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# Transcriptome analysis reveals various genes involved in the regulation of potato to late blight

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## Abstract

**Background** Potato (*Solanum tuberosum* L.) production is seriously threatened by the oomycete *Phytophthora infestans* (*P. infestans*). However, it remains unclear how the two potato cultivars, Q9 (moderately resistant) and Atl (susceptible), initiate distinct defense responses after inoculation with *P. infestans*. To uncover the underlying process, we investigated the dynamic gene expression profiles and the functions of the differentially expressed genes (DEGs).

**Results** The results showed that the numbers of up-regulated DEGs were 1345, 1063 and 1694 in Q9 and 272, 603 and 2554 in Atl at 1 day post inoculation (dpi), 3 dpi and 5 dpi, respectively. Meanwhile, the down-regulated DEGs were 1557, 1417 and 389 in Q9 and 114, 364 and 2528 in Atl simultaneously. KEGG enrichment analysis revealed that plant-pathogen interaction, phenylpropanoid biosynthesis and MAPK signaling pathway were significantly enriched in Q9 at the later stage. Furthermore, DEGs associated with plant-pathogen interaction and phenylpropanoid biosynthesis showed higher expression levels in Q9 compared to Atl, especially at the later stage. The expressions of eight DEGs were validated by quantitative real-time PCR (qRT-PCR), which further verified the accuracy of the transcriptomics analysis. In addition, DEGs that were exclusively up-regulated and down-regulated in Q9 were analyzed, which may contribute to the resistance of Q9. Transient expression analysis revealed that four DEGs, including *StHP1*, *StMYB2*, *StHSP3* and *StNAC5*, exhibited increased tolerance to *P. infestans*, indicating a positive role in enhancing the resistance of Q9.

**Conclusion** Therefore, the DEGs associated with plant-pathogen interaction, phenylpropanoid biosynthesis and MAPK signaling pathway were involved in regulating late blight, and the expression level of the related genes significantly increased in Q9. In addition, *StHP1*, *StHSP3* and the transcription factors, including *StMYB2* and *StNAC5*, played positive roles in improving the resistance of potato Q9 to *P. infestans*. These findings provide useful information for further understanding the molecular mechanism of potato resistance to late blight. Furthermore, our study identified novel resistance genes that can be used in resistance breeding and functional research.

**Keywords** Potato, Transcriptome, Late blight, Plant-pathogen interaction, Phenylpropanoid biosynthesis, Tolerance; susceptibility

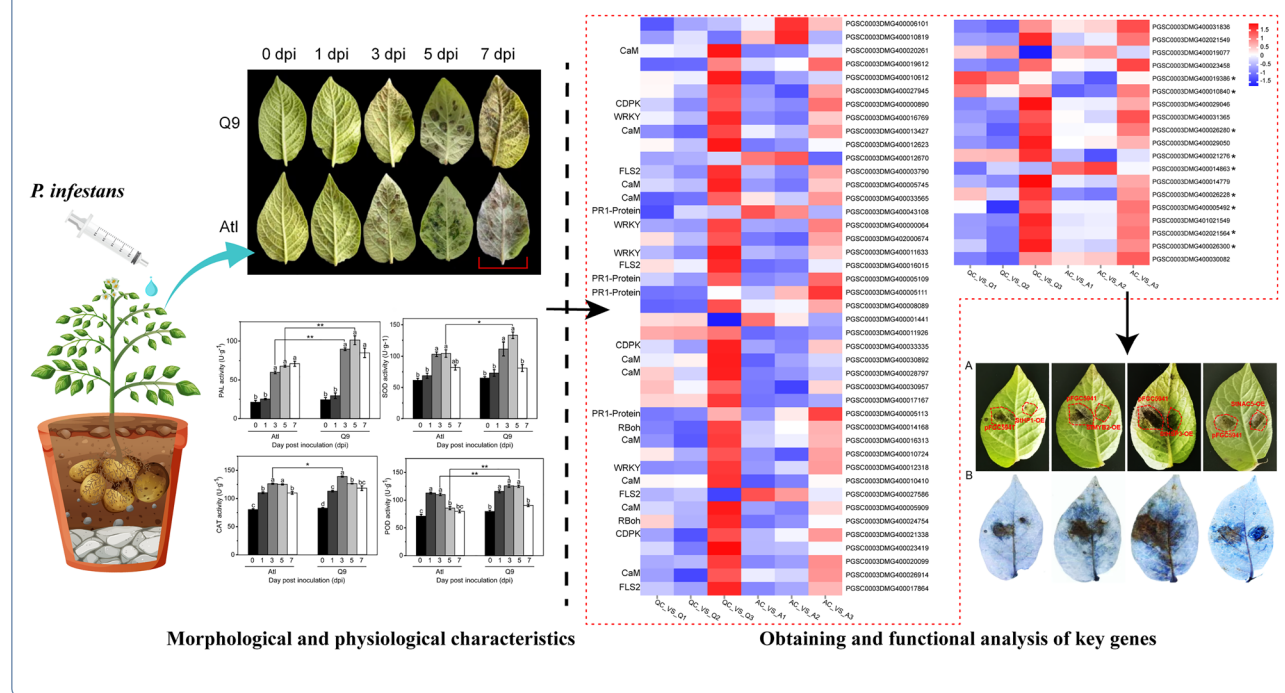
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## Graphical abstract



