Research article

Transcriptomic insights into the molecular mechanism of wheat response to stripe rust fungus

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ABSTRACT

The wheat crop (*Triticum aestivum* L.) is the widely cultivated and most important staple foods of worlds. Stripe (yellow) rust is prompted by *Puccinia striiformis* f. sp. *tritici* (*Pst*) to reduces the yield and grain quality of the wheat significantly. Although many resistant cultivars have been successfully used in wheat breeding, the size of the regulating network and the underlying molecular mechanisms of wheat to response *Pst* still unknown. Therefore, in order to identify differentially expression genes (DEGs) and the regulate network related to *Pst* resistance, 15 cDNA libraries were constructed from wheat with CYR34 infection. In this study, a highly susceptible cv. Chuanyu12 (CY12) was used to study the transcriptome profiles after being inoculated with *Pst* physiological race CYR34. The DEGs were investigated at 24h, 48h, 72h, and 7 days post-inoculation. Certain key genes and pathways of response for *Pst*-CYR34 in CY12 were identified. The results revealed that *Pst*-CYR34 inhibited the DEGs related to energy metabolism, biosynthesis, carbon fixation, phenylalanine metabolism, and plant hormone signaling pathways after post-inoculation at 24h, 48h, 72h, and 7d. Light-harvesting chlorophyll protein complex in photosystem I and photosystem II; F-type ATPase, cytochrome b6/f/complex, and photosynthetic electron transport; ethylene, salicylic acid (SA), and jasmonic acid (JA); and lignin and flavonoids biosynthesis in CY12 are among the down-regulated DEGs. The expression patterns of these DEGs were verified via Quantitative Real-time PCR analysis. Our results give insights into the foundation for further exploring the molecular mechanisms regulating networks of *Pst* response and opens the door for bread wheat *Pst* resistance breeding.

1. Introduction

Triticum aestivum L. (Wheat) is the most widely grown and a major staple food crop in the world (Prasad et al., 2019). Wheat yellow rust is one of the most globally devastating fungal diseases caused by *Pst* (*Puccinia striiformis* f. sp.) (Wellings, 2011; Wang et al., 2019), which has become a major threat, causing massive yield losses of wheat. The *Pst* races can of spreading long distances due to seasonal wind or the movement by human-assisted to other regions or continents (Prasad et al., 2019). In China, yellow rust has been considered as the most crucial fungal disease to decrease wheat production, which threatens all wheat cultivars (Wan et al., 2004; Zeng and Luo, 2006; Zhou et al., 2019). Because of its strong adaptive capacity, wheat *Pst* evolved fast to become a new virulent race and overcome resistant cultivars within a short time, so that wheat cultivars have race-specific resistance to yellow rust can

retain resistance only for several years (Liu et al., 2020). Previously reported yellow rust resistance genes already lose effectiveness and with yield losses caused by the emergence of new *Pst* races (Schwessinger, 2017). A new stripe rust race (V26/CYR34) was virulent to the resistance gene Yr26 and spread in wheat breeding programs in many major wheat-planting regions (Liu et al., 2010; Wang et al., 2017). According to recent research, approximately 76% of universal wheat cultivars and wheat breeding lines are susceptible to Pst-CYR34 (Han et al., 2015; Bai et al., 2018; Wang et al., 2019). Therefore, it is essential to breed novel resistant cultivars or understand the defense response mechanism of wheat against Pst pathogen before CYR34 causes more loss in wheat production.

Global gene expression methods can be used to elucidate the molecular mechanisms for wheat-Pst (fungal) interactions, specifically by using the next-generation sequencing method to research crucial non-model

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plant host-pathogen systems such as wheat rust (Cantu et al., 2011). The Affymetrix Gene Chip Wheat Genome Array (Affymetrix, Santa Clara, CA, USA) has been used in a large number of studies on wheat-rust interactions (Hulbert et al., 2007; Coram et al., 2009), as well as cDNA-AFLP (Wang et al., 2010) and RNA-seq analysis on the Pst-wheat response (Cantu et al., 2011; Hubbard et al., 2015). However, a whole gene expression profile for wheat to response stripe rust pathogens is still lacking. Many studies have reported that photosynthetic activity is closely related to wheat to response of Pst (stripe rust). The Pst infection is easy to damage the PSII of wheat, resulting in the decline of photosynthetic capacity (Chen et al., 2015; Li et al., 2015). In addition, the antioxidant enzyme activities and chlorophyll fluorescence of stripe rust-resistant wheat were higher than in susceptible wheat during *Pst* infection (Chen et al., 2015). Chen et al. (2019) found that the activity of antioxidant enzymes and reactive oxygen species, energy dissipation increased and cell death after *Pst* infection in wheat. Compared with smaller genomes plants, most of the genetic and molecular techniques used to study the genes involved in wheat-Pst interactions have been limited. Due to the hexaploid wheat has a large and complex genome with difficult transformation (Wang et al., 2010), and fungi show sexual reproduction as well as irreversible deletion of genes not indispensable for biotrophy.

Therefore, the main objective of this study is to provide a better understanding for susceptibility and resistance mechanisms of wheat in response to stripe rust infections and to enhance the wheat yield. We investigated the phenotypic and co-regulated mRNA transcriptomic responses of wheat cv. Chuanyu12 (CY12), which shows a variation in expression mode after *Pst*-CYR34 infection to ascertain DEGs and related signal pathways to the fungal (Pst) response. This is the first study to reveal susceptible mechanisms using an overall expression profiling strategy.

2. Material and methods

2.1. Plant materials and fungal treatment

The wheat cv. CY12 was used in this study, which was acquired from Chengdu Biology of Sciences, Chinese Academy of Sciences. CY12 is developed by our research group and is highly susceptible to the currently predominant stripe rust, such as the new *Pst*-race CYR34 (Liu et al., 2020). Pst-CYR34 was inoculation artificially at the seedling period was conducted under the controlled greenhouse conditions at the Gansu Academy of Agricultural Sciences (GAAS). Two-week-old CY12 seedlings were inoculated with *Pst*-CYR34. The wheat cv. Mingxian169 was used to monitor inoculation efficiency. The wheat leaves of CY12 were collected at early stages of 0h, 24h, 48h, and at late stages of 72h, 168h (7 days) post inoculation, with each time point having 3 biological replicates

(Sharma et al., 2018a). For transcriptome sequencing, all leaf samples were quickly frozen in liquid nitrogen and to kept in an -80 °C refrigerator.

2.2. RNA isolation and cDNA library construction for RNA sequencing

The total RNA was extracted in wheat leaves by using the mirVana miRNA Isolation Kit (Ambion) according to manufacturer's instruction. The Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to evaluate RNA integrity (number ≥7) for subsequent study. Then, the total wheat leaf RNA was broken into short fragments, then purified in the thermomixer at a suitable temperature for the first-strand cDNA and second-strand cDNA synthesizing. The TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) is used to the libraries construction that followed by the manufacturer's protocol. Fifteen cDNA libraries of wheat were then sequenced to generate 125 bp/150 bp paired-end reads on the Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeq X Ten). The transcriptome sequencing was analyzed by OE Biotech (Shanghai, China). Raw data (raw reads) was processed using Trimmomatic (Bolger et al., 2014). The ploy-N and the low-quality reads were removed to obtain clean reads. Then, all clean reads were used for subsequent analyses and mapped into the reference genome by using hisat2 (Kim et al., 2015).

