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Research Article

Colletotrichum siamense infection caused transcripts involved plant hormone signal transduction and phenylpropanoid biosynthesis varied in strawberry

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Abstract

Colletotrichum siamense infected the petiole and crown of strawberry (*Fragaria* × *ananassa*), and this anthracnose threatens strawberry production heavily in Hubei province. Characteristic the variation of transcript caused by *C. siamense* is important for anthracnose control of strawberry. In this study, RNA-seq analysis was used to monitor the strawberry transcripts in response of *C. siamense* at 0- and 6-days post-inoculation based on lesion measurement and hypha observation. The number of clean reads achieved for each sample was ranged from 5.68 to 8.12 Gb. The 0DPM vs 0DPI group had seven significantly differentially expressed transcripts, whereas the 6DPM vs 6DPI group exhibited 12,097 significantly differentially expressed transcripts. The differentially expressed transcripts of 6DPM vs 6DPI group were enriched in 'metabolic process' and 'biological process' in the biological process category and 'oxidoreductase activity' in the molecular function category by GO analysis. While 'Starch and sucrose metabolism', 'Plant hormone signal transduction' and 'Phenylpropanoid biosynthesis' pathways were significantly enriched by KEGG analysis. The expression of MYC2 in JA, the ETR in Eth and, the SnRK2 and ABF in ABA signal transduction pathways were promoted, whereas expression of the PR1 in SA, JZA1 in JA, and ERF in Eth signal transduction pathways were inhibited. In addition, the transcripts that encode enzymes involved in the 'Phenylpropanoid biosynthesis' pathway, such as PAL, CYP73A, CSE, 4CL, HTC, GSE and COMT were all inhibited showed by RNA-seq and qRT-PCR. This study provided basic information of strawberry responding to *C. siamense* infection.

Keywords: 'Benihoppe' strawberry; gene expression; petiole; phenylpropanoid; signal transduction

Introduction

Colletotrichum siamense infected the petiole and crown of strawberry (*Fragaria* × *ananassa*), and anthracnose caused by *C. siamense* infection threatens strawberry production heavily in Hubei province, China (Han *et al*., 2016). *C. siamense* as hemibiotrophic pathogen of strawberry anthracnose, starting from the suppression of host immune system in a biotrophic phase, followed by a later necrotrophic phase during which the pathogen induces host cell death by its toxin production (Rahman *et al*., 2013). To date, there are no

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cultivars that exhibiting complete resistance to petiole and/or crown rot caused by *Colletotrichum.* (Anciro *et al*., 2018; Miller-Butler *et al*., 2019).

Plants microbe interaction showed two situations, which plant has evolved complex defense system to overcome pathogen infection or pathogens resist defense system successfully and complete life cycle (Castro-Moretti *et al*., 2020). Either plant or pathogen win the war, plant metabolism was completely changed during pathogen infection (Berger *et al*., 2007; Fagard *et al*., 2014). More complex changes in metabolism for plant responding to hemibiotrophic pathogen for its biotrophic and necrotrophic phase. The transcripts involved in carbohydrates, amino acid and lipids biosynthesis, plant hormone biosynthesis of primary metabolism was key response to infection by pathogens for requiring nutrition from the host in pathogens successful colonization and energy supplying in plant defense responses to pathogens requiring energy supply (Rojas *et al*., 2014; Wang *et al*., 2019). While the salicylic acid (SA) pathway is normally associated with resistance to biotrophic pathogens and the jasmonic acid (JA) pathway generally provides protection against necrotrophic pathogens in regulating plant defense for hemibiotrophic pathogens (He *et al*., 2019; Fang *et al*., 2021). In addition, several secondary metabolites involve in plant responding to pathogen infection. These secondary metabolites like phenylpropanoids are present in plant with high concentrations and take part in plant defense against disease resistance for it provides flavonoids that function in scavenging the ROS and provides plants with lignin for physical barriers against pathogen infection (Zaynab *et al*., 2018; Dong and Lin, 2021).

Our previous study observed transcripts involved in phenylpropanoid biosynthesis were induced in 'Benihoppe' strawberry crown by *C. siamense* causing crown rot and transcripts of SA, JA, ethylene (Eth) and abscisic acid (ABA) biosynthesis were not upregulated (Shu *et al*., 2022). In contrast, transcripts of SA, sesquiterpenes, and terpene biosynthesis were enriched in the genus which was less susceptible to anthracnose, according to previous reports (Zhang *et al*., 2016; Mehmood *et al*., 2021). We proposed that the strawberry showed tissue-specific expression (leaf or crown) for responding anthracnose in different strawberry genus. What are transcripts induced in the strawberry petiole for responding to *C. siamense* infection? Whether those transcripts were different from crown responding to *C. siamense*? To understand these two unknowns, an experiment was designed as follows: (1) verification of the infection process via phenotypic and histopathological analysis; (2) analysis of the transcriptome for identifying differentially expressed transcripts during petiole rot process.