## Introduction

The late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary (*P. infestans*), is one of the most destructive diseases in potato production worldwide [1]. Extensive efforts have been made to prevent and control the disease for a long time. To effectively control the occurrence of the disease in the field, various practices have been implemented, such as promoting disease-resistant seeds, optimizing cultivation models, and screening chemical fungicides [2–4]. Nevertheless, along with developing high-quality agricultural products, it is crucial to develop green, safe and efficient prevention and control strategies [5]. Resistant breeding is a durable, environmentally friendly, and convenient practice that is widely feasible, but it has been hindered by a lengthy breeding cycle and low selection efficiency. To date, it is crucial to accelerate the molecular breeding process by rapidly anchoring the critical genes related to resistance regulation and systematically elucidating the molecular mechanisms.

When plants are subjected to biotic or abiotic stresses, several antioxidant enzymes play central roles in stress tolerance. Previous studies have shown that an increase in the level of peroxidase (POD) can enhance plant tolerance to cadmium (Cd) and zinc (Zn) stress [6] and alleviate the oxidative damage induced by aluminum (Al) stress [7]. When tomatoes are infected with the

necrotrophic fungus *Botrytis cinerea*, catalase (CAT) activity increases during the initial stages of infection. However, there is a significant decline in the later stages [8]. In soybean, superoxide dismutase (SOD) activity was significantly increased when the seedlings were infected with *Rhizoctonia solani* [9].

Moreover, to defend themselves against pathogenic microorganisms, plants have evolved complex and organized immune responses that mainly consist of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) [10–12]. After infection, membrane-localized pattern recognition receptors (PRRs) can recognize pathogen-associated molecular patterns (PAMPs) and activate PTI. PRRs are primarily composed of receptor-like kinases (RLK) or receptor-like proteins (RLP) [11]. To facilitate infection, pathogens can secrete effectors that can be recognized by resistant (R) genes and then trigger ETI [12]. Apart from several RLPs, most identified R genes belong to the Nucleotide Binding Site-Leucine-Rich Repeat (NBS-LRR) superfamily [13]. In the standard case, PTI is controlled by micro-effect multi-genes with quantitative characteristics, a broad-spectrum and durable resistance [14]. On the contrary, ETI is triggered by a single gene and displays species- or race-specific immunity. Recent investigations have suggested that PTI and ETI are involved in mediating plant immunity. For instance, several FLS2/EFR/CERK1 and

BAK1/BKK1/CERK1 complexes are required for NLR-mediated immunity in *Arabidopsis* [15]. Initiation of PTI and ETI further transfers the local signals to global defense responses, known as systemic acquired resistance (SAR) [16]. Salicylic acid (SA) is a critical regulator in the model SAR systems. The SA level remains low but rises after corresponding biotic or abiotic stimulus [17]. Methyl salicylate (MeSA) can also be used as a plant SAR signal [18]. The tobacco plants can produce and volatilize gaseous MeSA when infected with TMV. Then, MeSA acts as an airborne signal between uninvaded leaves or plants to activate defense responses. MeSA can be transported to the leaves to convert into SA, thus inducing SAR [19]. Núñez-Pastrana et al. (2011) reported that ethylene (ET) could induce resistance responses against *Phytophthora capsici* in peppers [20]. In potato SD20, a tetraploid potato with a high disease-resistant genotype, multi-signaling pathways are involved in resistance and defense against *P. infestans*, including SA, JA, and ET signaling pathways [21, 22].