2.3. Differentially expression genes (DEGs), gene ontology (GO) and KEGG analysis

The FPKM value and read count of each gene were calculated by cufflinks and htseq-count, respectively (Trapnell et al., 2010; Roberts et al., 2011; Anders et al., 2015). DEGs were analyzed and identified by using the DEGseq R package. The DEGs were chosen with the |log2 FC| > 1 and Q < 0.005 thresholds in order to test whether each gene was affected by genotype and time point. In order to explore the changes of gene expression pattern, we performed the hierarchical cluster analysis on these DEGs. P values were adjusted by using the Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995). GO enrichment and KEGG pathway enrichment analysis (Kanehisa et al., 2008) of DEGs were performed by using R based on the hypergeometric distribution during CYR34 infection.

2.4. qRT-PCR analysis

Twelve DEGs were casually chosen for qRT-PCR detection, and using *TaGAPDH* as the internal reference. Primer 5.0 was used for primer design according to each gene sequence (Table 1). The RNA extracted from CY12 at 0 h, 24 h, 48 h, 72 h and 7 days. HiScriptII Q RT SuperMix (Vazyme, R223-1) were used to synthesize the first-strand cDNA. The

Table 1. List of primers used for the relative quantification of gene transcripts.

| Gene id	Forward primer	Reverse primer	Product length (bp)	Tm (°C)
TraesCS6B02G312200	GGGAAGGAAGGAAGGGTG	GCCCTCTTCGTGTCCCTTA	87	60
TraesCS2B02G040600	TCAAAGTCACAGTACATCGGTT	AGCTGAGCATGTGATACTAGG	98	60
TraesCS2D02G568300	CCTAGAGTACACCAAGTAGCAA	GCTGACCGTGTCATCCCA	110	60
TraesCS1A02G349900	CAAACTTCCAGATGCAGTAGG	ATCAAATGGTATCAGCAGGAAT	83	60
TraesCS3A02G516500	CCTCGTCAGCTTCCTCAG	CTACGAGGGAGAGACTTGC	82	60
TraesCS2B02G050600	CAGGTCAAGGAGGAGCAG	TCCGTGTCCAGGAAGAAC	98	60
TraesCS2D02G572300	CTGGTGCTGAGGATCGAG	CCGACCTTGAACTGGTTG	176	60
TraesCS2B02G048400	ACTACCCGGACTACTACTTC	TGATCTGGGACTTGTCACAC	93	60
TraesCS1B02G135800	TGCGTGTGGTTTACTTCG	CACTCCATCCATAGTGGAGATA	116	60
TraesCS6B02G412700	ACAAGAGGAACACCTGGTAA	GCCTTGGACACTGAGGAT	83	60
TraesCS6D02G359300	TCGTTGGTGCTGGTCTATAC	TGCAGGTCTCTTGTCATCC	81	60
TraesCS7A02G113600	GGAGGGAATGGGTCACTAT	TGATGCTTATTCTCGCAACAC	81	60
GAPDH (Wheat)	AAGGCTGTTGGCAAGGTG	GTGGTCGTTCAGAGCAATCC	192	60

Table 2. Read statistics in 15 RNA sequencing libraries.

Sample	Raw reads (M)	Clean reads (M)	Q30 (%)	GC (%)	Total mapped (%)	Multiple mapped (%)	Uniquely mapped (%)
CY12_0h	49.2 ± 0.06	47.85 ± 0.19	94.8 ± 0.17	54.38 ± 0.98	97.23 ± 0.16	10.3 ± 0.49	86.92 ± 0.35
CY12_24h	49.51 ± 0.9	47.97 ± 1.5	94.68 ± 0.72	53.07 ± 0.24	96.61 ± 0.03	9.97 ± 0.23	97.64 ± 0.2
CY12_48h	49.18 ± 0.38	47.67 ± 0.56	94.46 ± 0.75	54.28 ± 0.39	96.83 ± 0.37	10.14 ± 0.62	86.7 ± 0.64
CY12_72h	49.59 ± 0.91	48.05 ± 0.52	94.41 ± 0.83	53.81 ± 0.66	96.87 ± 0.26	9.72 ± 0.61	87.14 ± 0.77
CY12_7d	49.56 ± 0.87	48.4 ± 0.75	94.83 ± 0.34	54.09 ± 0.64	97.02 ± 0.31	10.47 ± 0.99	86.55 ± 0.81
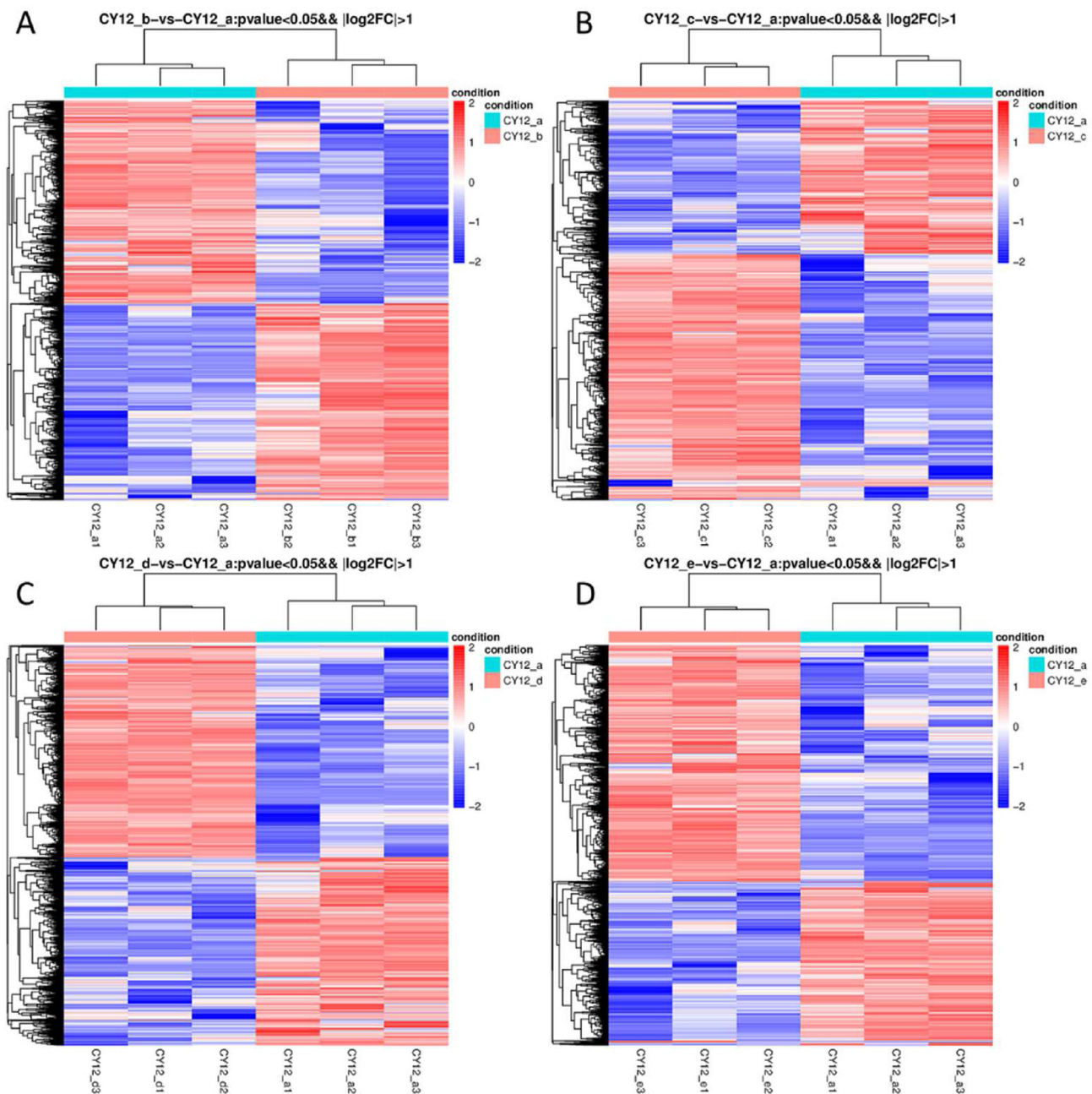
Figure 1. Hierarchical clustering analysis of differentially expressed genes (DEGs) in CY12 at 24h (A), 48h (B), 72h (C) and 7d (D) under *Pst-CYR34* infection, respectively. Red color represents upregulated genes, and blue color represents downregulated genes.

