGs) in Mot _H$_2$O vs. Mot _Foxy library (Figure 6). When compared with Mot _H$_2$O vs. Mot_Foxy library, 19 DEGs were presented in MM_H$_2$O vs. MM_Foxy library.

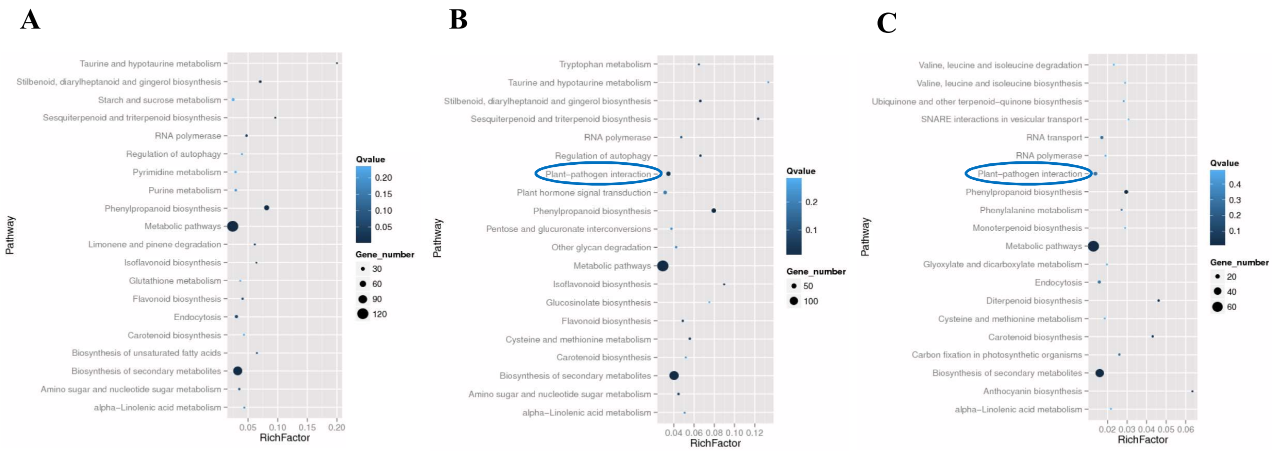

Figure 6 Top 20 of pathway enrichment analysis of DEGs. A MM_H2O *vs* MM_Foxy. B Mot_H2O *vs* Mot_Foxy. C MM_Foxy *vs* Mot_Foxy. Blue circle in B and C highlighted the plant-pathogen interaction.

Expression profiles of DEGs selected in plant-pathogen interaction by qRT-PCR

To verify the DEGs in plant-pathogen interaction pathway, ten disease related DEGs were selected to characterize the gene expression profiles between water and FOL treated Moneymaker and Motelle by qRT-PCR using primers listed in table 3. These DEGs were Solyc00g174330 (Pathogenesis related protein PR-1), Solyc09g007010 (Pathogenesis related protein PR-1), Solyc02g084890 (Cc-nbs-lrr, resistance protein), Solyc07g054120 (LRR receptor-like serine/threonine-protein kinase, RLP), Solyc10g011910 (WRKY transcription factor 23), Solyc03g124110 (Pathogenesis-related transcriptional factor and ERF, DNA-binding), Solyc03g026280 (Pathogenesis-related transcriptional factor and ERF, DNA-binding), Solyc12g009240 (Pathogenesis-related transcriptional factor and ERF, DNA-binding) and Solyc02g080070 (RLK, Receptor like protein, putative resistance protein with an antifungal domain).

The results of qRT-PCR showed the similar pattern with sequencing results with minute difference. Among these DEGs, Solyc00g174330, Solyc10g011910, Solyc03g124110, Solyc02g084890 and Solyc12g009240 were induced greatly in Motelle affected by FOL, however, no significant changes were present in Moneymaker between water and FOL treatment (Figure 7).

DISCUSSION

In this study, we explored the availability of near-isogenic susceptible and resistant cultivars of tomato infected by FOL to uncover a global transcriptomic profile of tomato-FOL interaction using Illumina sequencing. The components of plant responding to pathogen challenging may lead to understand the underlying defense mechanisms. Plants have evolved a complicate defense system against pathogens including cascade signaling activation, the regulation of gene expression, synthesis of defensive metabolites as well as hormone balancing (Mukhtar *et al.* 2011; Andolfo *et al.* 2014). So far, by taking advantage of high-throughput RNAsequencing (RNA-seq) approach, a few of transcriptome studies discovering the *F. oxysporum*-host interaction have been reported in plants such as banana, watermelon, mango and Arabidopsis (Chen *et al.* 2014; Guo *et al.* 2014; Gupta *et al.* 2014; Liu *et al.* 2015; Liu *et al.* 2016; Xing *et al.* 2016), shedding light on the cross-talking among different signaling pathways involving in plant-pathogen interaction.