Materials and Methods

Plant and pathogen material

The plant materials in a single factor experiment (inoculation with *C. siamense* SCR-7 and control medium) were used for histopathological analysis, transcriptomic and qRT-PCR analysis. Twenty-four pots were divided into inoculation group and control group equally. Each pot cultivated three half a year old healthy and consistent 'Benihoppe' strawberry seedlings. The seedlings were exposed to conditions of 900 μM $m^{-2}s^{-1}$ photon flux density, 28/20 °C day/night temperature under greenhouse. The seedlings of inoculation group were inoculated with *C. siamense* SCR-7 by using a sterilized needle as described in Han *et al.* (2016), while the seedlings of control group were inoculated with sterilized PDA solid medium by using the same method. The seedlings were sampled on 0 and 6 days after inoculation, resulting in four treatment groups, which were 0-day post inoculation with medium (0DPM), 6 days post inoculation with medium (6DPM), 0-day post inoculation with *C. siamense* SCR-7 (0DPI) and 6 days post inoculation with *C. siamense* SCR-7 (6DPI). The seedlings of each pot were one replicate for length and width of lesion measure and pathological analysis. Meanwhile, the seedlings of four treatments (0DPM, 0DPI, 6DPM, 6DPI) were used for transcriptomic and qRT-PCR analysis. The seedlings of two different pots in the same treatment were mixed as one replicate, so each treatment had three biological replicates in transcriptomic and qRT-PCR analysis.

Validation of the infection of C. siamense SCR-7 in 'Benihoppe' strawberry seedlings petiole

The length and width of lesion were measured directly. The method of pathological analysis was according to Redkar *et al*. (2018) with minor modifications. Dye preparation: 10 μL WGA storage solution and 20 μL PI stock solutions were added to 970 μL 0.2% Tween-PBS and mixed thoroughly. Fix: Carnot fixative was used to fix petiole samples. The petioles were transferred into 10% KOH solution, and the tube was sealed with a parafilm to prevent the tube from collapsing, and incubated at 85 °C for 5h. Staining pretreatment: Petioles were washed 4 or 5 times with PBS. Staining: dye solution was added to the centrifuge tube containing blades, and filtered 4 times with vacuum filter where each time lasted for 5min and each interval was 5 min under normal pressure. Photographing: The petioles were washed 2 or 3 times with PBS, and sealed with anti-fluorescence quenching to be stored at 4 °C in the dark, and photographed with a fluorescence microscope (Nikon E400, Melville, NY).

Transcriptome analysis

RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). A total amount of 1 μg RNA per sample was used as input material for the RNA sample preparations. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in First Strand Synthesis Reaction Buffer. First strand cDNA was synthesized using random hexamer primer and RNase H. In order to select cDNA fragments of preferentially 100-200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Adapter ligation at 25°C for 10min before PCR. Then PCR was performed with Phusion HighFidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. Clustering and sequencing (Novogene Experimental Department). The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina platform and 150 bp paired-end reads were generated (Shu *et al*., 2016)

Raw data (raw reads) of fastq format were firstly processed through in-house perl scripts. In this step, clean data (clean reads) were obtained by removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data. At the same time, Q20, Q30 and GC content of the clean data were calculated. The UMI (Unique Molecular Identifiers) was extracted by UMI-tools v1.0.0. All the downstream analyses were based on the clean UMI reads with high quality. Mapping reads to reference genome and deduplicating reads by UMI mapping coordinates Reference genome and gene model annotation files were downloaded from genome website directly. Index of the reference genome was built using Hisat2 v2.0.4 and paired- end clean reads were aligned to the reference genome using Hisat2 v2.0.4. Hisat2 was selected as the mapping tool for that Hisat2 can generate a database of splice junctions based on the gene model annotation file and thus a better mapping result than other non-splice mapping tools. UMI-tools v1.0.0 was used to deduplicate reads based on the mapping coordinate and the UMI attached to the read. Quantification of gene expression level HTSeq v0.9.1 was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. FPKM, expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced, considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels (Trapnell *et al*., 2012). Differential expression analysis of two conditions/groups (two biological replicates per condition) was performed using the DESeq R package (1.18.0). DESeq provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOseq R package, in which gene length bias was corrected. GO terms with corrected P-value less than 0.05 were considered significantly enriched by differential expressed genes (Young *et al*., 2010). KOBAS software was used to test the statistical enrichment of differential expression genes in KEGG pathways (http://www.genome.jp/kegg/) (Shu *et al*., 2016).

qRT-PCR analysis

The qRT-PCR was performed as described in Luo *et al*. (2020). Six genes were selected for RNA-seq verification, and the primers used for qRT-PCR were shown in Supplementary Table 1. The relative gene expression was calculated using the $2^{-\triangle\triangle Ct}$ method, where β-actin was taken as the reference gene. The measured transcripts were normalized to the relative expression value of negative control.

Statistical analysis

Experimental data were statistically analyzed using ANOVA through SAS 8.1 software (SAS Institute, Cary, NC, USA). The probabilities of significance were used to determine significance among the treatments, and the least-significant difference ($p < 0.05$) was used to compare the data.