qRT-PCR was performed with the SYBR Green PCR kit (Qiagen, 204054). All DEGs were repeated 3 times in this study, using $2^{-\Delta\Delta ct}$ method to calculate the mRNA expression level of each genes (Schmittgen and Livak, 2008). Each wheat leaf sample was repeated three times. The correlation between RNA-seq and qRT-PCR results was analyzed using these values in the R package version 3.1.3 (http://cran.r-project.org/). The normalized values of relative expression and RPKM values were calculated using log2 (fold change) measurements.
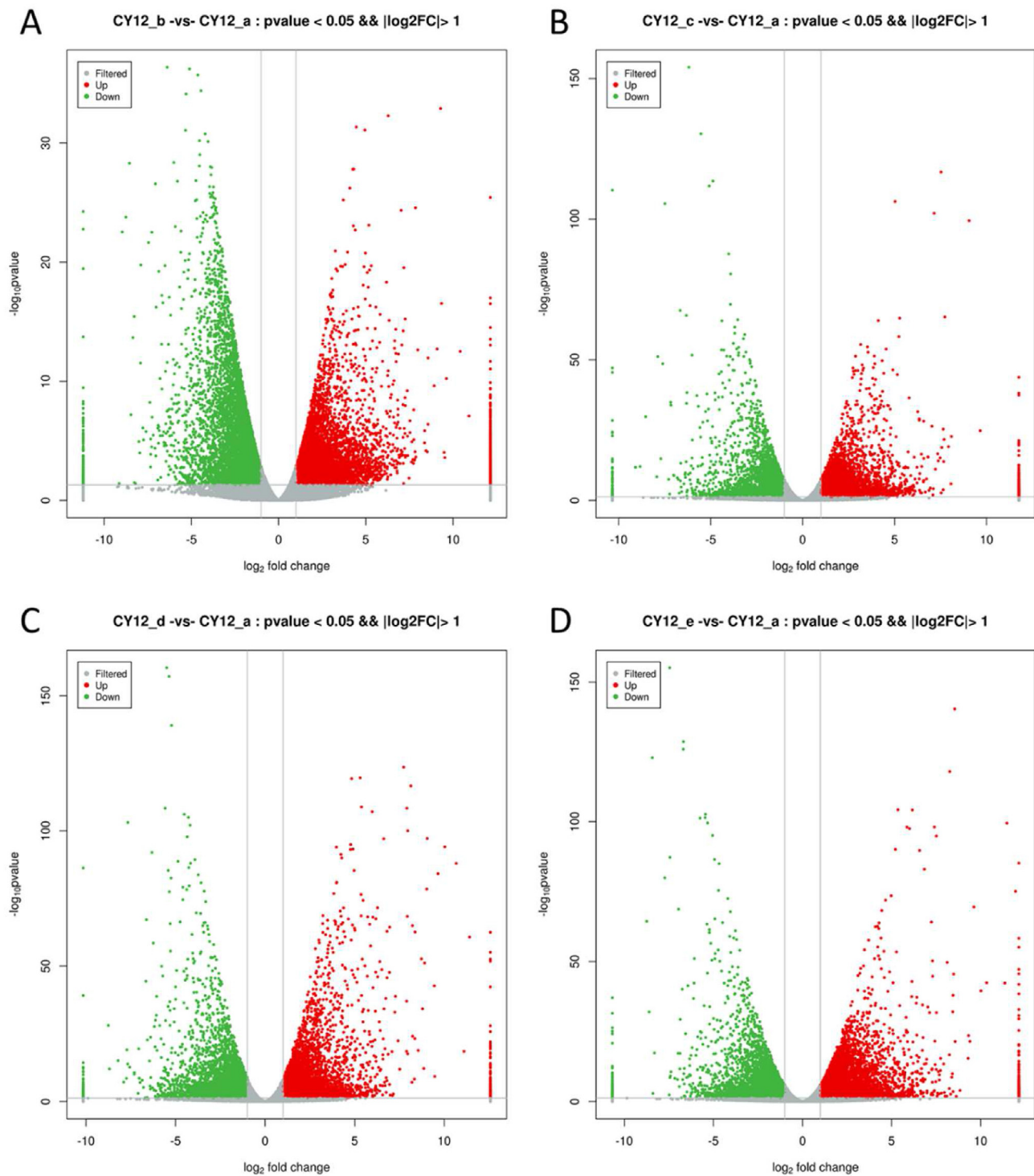
Figure 2. Volcano plots of differentially expressed genes with *Pst-CYR34* infection at 0h, 24h, 48h, 72h and 7d time points in CY12: (A) 24h of *Pst-CYR34* infection; (B) 48h of *Pst-CYR34* infection; (C) 48h of *Pst-CYR34* infection; and (D) 7d of *Pst-CYR34* infection. In this figure, CY12_a, CY12_b, CY12_c, CY12_d and CY12_e represents different time points of collecting sample. a-0h, b-24h, c-48h, d-72h and d-7days after CYR34 infection.

3. Results

3.1. mRNA transcriptome and analysis of CY12

In order to clarify the molecular basis for CY12 response to stripe rust, we conducted comparative transcriptome analysis through RNA sequencing. Leaves from the 0 h, 24 h, 48 h, 72 h, and 7 d Pst treatments were used to study the early and late responses of wheat to *Pst-*CYR34. Fifteen cDNA libraries, each for CY12 inoculated with *Pst-*CYR34 and a non-infected control at five time points, were characterized by Illumina HiSeq to explore gene expression pattern on the transcriptome level (Table 2). Removed low-quality reads and ploy-N, we obtained about 48.0 million (47.67–48.40) clean reads per library to mapped (>96.61% per library) to the wheat reference genome (http://ftp.ensemblgenomes.org/pub/plants/release-53/fasta/triticum_aestivum/dna/).
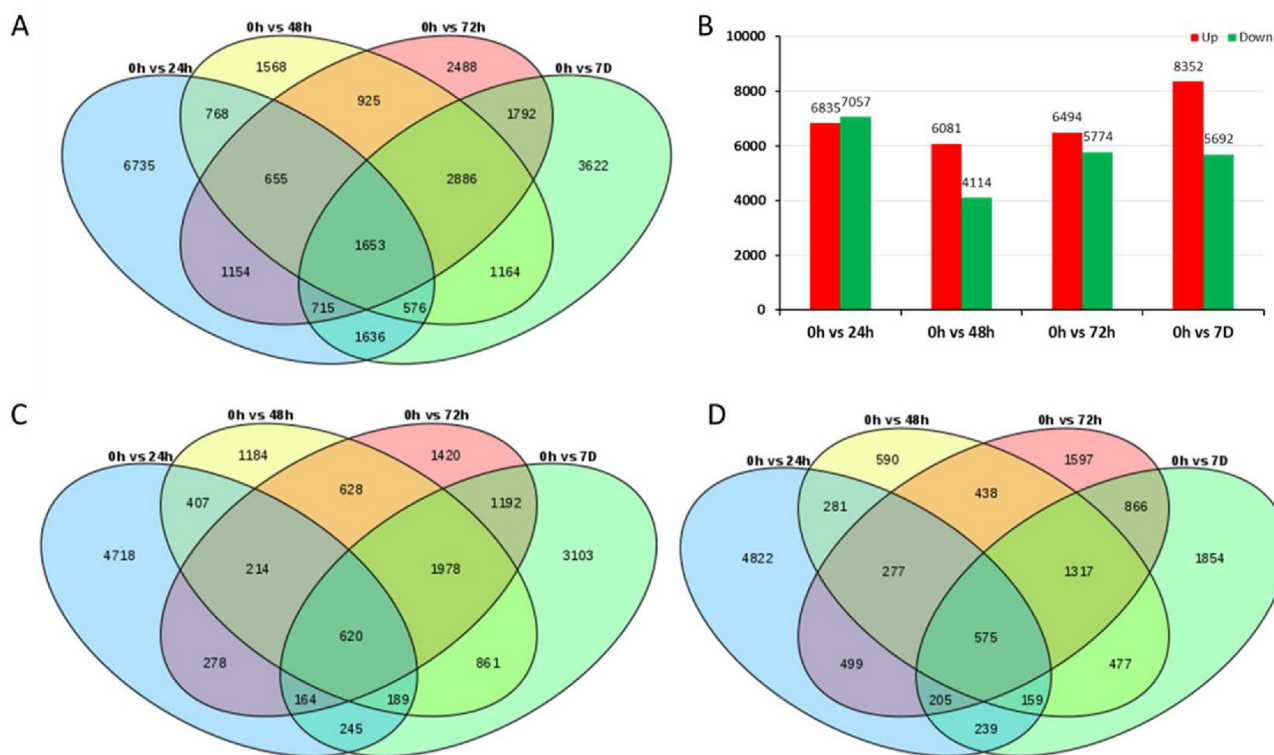
Figure 3. Venn diagrams of differentially expressed genes (DEGs) in CY12. Venn diagram showing the DEGs between *Pst-CYR34* treatment at 0hpi, 24hpi, 48hpi, 72hpi and 7dpi time points in CY12 (A), the number of DEGs up- or down-regulated (B) and the Venn diagrams of up-regulated DEGs (C) and down-regulated DEGs (D) at each time points.