Table 3 Annotation of pathogenesis related genes and primers used for qRT-PCR in this study

Gene ID	Sequence (5’ – 3’)	Gene annotation
Solyc00g174330	F: AAGTGGATCGGATCGATCTC R: GAACCTAAGCACGATACCATG	Pathogenesis related protein PR-1
Solyc09g007010	F: ATTTCACGTAAGGACGGTTC R: GGACTCAAGATCTCTGATCAAG	Pathogenesis related protein PR-1
Solyc02g084890	F: AAGAGCACTATGGACAACGC R: GCTGTGACTTTTCATGCCCA	Cc-nbs-lrr, resistance protein
Solyc07g054120	F: CCGATTAGGAGAAAGGTCTG R: CAGAGAAGATTAGCATGGCC	LRR receptor-like serine/threonine-protein kinase, RLP
Solyc10g011910	F: ACTGATAAAGGACACGTGGC R: TCTTCCAATCTCTAACGTAC	WRKY transcription factor 23
Solyc03g124110	F: TTTTACCCCGTACCCAACTC R: GGCGGTGATTTGAGTGTTAC	Pathogenesis-related transcriptional factor and ERF, DNA-binding
Solyc03g026280	F: TTATGGGGATTCAATGG R: TGGTGGCACTACTATCTACC	Pathogenesis-related transcriptional factor and ERF, DNA-binding
Solyc12g009240	F: ACACACGGTTTACGCTACTC R: GACGATGCAAAATATTGTTGC	Pathogenesis-related transcriptional factor and ERF, DNA-binding
Solyc02g080070	F: ACGTGTATTCTAGCTAGCAG R: ATGGAATGGAAGGAGGTTCC	RLK, Receptor like protein, putative resistance protein with an antifungal domain

When plant is attacked by pathogen, the host reprograms metabolism balance between development and the resources to support defense to pathogen, involving biological process, cellular components and molecular functions (Mithöfer and Boland 2012). Upon to our results, the tomato–FOL interaction basically followed