Results

Effects of C. siamense infection

Results showed that the wound length and width of seedlings of 0DPI, 0DPM and 6DPM ranged 0.09 cm to 0.11 cm. *C. siamense* successfully invaded strawberry petioles after 6D inoculation, and the length and width of lesions increased to 0.4 cm and 0.6 cm in 6DPI (Figure 1a). Corresponding to this, the strawberry seedlings petiole of 0DPM, 6DPM and even 0DPI exhibited no hypha infection, and petioles of 6DPI showed *C. siamense* infection (Figure 1b).

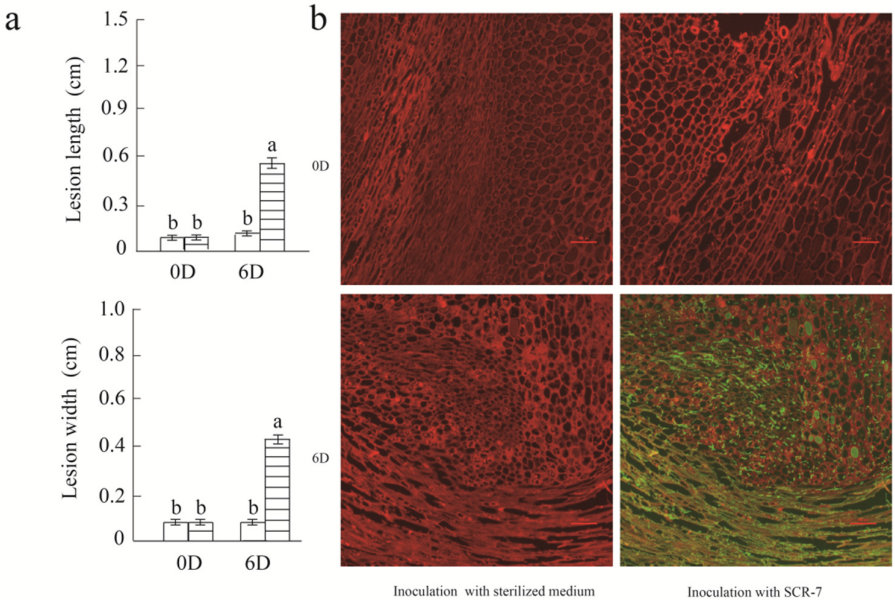

Figure 1. Infection of *Colletotrichum siamense* SCR-7 in 'Benihoppe' strawberry petiole

a. The length and width of *C. siamense* SCR-7; b. The hypha of SCR-7 in 'Benihoppe' petiole (green color) 0- and 6-days post pathogen inoculation, respectively. Data (Means ± SE, n = 6) followed by different letters above the bars among treatments indicate significant differences at the 5% level.

Basic information of RNA-seq and identification of differentially expressed transcripts

From the RNA-seq data, the GC content of each sample was > 46% and the number of clean reads achieved for each sample was 5.68-8.12 Gb after filtration (Table 1). All clean reads were compared to the reference genome, and the proportion of reads mapped onto the reference genome ranged from 94.92-98.00% under Q20 level. While the clean reads of unique molecular identifiers pattern ranged from 91.47 to 92.50. The transcriptomic data were uploaded to the NCBI Sequence Read Archive under the accession number PRJNA851933 and PRJNA852286.

The Pearson index of three biological repeats in 0DPI, 0DPM, 6DPI and 6DPM was 0.878-0.928, 0.871-0.917, 0.933-0.971 and 0.913-0.962, respectively, and the Pearson correlation showed the 0DPM and 0DPI were more similar than 6DPM and 6DPI. The Pearson index of samples of 0DPI and 0DPM were higher than the 6DPI and 6DPM (Figure 2). The 0DPM vs 0DPI group nearly had no significantly differentially expressed transcripts (seven differentially expressed transcripts), whereas the 6DPM vs 6DPI group exhibited 12097 significantly differentially expressed transcripts (5666 downregulated and 6431 upregulated) (Figure 3).