3.2. DEGs identification

The DEGs were identified by comparing the FPKM values of each gene in CY12 with the criteria of fold change≥2 and P < 0.05. Then DEGs involved in the *Pst*-CYR34 infection process were screened. The result of sample to sample clustering (sample correlations) showed that gene expression level among 15 wheat samples were reproducible between each biological replicates and batch effects controlled well (Figure 1). The DEGs of CY12 with *Pst*-CYR34 infection were found to have 13892 DEGs (6835 up-regulated and 7057 down-regulated) at 24hpi, 10195 DEGs (6081 up-regulated and 4114 down-regulated) at 48hpi, 12268 DEGs (6494 up-regulated and 5774 down-regulated) at 72hpi, and 14044 DEGs (8352 up-regulated and 5692 down-regulated) at 7dpi (Figure 2, Figure 3B).

We constructed the Venn diagram to examine the unique and shared DEGs in wheat following Pst-CYR34 infection. All DEGs at 0hpi, 24hpi, 48hpi, 72hpi, and 7dpi were shown in Figure 3. A total of 1653 specific DEGs were identified and shared in each group (Figure 3A). Among them, 620 shared DEGs were up-regulated (Figure 3C) and 575 DEGs were down-regulated (Figure 3D) at all time points in CY12. All 1653 DEGs were shown in the heatmap (Figure 4). As shown in Figure 4, compared with 0hpi, there were a significant change in the expression of the DEGs after *Pst*-CYR34 inoculation. Therefore, we conducted the following analysis on these differentially expressed genes.

3.3. Verification of RNA-seq by qRT-PCR

To study the validity of transcriptome data from the RNA sequencing results, the expression level of 11 genes (with 12 IDs of wheat) were randomly screened for qRT-PCR analysis. Trace annotation results show that TraesCS2B02G040600 was UDP-glucosyltransferase, TraesCS2D02G568300 (Tricetin 3',4',5'-O-trimethyltransferase), and TraesCS1A02G349900 (Ferredoxin). The uncharacterized proteins

involved in energy metabolism were TraesCS3A02G516500 and TraesCS2B02G050600. TraesCS2D02G572300 (30S ribosomal protein S17), TraesCS2B02G048400 (chalcone synthase 2-like), and TraesCS1B02G135800 (metallothionein) are the three genes involved. TraesCS6B02G412700 and TraesCS6D02G359300 were protein SRG1-like, and TraesCS7A02G113600 was GDSL esterase/lipase At5g45910-like. Among them, TraesCS6B02G412700 and TraesCS6D02G359300 were homologous genes (the alignment rate was 96.6%), which had similar expression patterns in this study. The correlation between qRT-PCR and RNA-Seq (FPKM) are exhibition in Figure 5. The genes relative expression levels from the qRT-PCR were consistent with those from the RNA-Seq data (P < 0.001), which confirms the reliability of the data of RNA-Seq.

3.4. Gene ontology (GO) and KEGG analysis

To identify the major functional terms under the *Pst*-CYR34 infection, the analysis of GO enrichment was carried out on DEGs (Figure 6). The results of GO analysis showed that *Pst*-CYR34 response DEGs were mainly enriched in signal transduction, energy metabolism, translation, carbohydrate metabolism, and amino acid metabolism in CY12 at 24h, 48h, 72h, and 7dpi.

For KEGG analysis, *Pst*-CYR34 induced DEGs in CY12 were notably down-regulated in photosynthesis, carbohydrate metabolism, plant hormone signal transduction, and amino acid metabolism at 24h, 48h, 72h, and 7d time points (the early and late responses of wheat to Pst) (Figure 7A-D). The up-regulated genes in CY12 were enriched in vitamin B6 metabolism, ribosome and RNA degradation significantly (Figure 7E-H). Those results indicate that energy metabolism (including photosynthesis and carbohydrate metabolism) and plant hormones are important for disease resistance in wheat. Therefore, the down-regulation of the relevant DEGs may cause the cv. CY12 to be susceptible to *Pst*.
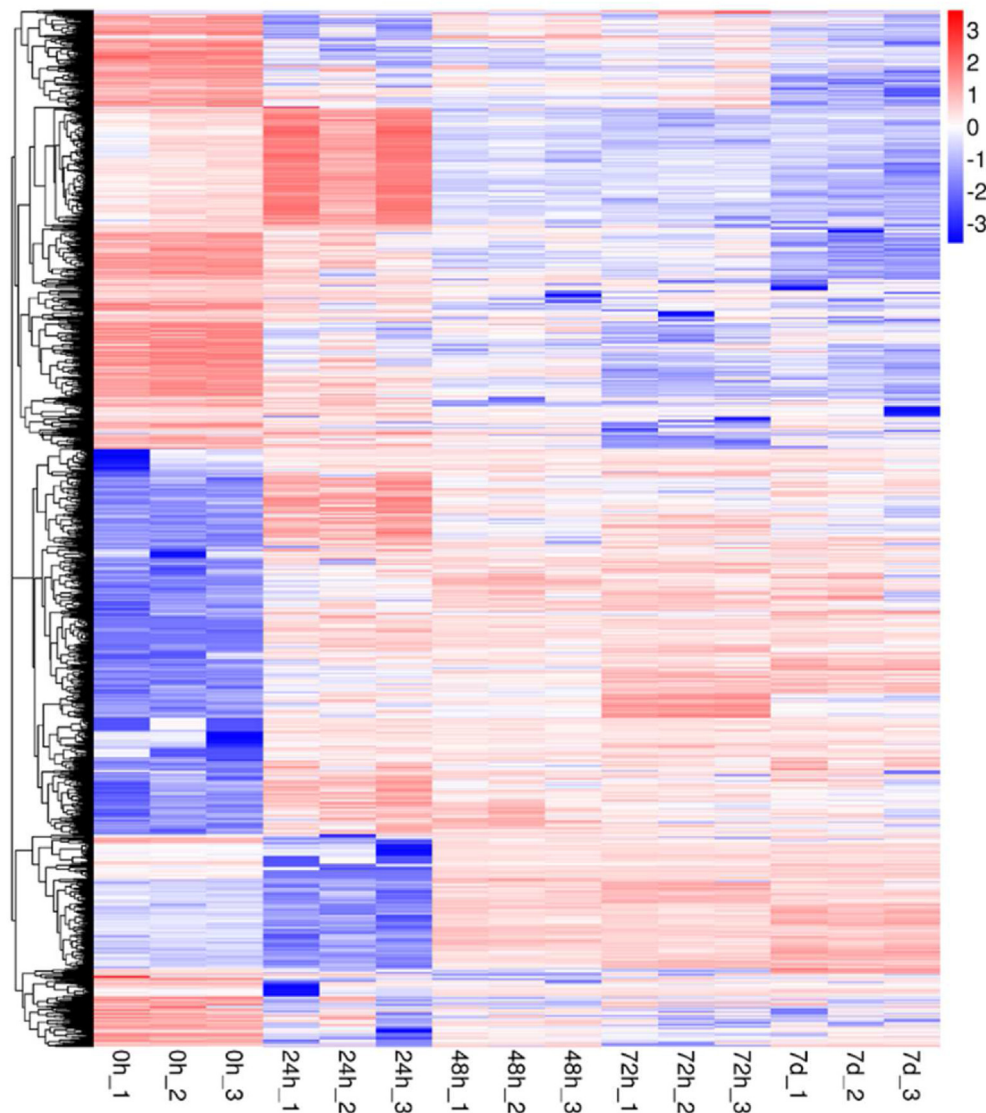
Figure 4. Heatmap of 1653 differentially expressed genes shared in each group at 0h, 24h, 48h, 72h and 7d time points.