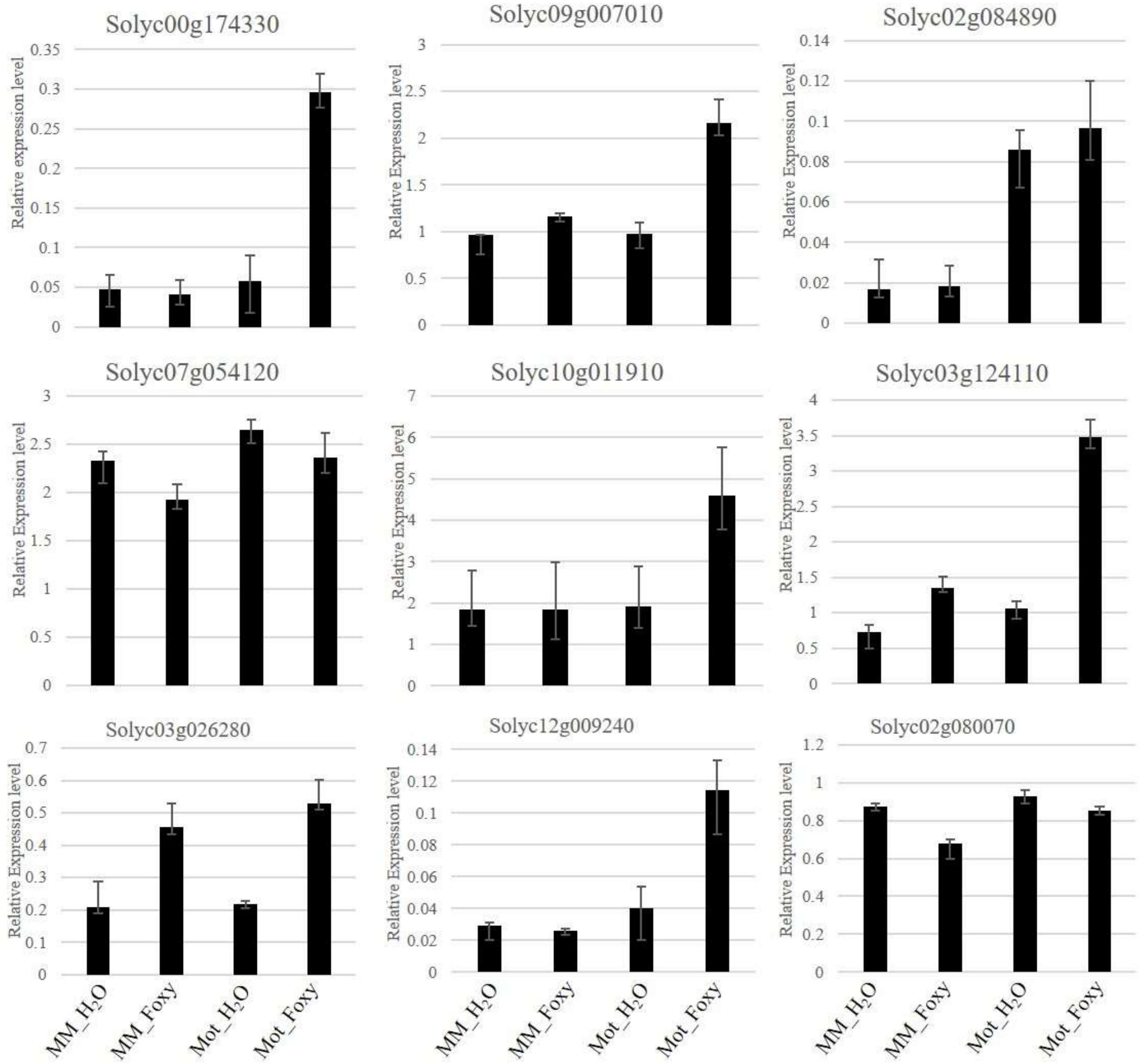

Figure 7 Validation of DEGs selected in plant-pathogen interaction pathway by qRT-PCR. Total tomato root RNA was used for qRT-PCR with gene-specific primers. Each column represents an average of three replicates, and error bars represent the standard error of means.

the typical reaction of biotrophic phase pathogens infection. Gene Ontology analysis of DEGs between two tomato cultivars revealed specific enriched categories in both interactions. In resistant tomato cultivar Motelle, cellular component organization or biogenesis, signaling, molecular transducer activity, and signal transducer activity were evidenced when compared to susceptive tomato cultivar Moneymaker. Among them, cellular component organization or biogenesis was a critical metabolic activities required by plants to survive under fungus-inflicted stresses (Paul *et al.* 2011). Generally, the genes involved in GO analysis presented in Motelle more than in Moneymaker upon FOL infection which was due to different resistant cultivar.

Two main mechanisms, pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) (Boller and Felix 2009; Cui *et al.* 2014; Yang and Huang 2014) and the adaptive immune system composed of resistance (R) genes (Dangl and Jones 2001; Van Ooijen *et al.* 2007; Marone *et al.* 2013), are involved in plant responses to pathogenic microorganisms in plant. At least five different classes of *R* genes have been classified based on functional domain (Van Ooijen *et al.* 2007). Among these classes, a nucleotide-binding site (NBS) and leucine-reach repeats (LRRs) (NBS-LRR) is known as the most numerous *R*-gene class (Dangl and Jones 2001). Previously, we reported that tomato endogenic microRNA slmiR482f and slmiR5300 conferred to tomato wilt disease resistance. Two predicted mRNA targets each of slmiR482f and slmiR5300, encoded protein with full or partial NBS domains respectively, confirmed to exhibit function of resistance to FOL (Ouyang *et al.* 2014). A few of investments have been demonstrated that NB-LRR proteins are required for the recognition of a specific Avr and disease resistance in several plant species, including rice, *N. benthamiana*, Arabidopsis and wheat (Sinapidou *et al.* 2004; Peart *et al.* 2005; Lee *et al.* 2009; Loutre *et al.* 2009; Narusaka *et al.* 2009; Okuyama *et al.* 2011; Ouyang *et al.* 2014). The corresponding *R* genes were located tightly in physical linkage. However, in spite this physical linkage, not all these *R* gene pairs were homologous (Sinapidou *et al.* 2004; Lee *et al.* 2009). We found that genes related to plant-pathogen interaction were activated in resistant cultivar Moltelle once treated with FOL. Our qRT-PCR results demonstrated that some of these genes were up-regulated specifically in Motelle but not in Moneymaker. In particular, most of these genes were NBS-LRR or like genes which may imply that NBS-LRR genes played a critical role in resistance to FOL in tomato. Investigation of differentially regulated pathogen-induced NBS-LRR genes could lead to uncover the specific modulation patterns upon FOL infection in tomato.

To conclude, our abroad genome transcriptome RNA-seq data provided a comprehensive overview of the gene expression profiles between two different tomato cultivars Moneymaker and Motelle treated with FOL. Our results will facilitate further analysis of putative molecular mechanism of resistance in tomato upon to FOL, which eventually lead to improvement of Fusarium wilt disease resistance in tomato. It remains to be determined whether or how these candidate pathogen-related genes confirmed by qRT-PCR are overexpressed/knockouted in Moneymaker/Motelle plant to reveal the Fusarium wilt disease resistance. In this scenario, we would expect that overexpressing of these candidate pathogen-related genes will enhance resistance to *F. oxysporum* and would therefore develop a useful molecular tool to uncover functional roles for the increasing number of discovered genes in tomato.

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