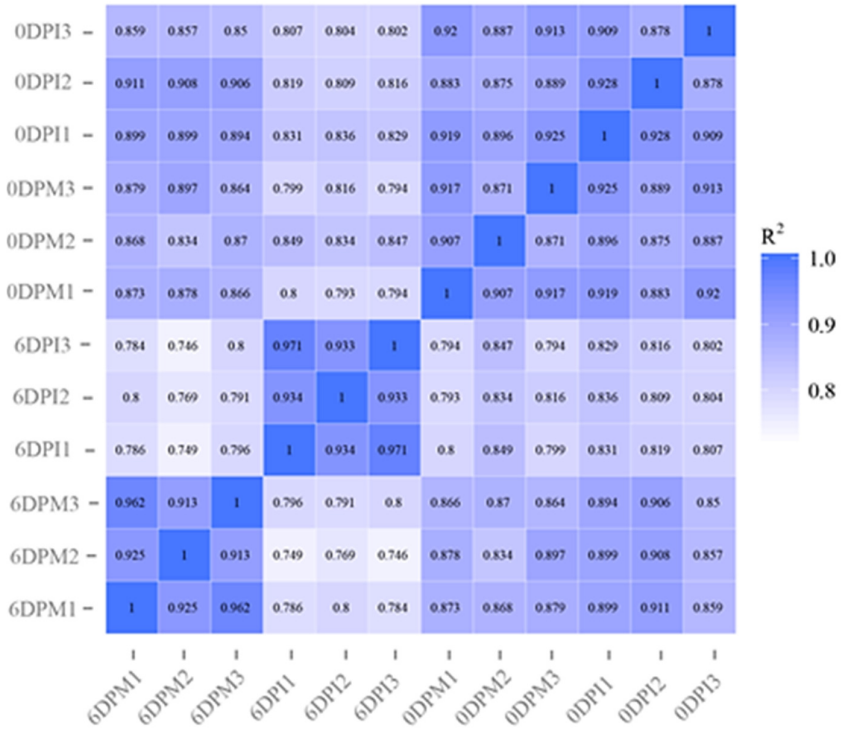

Figure 2. Pearson correlation between samples

0DPM and 6DPM represents 0 day or 6 days post inoculation with medium, 0DPI and 6DPI represents 0 day or 6 days post inoculation with *Colletotrichum siamense* SCR-7. 1, 2 and 3 was the biological repeats of same treatment.

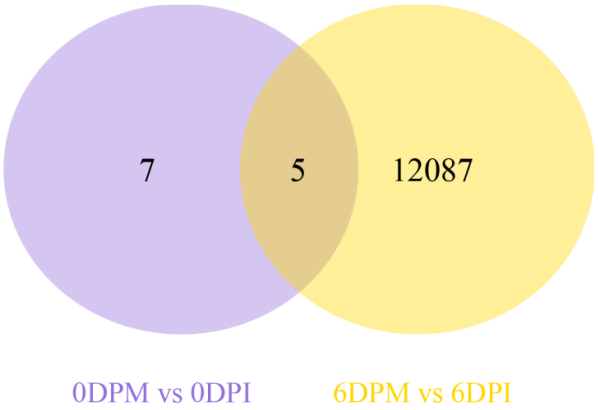

Figure 3. Venn diagram of differentially expressed transcripts of 0DPM vs 0DPI and 6DPM vs 6DPI comparison groups
0DPM and 6DPM represents 0 day or 6 days post inoculation with medium, 0DPI and 6DPI represents 0 day or 6 days post inoculation with *Colletotrichum siamense* SCR-7.

Table 1. Summary of basic information of the RNA-Seq data of 0DPM, 0DPI, 6DPM and 6DPI strawberry petiole samples

Sample name	Raw reads	Clean reads	UMI reads	Raw bases	Clean bases	Error rate (%)	Q20 (%)	Q30 (%)	UMI2Clean (%)	Dedup2Mapped UMI (%)	Deduped ratio (%)
0DPM1	40321610	37877308	34896170	6.05G	5.68G	0.03	97.48	93.31	92.13	71.98	28.02
0DPM2	40279108	37965446	34970446	6.04G	5.69G	0.03	97.93	94.03	92.11	73.38	26.62
0DPM3	39232304	35649464	32609044	5.88G	5.35G	0.03	97.87	94.08	91.47	61.36	38.64
0DPI1	44011994	42066376	38731760	6.6G	6.31G	0.03	97.98	94.15	92.07	71.97	28.03
0DPI2	43981292	41286738	38188612	6.6G	6.19G	0.03	97.11	92.33	92.50	78.89	21.11
0DPI3	44282866	41544894	38464280	6.64G	6.23G	0.03	97.55	93.34	92.58	74.09	25.91
6DPM1	44865542	42317454	39134014	6.73G	6.35G	0.03	97.06	92.34	92.48	78.05	21.95
6DPM2	45708226	41960902	38555074	6.86G	6.29G	0.03	94.92	88.32	91.88	73.76	26.24
6DPM3	58458146	51107142	47286498	8.77G	7.67G	0.03	97.84	93.86	92.52	83.56	16.44
6DPI1	58589162	54121520	49910136	8.79G	8.12G	0.03	97.97	94.06	92.22	68.41	31.59
6DPI2	43664024	41608770	38268344	6.55G	6.24G	0.03	97.31	92.73	91.97	77.14	22.86
6DPI3	52068494	47836300	44070878	7.81G	7.18G	0.03	98.00	94.07	92.13	78.24	21.76

0DPM and 6DPM represents 0 day or 6 days post inoculation with medium, 0DPI and 6DPI represents 0 day or 6 days post inoculation with *Colletotrichum siamense* SCR-7.1, 2 and 3 was the biological repeats of same treatment

GO enrichment analysis of differentially expressed transcripts

Normally, biological process, molecular function and cellular component showed more repressed transcripts than induced. The differentially expressed transcripts were enriched in 'metabolic process' and 'biological process' in the biological process category and 'oxidoreductase activity' in the molecular function category had the largest differentially expressed transcript numbers in the 6DPM vs 6DPI group. In addition, the 'intracellular non−membrane−bounded organelle' and 'non−membrane−bounded organelle' in the cellular component category had the largest differentially expressed transcript number in the 6DPM vs 6DPI group (Figure 4).