3.5. Energy metabolism involved in wheat response to Pst

The *Pst*-induced DEGs involved in energy metabolism were examined in this study, such as photosynthesis and carbon fixation. Figure 8 shows the pathways of photosynthesis, carbon fixation, and DEG enrichment in those pathways at 0hpi, 24hpi, 48hpi, 72hpi, and 7dpi in CY12 (the early and late responses of wheat to Pst). Compared with non-infection control and the early stage, all enrichment DEGs were down-regulated in photosynthesis (KO00195, KO00196) and carbon fixation photosynthetic organisms (KO00710) after the late stage of responses to *Pst* inoculation (Figure 8C, D, E), including DEGs of F-type ATPase, cytochrome b6/f/ complex, photosynthetic electron transport, and light-harvesting chlorophyll protein complex (LHC) in photosystem I and photosystem II (Figure 8A, B). Therefore, we suggest that the photosynthesis-related pathway and the *Pst*-induced the expression pattern of those DEGs changes to play a necessary role in wheat for defense response to *Pst*-CYR34 infection.

3.6. DEGs in plant hormone signaling pathway

KEGG enrichment analysis exhibited the *Pst* response of plant hormone signaling interaction in CY12. Compared with control (0h and 24h), the DEGs enrichment in plant hormone signaling transduction (KO

04075) was almost down-regulated in CY12 after *Pst* infection at 72h and 7d. The down-regulated DEGs were mainly in cysteine and methionine metabolism, α-Linolenic acid metabolism and phenylalanine metabolism, which were related to ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) signaling pathways. Then the heatmap and detailed flow of DEGs in the plant hormone signaling pathway are shown in Figure 9. Wheat PST resistance was mediated by ethylene (ET), jasmonic acid (JA), and salicylic acid (SA). The down-regulated genes are eventually related to regulating disease resistance, senescence, and stress response of CY12. This result indicates that these plant hormones were the key factors in the plants' response to defense after *Pst* inoculation. Otherwise, the plant will lose resistance and the pathogen will successfully invade.

3.7. DEGs involved in phenylalanine metabolism

In this study, the phenylalanine pathway was also found important for wheat's *Pst* response. Those DEGs were also down-regulated in CY12 at 24hpi, 48hpi, 72hpi, and 7dpi (Figure 10A). Although there were some cases of up-regulation or non-significant down-regulation for some DEGs at different time points, most of the genes in this pathway were down-regulated in CY12 within *Pst* infection compared with the non-inoculated control.
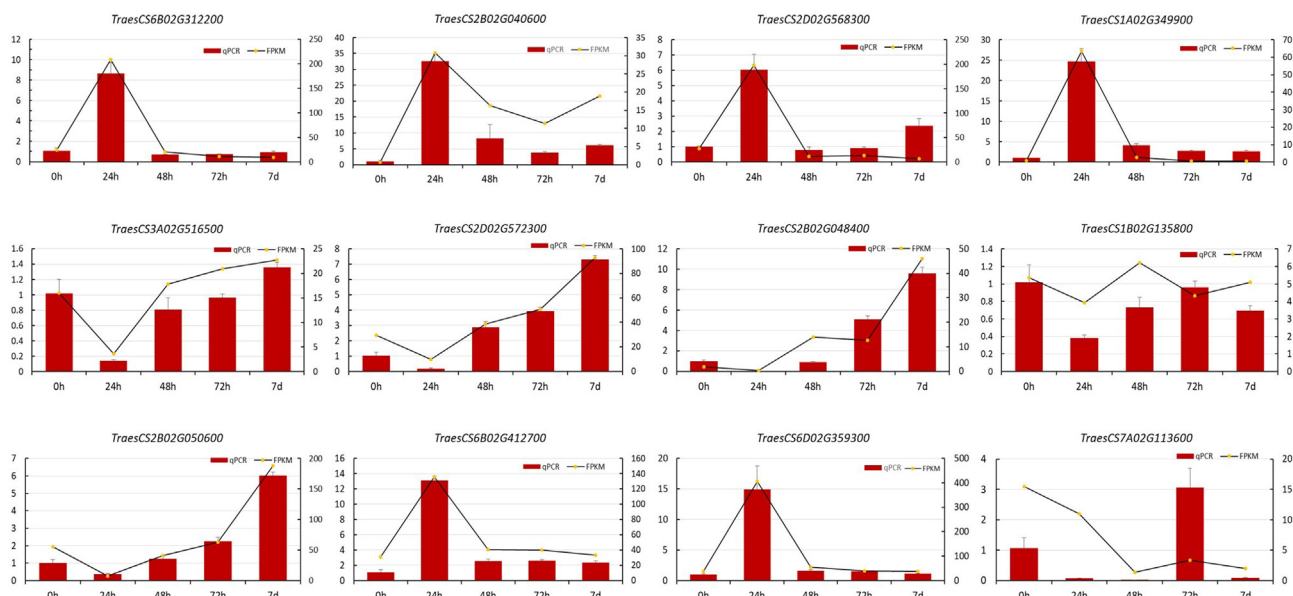
Figure 5. Expression patterns between qRT-PCR and RNA-seq for the 12 genes. The heights of the columns and points stand for the log2 (fold change) computed from both qRT-PCR and RNA-seq profile.
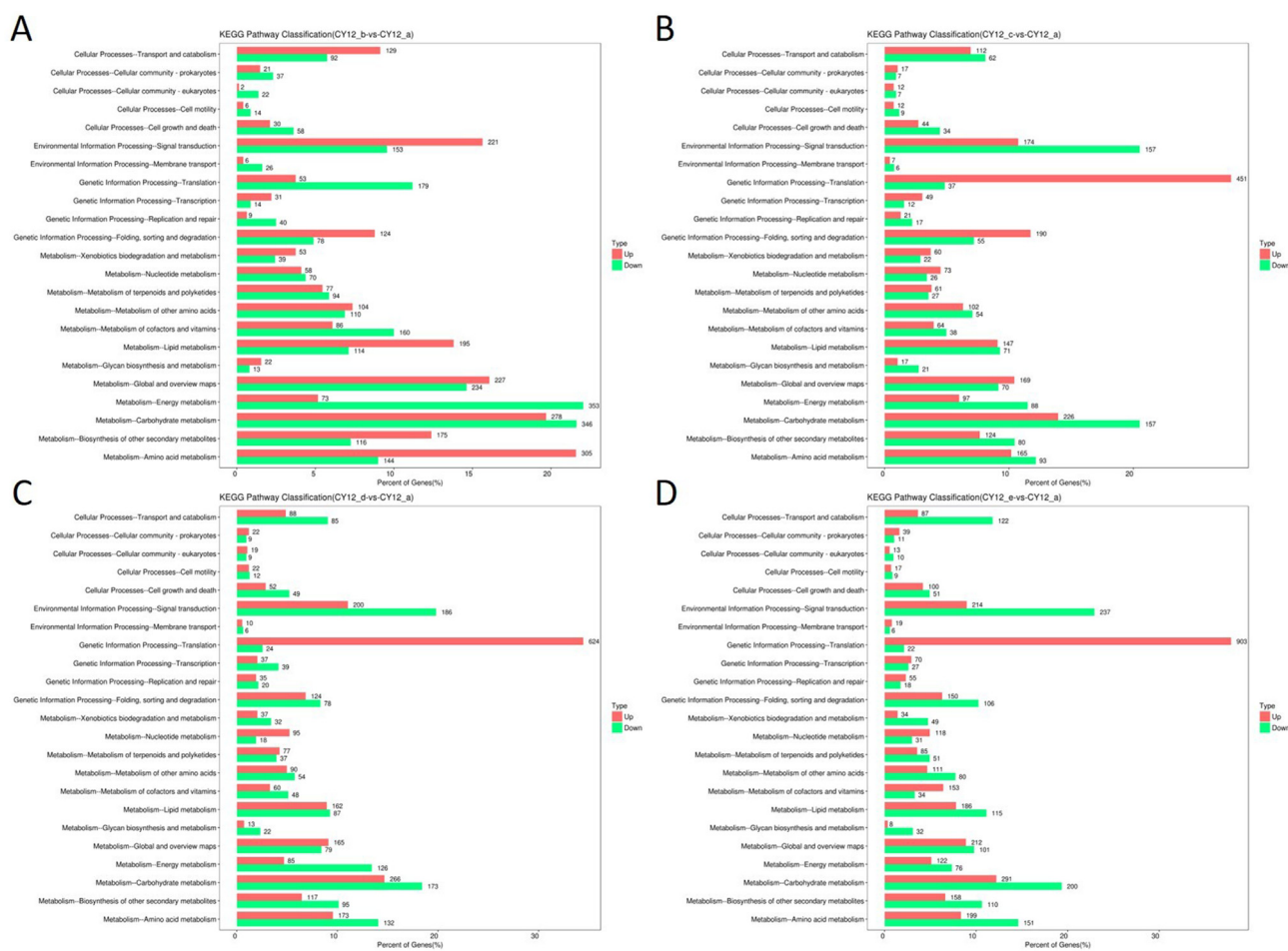

Figure 6. Gene ontology (GO) functional classifications of differentially expressed genes (DEGs) in CY12 at 24hpi (A), 48hpi (B), 72hpi (C) and 7dpi (D).

The enrichment and trend flow of DEGs in the phenylalanine metabolism pathway are shown in Figure 10B. Phenylpropanoid is synthesized first from phenylalanine and then through a series of steps

catalyzed by four different enzymes: ammonia-lyase (PAL), hydroxycinnamoyl transferase (HCT), cinnamoyl alcohol dehydrogenase (CAD), and E1.11.1.7. Finally, the pathway produces monolignols with three
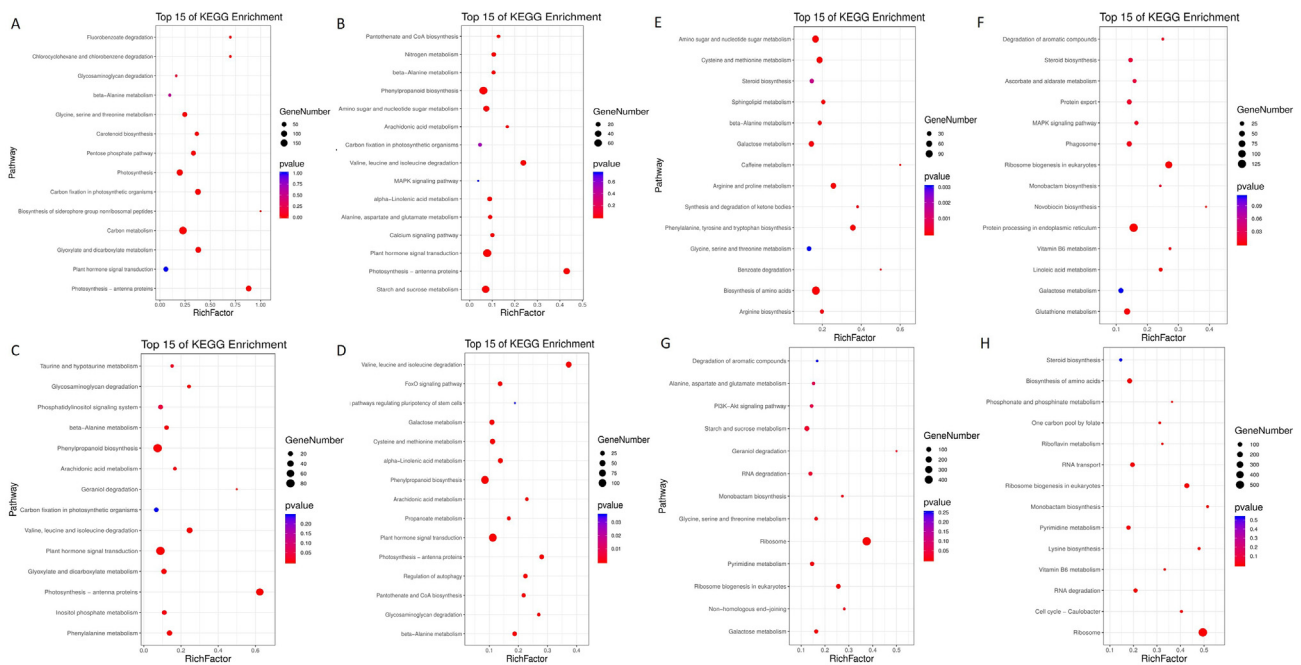
Figure 7. KEGG analysis of DEGs in CY12 at all time points. A-D are down-regulated DEGs enrichment in top 15 KEGG enrichment pathways at 24h, 48h, 72h and 7d. E-H are up-regulated DEGs enrichment top 15 KEGG enrichment pathways at 24h (E), 48h (F), 72h (G) and 7d (H). A and E: 0h vs 24h, B and F: 0h vs 48h, C and G: 0h vs 72h, D and H: 0h vs 7d.

types that polymerize to form lignin, including guaiacyl (G), p-hydroxyphenyl (H), and syringyl (S) lignin. Another branch of this pathway was the synthesis of flavonoids and anthocyanins catalyzed by cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), chalcone isomerase (CHI) and chalcone synthase (CHS). These results show that *Pst*-CYR34 inoculation decreased lignin and flavonoids biosynthesis in CY12. Therefore, the study indicated that lignin and flavonoids played a vital role in response defenses and enhancing the resistance of wheat.