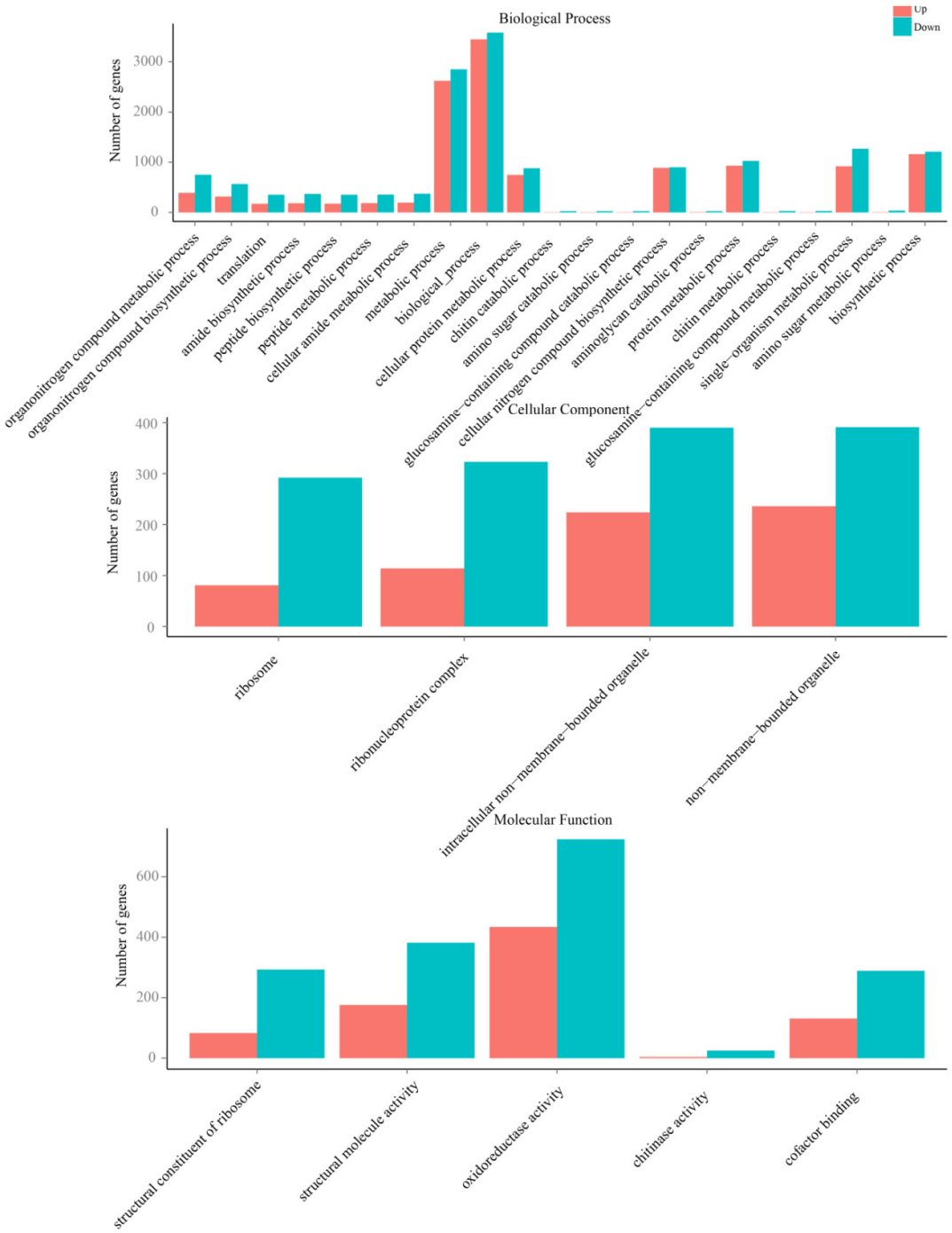

Figure 4. The GO enrichment analysis of differentially expressed transcripts of 6DPM vs 6DPI comparison group

6DPM and 6DPI represents 6 days post-inoculation with medium or *Colletotrichum siamense* SCR-7 on strawberry petiole, respectively.

KEGG pathway enrichment analysis of differentially expressed genes and qRT-PCR verification

The 'Biosynthesis of secondary metabolites', 'Biosynthesis of amino acids' and 'Carbon metabolism' were the significantly enriched KEGG pathways in the 6DPM vs 6DPI comparison group. While 'Starch and sucrose metabolism', 'Plant hormone signal transduction' and 'Phenylpropanoid biosynthesis' KEGG pathways were significantly enriched in the 6DPM vs 6DPI comparison group also, and the three pathways had higher q-value (Figure 5).

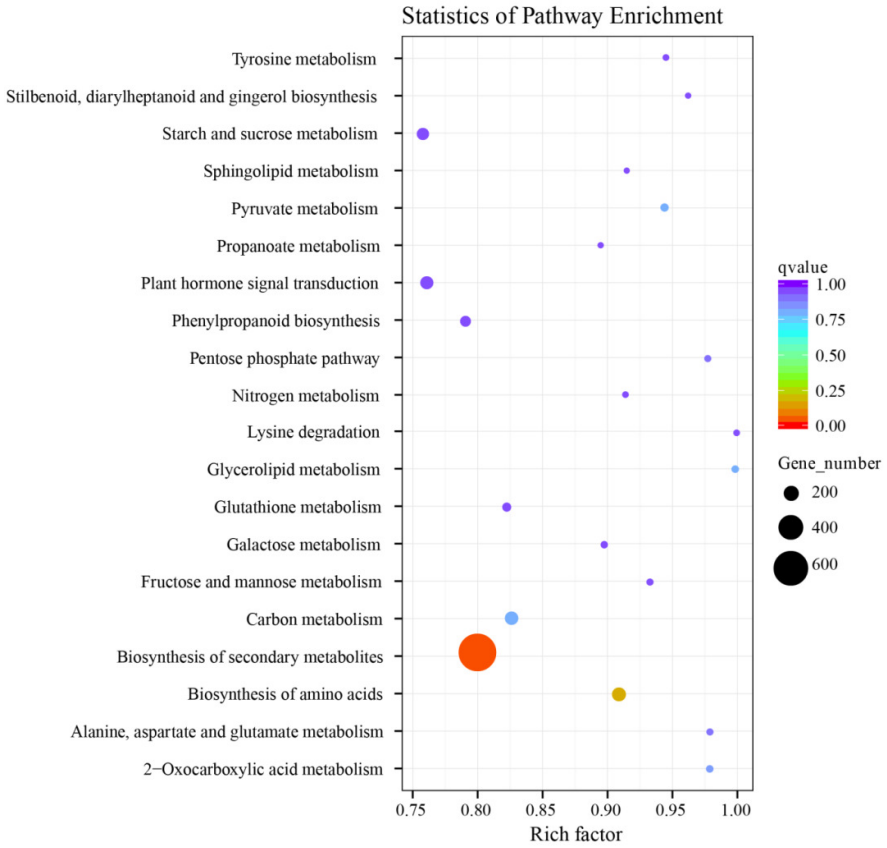

Figure 5. The KEGG enrichment analysis of differentially expressed transcripts of 6DPM vs 6DPI comparison group.
6DPM and 6DPI represents 6 days post-inoculation with medium or *Colletotrichum siamense* SCR-7 on strawberry petiole, respectively

Following the KEGG pathway, the biosynthesis of SA, JA, and Eth and ABA were not significantly enriched, but signal transduction were enriched in the 6DPM vs 6DPI group. The expression of MYC2 in JA, the ETR in Eth and, the SnRK2 and ABF in ABA signal transduction pathways were promoted, whereas expression of the PR1 in SA, JZA1 in JA, and ERF in Eth signal transduction pathways were inhibited. The ABA signal transduction pathway had the greatest numbers of transcripts, and PYR/PYL and PP2C were also enriched in ABA signal transduction pathway (Figure 6).