4. Discussion

To meet the needs of future world food security, the widespread occurrence of plant diseases has been effectively controlled, which makes wheat production face greater challenges (Sharma et al., 2018b). Stripe rust (Pst) is the greatly destructive disease in wheat that causes severe yield losses in the field (Wan et al., 2004). However, the information on wheat defense regulatory networks is still limited, such as *Pst*-induced activation and downregulation of DEGs to fine-tune wheat plant defense responses remains not clear. Due to the rapid evolution of *Pst* virulence races, wheat cultivars with race-specific resistance generally develop susceptibility within a few years (Line et al., 1995). Therefore, it is essential to understand the susceptibility mechanism of wheat by Pst infection for developing new strategies to promote wheat disease resistance breeding.

Plants have a complex regulatory network regulation growth and defense responses to various biotic and abiotic stresses. Phytohormones such as gibberellins, auxin, cytokinins, jasmonic acid (JA), ethylene (ET), brassinosteroids (BRs), salicylic acid (SA), abscisic acid (ABA), and strigolactone play essential roles throughout whole plant life (Chow et al., 2006). During defense, plant hormone-mediated signal transduction is critically important to increase plant resistance (Hussain et al., 2018; Verma et al., 2016). Compounds such as JA, SA, ET, ABA, and gibberellin can provide a wide range of biochemical resistance and reduce pathogen growth in plants (Robert-Seilaniantz et al., 2011). JA and ET have been shown to play a role in plant development as well as stress responses such as herbivore attack and pathogen infection (Wasternack et al., 2007; Wu

et al., 2009; Browse, 2009; Ozimek et al., 2018). In the present results, we found that ethylene and jasmonic acid were involved in the *Pst* response of wheat. And all of the DEGs were down-regulated in those pathways, which eventually involved regulating the senescence and stress response of wheat to defend against the CYR34 infection.

The small plant signaling hormone molecule salicylic acid (SA) is essential to alleviate biotic (pathogens and insects) and abiotic stresses in plants (Wang et al., 2010; Pregelj et al., 2010), which is required for both systemic acquired resistance and local defense by microbial pathogen infections (Loake et al., 2007; Vlot et al., 2008, 2009). Higher levels of SA are associated with higher disease resistance in the plant. Otherwise, plants are easily susceptible to virulent pathogens due to its failed to accumulate SA (Dempsey et al., 1999). Certain studies have reported that spot blotch resistant wheat particularly causes changes in content of SA after pathogen infection, thereby reprogramming the expression of each defense-related genes and finally conferring resistance (Sahu et al., 2016). However, susceptible wheat did not display a similar response, suggesting that salicylic acid accumulation is an important event in the regulation of wheat spot resistance. Similar to the previous research, we also found that the entire DEGs were down-regulated in the SA signal pathway with *Pst*-CYR34 infection in susceptible wheat cv. CY12. The SA induced by pathogenic bacteria is involved in the production of antimicrobial disease-related proteins, which can promote the immunity of plants to microbial pathogens by binding with antimicrobial proteins (Wang et al., 2005). According to the results from the RNA-seq profile, we suggested that the mechanism of wheat susceptible to CYR34 may be caused by ET, JA, and SA pathway suppression. They may confer resistance in wheat as a signaling molecule.

Photosynthesis and carbon metabolism dominate plant energy metabolism, and more than 90% of grain yield is produced by photosynthesis (Makino et al., 2011). Photosynthetic activities are influenced by numerous abiotic and biotic stresses, which include response to rust infection (Major et al., 2010). However, so far, masses of studies about wheat photosynthesis under environmental stress have focused on abiotic stresses (Yang et al., 2008). In contrast, only a few literatures have been illustrated on the relationship between biotic stresses and plant
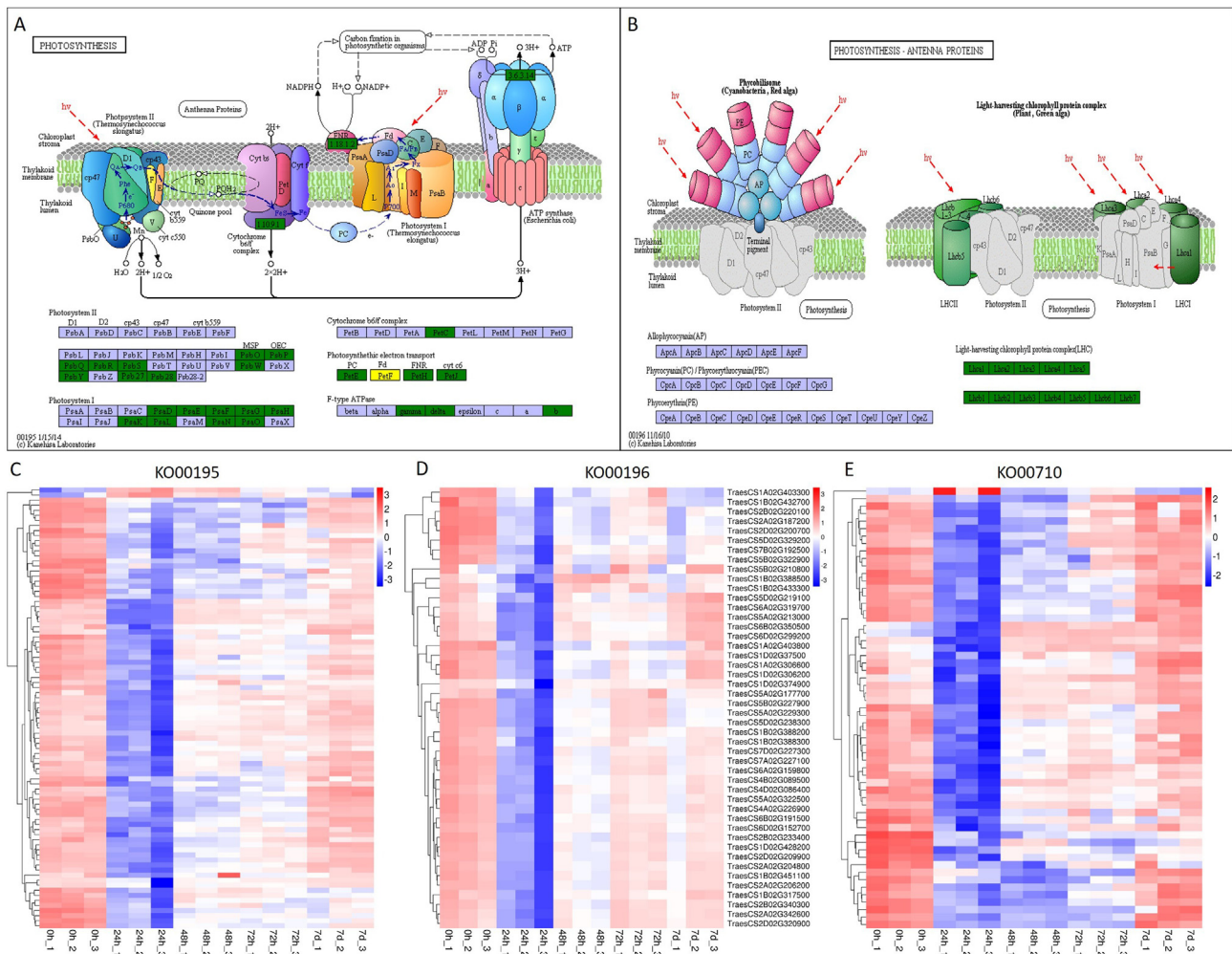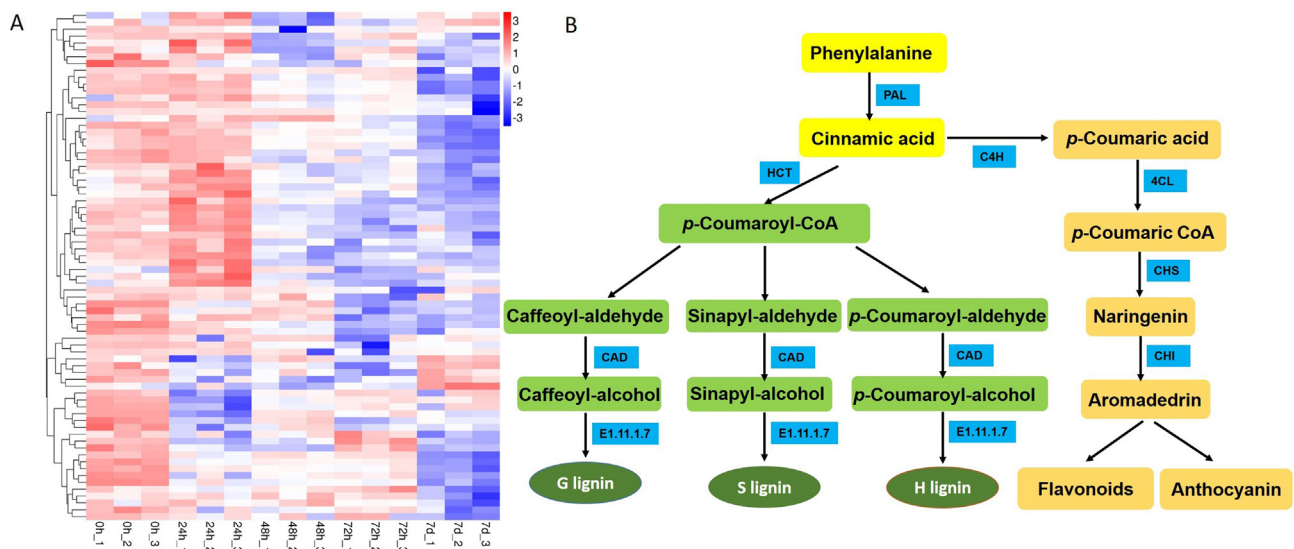
Figure 8. The DEGs enrichment in photosynthesis at 24hpi (A), photosynthesis-antenna proteins at 24hpi (B) and carbon fixation photosynthetic organisms; The heatmap of expression DEGs enrichment in KO00195 (C), KO00196 (D) and KO00710 (E) at 0h, 24h, 48h, 72h and 7d (B) after *Pst-CYR34* inoculation, the expression levels estimated using log2 (fold change) for each transcript. In figure A and figure B, genes marked with green was down-regulated in CY12.
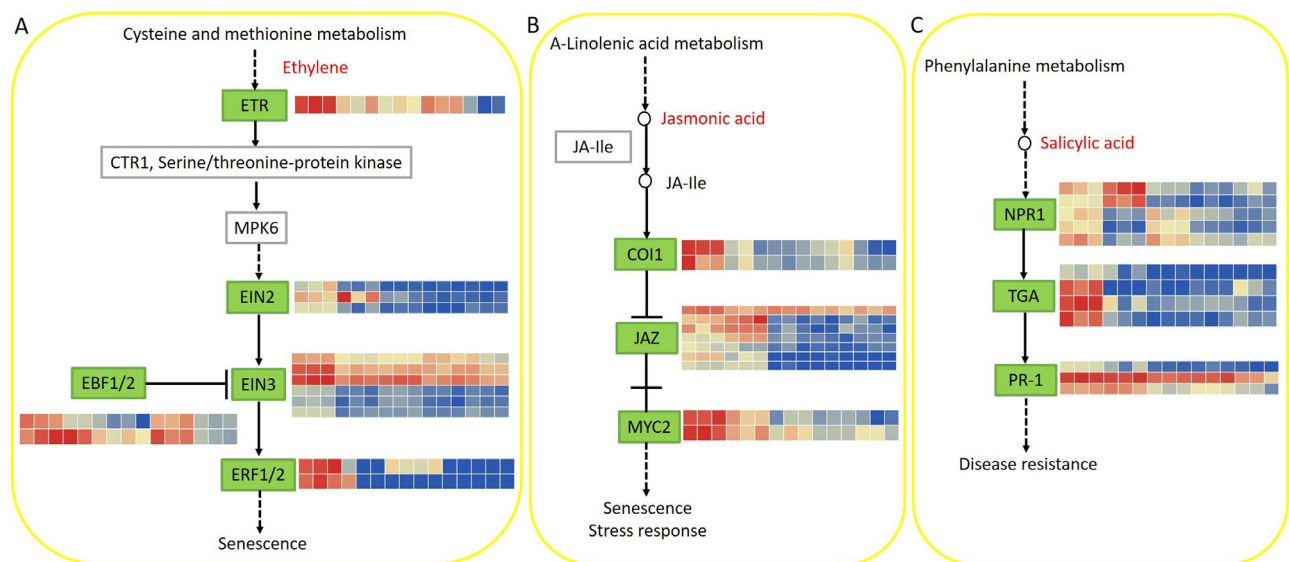
Figure 9. The DEGs expression enriched in ethylene (A), jasmonic acid (JA) (B) and salicylic acid (SA) (C) pathway, expression levels are indicated by the heatmap at 0h, 24h, 48h, 72h and 7d after CYR34 infection, estimated using log2 (fold change) for each transcript. Genes marked with green was down-regulated in CY12.

Figure 10. The heatmap of expression DEGs enrichment in phenylalanine pathway at 0h, 24h, 48h, 72h and 7d in CY12 after *Pst-CYR34* inoculation (A), and overview of specially DEGs enriched in phenylalanine pathway (B).

photosynthesis. Although previous studies have suggested that harmful pathogen infection may lead to changes in plant photosynthesis (Wang et al., 2000), the specific effects of wheat stripe rust on PS I and PS II are still not known. Therefore, in order to better understand the response mechanism of wheat stripe rust, the DEGs enrichment in photosynthetic and carbon fixation pathways in susceptible wheat cultivars were investigated after infected by new stripe rust races (CYR34). A previous study revealed that *Pst* infection would lead to a reduction in photosynthesis associated protein (D1) in susceptible wheat (Shen et al., 2008). The drastic changes in photosynthesis functions were amplified in pre-symptom wheat leaves through the decrease in gas exchange and chlorophyll fluorescence, and the inhibition of photosynthetic related gene expression during symptom development, which can trigger defense against mechanisms in response to the leaf stripe rust (Magnin-Robert et al., 2011). These findings were consistent with our results. In the current study, the DEGs of photosynthesis and carbon fixation pathways were entirely down-regulated in the susceptible wheat (CY12) after being inoculated with *Pst*-CYR34. The DEGs include F-type ATPase, cytochrome b6/f/complex, photosynthetic electron transport, photosystem I and photosystem II. These results indicate that DEGs in photosynthesis and carbon metabolism may participate in the regulation of wheat's resistance to stripe rust.

Phenylpropanoid metabolism pathway is the most crucial secondary metabolic pathway, which involved in plant defense against biotic and abiotic stresses (La et al., 2004). The main branches of the phenylpropanoid pathway are directed toward the production of lignin and flavonoids. Certain research suggests that lignin, flavonoids, isoflavones, and phenolic compounds play an crucial role in plant defense responses against harmful pathogens (Harborne, 1999; Pregelj et al., 2010). They accumulate in the plant and can enhance the plant's resistance. However, there are rarely studies on the universal analysis of the expression mode changes of those gene on transcriptome level in wheat. In this study, we found down-regulated DEGs in phenylalanine pathway in CY12 with *Pst* infection, which led to lignin and flavonoids' biosynthesis decreasing in CY12. Lignin can provide mechanical strength and reinforce cell walls to provide a physical barrier to limit pathogen infection (Bonello and Blodgett, 2003). And also preventing the transfer nutrients and water from the plant to the pathogen to restrict the pathogen developing and growth (Hu et al., 2018). Therefore, the plant will lose resistance and the pathogen will successfully invade because of decreased lignin and flavonoid biosynthesis.

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