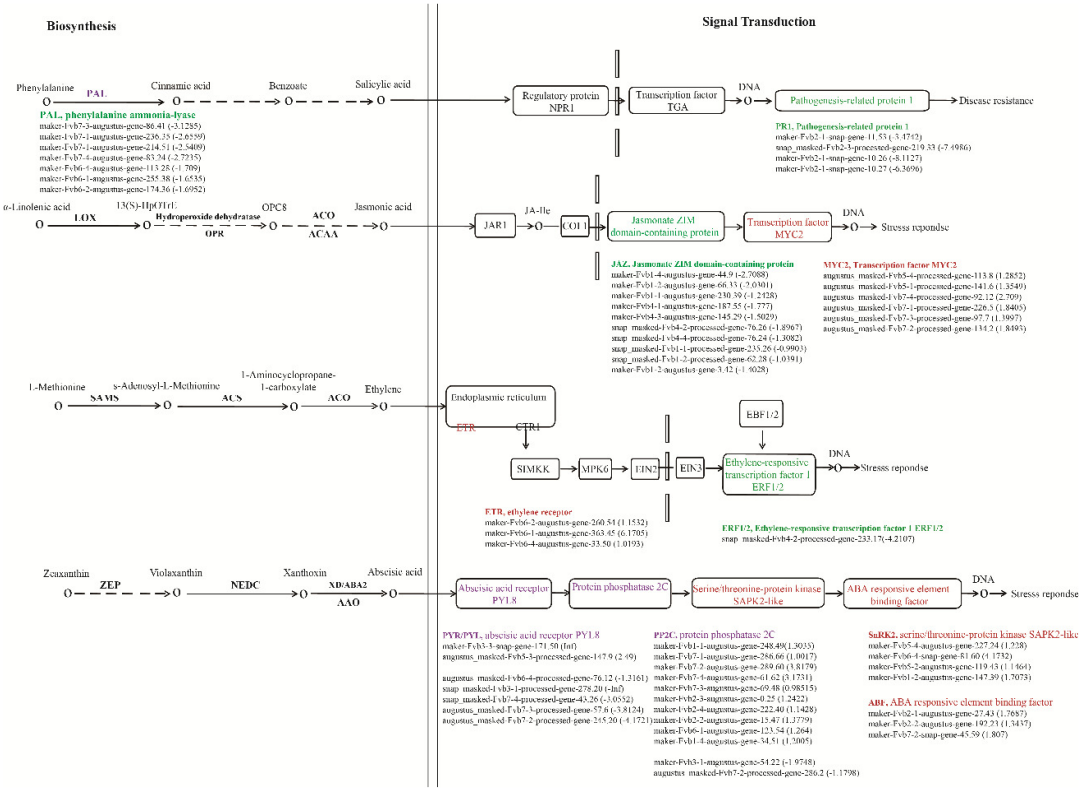

Figure 6. The effect *Colletotrichum siamense* SCR-7 infection on biosynthesis and signal transduction of salicylic acid (SA), jasmonic acid (JA), ethylene (Eth) and abscisic acid (ABA)
SA, JA Eth and ABA biosynthesis pathway based on 6DPM vs 6DPI data of transcriptomic analysis, up-expressed transcripts were shown in red and 6DPM and 6DPI represents 6 days post-inoculation with medium or *C. siamense* SCR-7, respectively.

The transcripts that encode enzymes involved in the 'phenylpropanoid biosynthesis' pathway such as PAL, CYP73A, CSE, 4CL, HTC, GSE and COMT were all downregulated (Figure 7a). The expression of six selected transcripts that encode enzymes involved in 'phenylpropanoid biosynthesis' were analyzed using qRT-PCR to confirm the transcriptome data. In accordance with the transcriptome data, qRT-PCR showed that the transcripts encoding PAL, CYP73A, CSE, and COMT were all inhibited by *C. siamense* inoculation (Figure 7b).

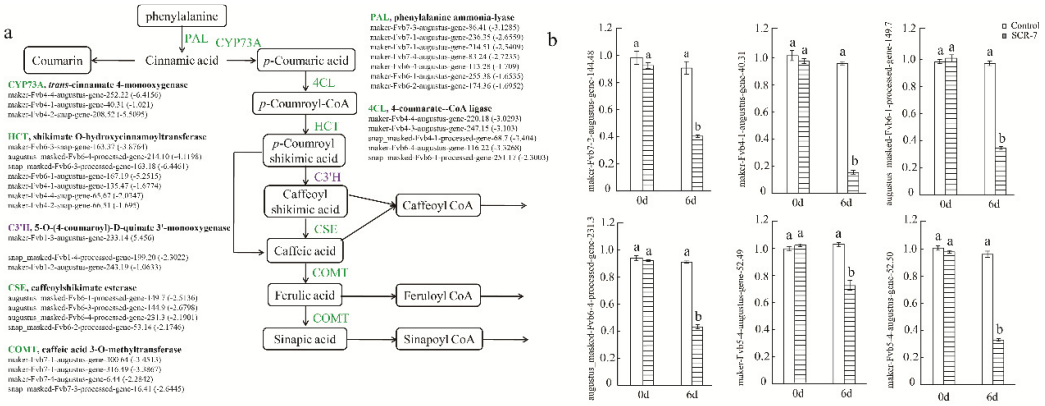

Figure 7. The effect *Colletotrichum siamense* SCR-7 infection on phenylpropanoid biosynthesis pathway
a. phenylpropanoid biosynthesis pathway based on 6DPM vs 6DPI data of transcriptomic analysis, the down-expressed transcripts were shown in green; b. verification of differentially expressed transcripts by qRT-PCR. 6DPM and 6DPI represents 6 days post-inoculation with medium or *C. siamense* SCR-7, respectively.

Discussion

RNA-seq is a technique can be used to detect low-expressing reads and identify novel transcripts in plant-microbe interaction (Shu *et al*., 2016). Here, we confirmed *C. siamense* successfully invaded strawberry petioles after 6D inoculation by the length and width of lesions and hypha infection (Figure 1b). The number of clean reads achieved for each sample was 5.68-8.12 Gb after filtration (Table 1). All clean reads were compared to the reference genome, and the proportion of reads mapped onto the reference genome ranged from 94.92-98.00% under Q20 level. The pearson index of three biological repeats in 0DPI, 0DPM, 6DPI and 6DPM was 0.878-0.928, 0.871-0.917, 0.933-0.971 and 0.913-0.962, respectively. The pearson index of samples of 0DPI and 0DPM were higher than the 6DPI and 6DPM (Figure 2). The 0DPM vs 0DPI group nearly had no significantly differentially expressed transcripts (seven differentially expressed transcripts), whereas the 6DPM vs 6DPI group exhibited 12,097 significantly differentially expressed transcripts (5,666 downregulated and 6,431 upregulated) (Figure 3). RNA-seq analysis identified 2,210 fungal and tomato sequence assemblies from mycorrhizal roots through comparing the wild-type and *rmc* root samples under field condition (Ruzicka *et al*., 2013); 3,641 genes differentially expressed during AM development in *Lotus japonicus* (Handa *et al*., 2015). The numbers of annotated sequences of strawberry in present study compared with those annotated sequences and DEGs in the biological process of fungi and other host plants suggested that RNA-seq is an effective method for identifying transcript variation in strawberry responding to *C. siamense* infection.

The SA normally associates with resistance to biotrophic pathogens and the JA generally provides protection against necrotrophic pathogens and the two plant hormones are well known in regulating plant defense for hemibiotrophic pathogen (Zaynab *et al*., 2018). Previous research showed transcripts involved in SA biosynthesis enriched in the genus leaf of less susceptible strawberry for responding to anthracnose (Zhang *et al*., 2016; Mehmood *et al*., 2021). The metabolites and trancripts in SA, JA, and Eth biosynthesis were not accumulated in crown for responding to *C. siamense* SCR-7 infection in our previous study. Following the KEGG pathway, this experiment showed the biosynthesis of SA, JA, and Eth and ABA were not significantly enriched in petiole for responding to *C. siamense* SCR-7 infection (Figure 6). We proposed that the different transcripts expression changes in this experiment and previous studies might be due to the differences of genus resistance, tissue-specific expression (fruit, runner, leaf, or crown), and the time post-anthracnose infection. 'Benihoppe' strawberry is susceptible to anthracnose thus the SA and JA might be inhibited by pathogen

(Zaynab *et al*., 2018). In addition, the expression of MYC2 in JA, the ETR in Eth and, the SnRK2 and ABF in ABA signal transduction pathways were promoted, whereas expression of the PR1 in SA, JZA1 in JA, and ERF in Eth signal transduction pathways were inhibited. The ABA signal transduction pathway have the most transcripts, and PYR/PYL and PP2C were also enriched in ABA signal transduction pathway (Figure 6). This result suggested that these plant hormones were also involved in petiole rot, and the function of SA and JA should be concerned based on the different regulation in biotrophic or necrotrophic phase.

Our results showed the differentially expressed transcripts were enriched in 'metabolic process' and 'biological process' in the biological process category and 'oxidoreductase activity' in the molecular function category in the 6DPM vs 6DPI group (Figure 4). This suggested oxidoreductase was important for strawberry responding to *C. siamense* infection. As expected, the 'Starch and sucrose metabolism' KEGG pathways which is associated to the energy required by the defense response (Swarbrick *et al*. 2006, Bednarek and Osbourn 2009), 'Plant hormone signal transduction' KEGG pathways which is associated to hormone activated downstream resistant reaction (Zhang *et al*., 2016), and 'Phenylpropanoid biosynthesis' KEGG pathways which were widely studied for their antimicrobial properties and their role in signaling during defense reactions (Yadav *et al*., 2020; Chen *et al*., 2021), were significantly enriched in the 6DPM vs 6DPI comparison group (Figure 5). As the 'phenylpropanoid biosynthesis' pathway that produces lignin-building monolignols is greatly triggered after the cell wall is attacked by pathogens (Jaeck *et al*., 1992), an intermediate in the phenolic acids, such as the SA biosynthesis pathway, also affects resistance (Zhou *et al*., 2018; Fang *et al*., 2021). The transcripts that encode enzymes involved in the 'phenylpropanoid biosynthesis' pathway, such as PAL, CYP73A, CSE, 4CL, HTC, GSE and COMT were all downregulated (Figure. 7ab). All these evidences suggested that metabolites relating to 'phenylpropanoid biosynthesis' were inhibited in petiole when *C. siamense* infection.

Conclusions

Colletotrichum siamense infected the petiole and crown of strawberry, which caused heavily anthracnose. *C. siamense* infected petioles 6 days post inoculation. Thus, two comparison groups 0DPM vs 0DPI and 6DPM vs 6DPI were used for identifying different transcripts after *C. siamense* infection. The number of clean reads achieved for each sample was 5.68-8.12 Gb after filtration. All clean reads were compared to the reference genome, and the proportion of reads mapped onto the reference genome ranged from 94.92-98.00%. The Pearson index of three biological repeats in 0DPI, 0DPM, 6DPI and 6DPM was 0.878-0.928, 0.871-0.917, 0.933-0.971 and 0.913-0.962, respectively. The Pearson index of samples of 0DPI and 0DPM were higher than the 6DPI and 6DPM. The 0DPM vs 0DPI group had seven significantly differentially expressed transcripts, whereas the 6DPM vs 6DPI group exhibited 12,097 significantly differentially expressed transcripts. The differentially expressed transcripts of 6DPM vs 6DPI group were enriched in 'metabolic process' and 'biological process' in the biological process category, and 'oxidoreductase activity' in the molecular function category by GO analysis. While 'Starch and sucrose metabolism', 'Plant hormone signal transduction' and 'Phenylpropanoid biosynthesis' KEGG pathways were significantly enriched in the 6DPM vs 6DPI comparison group also. The expression of MYC2 in JA, the ETR in Eth and, the SnRK2 and ABF in ABA signal transduction pathways were promoted, whereas expression of the PR1 in SA, JZA1 in JA, ERF in Eth signal transduction pathways were inhibited. In addition, the transcripts that encode enzymes involved in the 'phenylpropanoid biosynthesis' pathway, such as PAL, CYP73A, CSE, 4CL, HTC, GSE and COMT were all downregulated.

Authors' Contributions

Chun Luo: conceived the experiments and wrote the manuscript. Bo Shu, Rong Zheng: carried out the experiments and analyzed the data. Chun Luo: critically reviewed the manuscript. All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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