The crucial roles of PRRs and related mechanisms in potatoes against *P. infestans* infection are elusive. Although several PRRs, such as RLK (StLRPK1) and RLP (ELR), which confer late blight resistance in potato plants, had been confirmed [23, 24]. Additionally, numerous resistance genes (*R1*, *R2*, *R3a*, *R3b*, *R8*, *R9a*, *Rpi-blb1*, *Rpi-blb2*, *Rpi-blb3*, *Rpi-abpt* and *Rpi-bt1*) have been identified and isolated from the wild species *Solanum demissum* (*S. demissum*) and *S. bulbocastanum* [25, 26]. However, rapidly evolving pathogens can easily surmount all the above R genes that contribute to the race-specific resistance of potatoes [27]. At present, it is urgent to find more significant genes that provide durable resistance to potatoes.

Several investigations of ‘Omics’ have supplied more information on the dynamic molecular network of potato responses to *P. infestans* infection. Many putative hypersensitive and effector-target proteins are identified by combining quantitative proteomics with transcriptomics [28]. Other transcriptomic analyses have suggested the crucial role of hypersensitive response, nitrate reductase, photosynthesis, jasmonate and mevalonate pathway and numerous related genes for late blight resistance of potatoes [29–32]. Furthermore, multiple R genes have been successfully cloned using a combination of R gene sequence capture (RenSeq) and single-molecule real-time (SMRT) [33]. However, we still have a long way to go in identifying vital resistant genes and understanding the related mechanisms.

‘Qingshu 9’ (Q9) is widely cultivated in China because of its excellent traits such as adaptability, high yield and superior quality [34]. Importantly, this cultivar exhibited high tolerance to *P. infestans* infection in the field.

Although He et al. (2020) evaluated the high tolerance of Q9 to late blight and screened out some resistance genes [35], the specific mechanism remains unclear. In the present study, to understand global gene expression patterns and identify resistant genes, we compared the transcriptomics of Q9 and Atl when infected with *P. infestans*. The results showed that several pathways were involved in the responses of *P. infestans* signals, including phenylalanine metabolism and plant-pathogen interactions. Strikingly, based on functional investigations, four genes were determined as critical regulators for late blight resistance. Our results provided new insight into the resistant mechanism of potatoes against *P. infestans* infection and supplied candidate genes for further resistance breeding.

## Materials and methods

### Plant materials and pathogen

Qingshu No.9 (Q9, moderately resistant) and Atlantic (Atl, susceptible) were produced in our laboratory (Gansu Agricultural University, Lanzhou, China). The second or third compound leaf was used for the experiments when the plants were grown for two months. Purified *P. infestans* strain 88069 was kindly gifted by Prof. Govers Francine (Wageningen University) and maintained on rye Suc agar medium at 18 °C in darkness [36]. *Phytophthora* zoospores were isolated, and detached leaf assays were performed as described by Wang et al. (2023) [37].

### Determination of enzyme activities

The leaves inoculated with *P. infestans* (6 leaves per treatment) were collected at 0, 1, 3, 5 and 7 days post-inoculation (dpi), respectively. Then, the samples were kept in liquid nitrogen during sampling and stored at −80 °C for further analysis. The activity of antioxidant enzymes was determined by referring to our previous method [38, 39].

### RNA extraction, cDNA preparation and RNA-sequencing

20 µL of the *Phytophthora* zoospores suspension above prepared ( $7 \times 10^4$  spores/ml) was inoculated onto the lower surface of the second or third compound leaves by an injection syringe. Subsequently, the inoculated leaves were kept in the dark, at 100% humidity for different times (0, 1, 3 and 5 dpi, denoted as QC, Q1, Q2, Q3 and AC, A1, A2 and A3, respectively) at 18 °C. In the detected time, the leaves were harvested, immediately frozen with liquid nitrogen and placed at −80 °C.

According to the manufacturer's instructions, total RNA was extracted from Q9 and Atl leaves using the SteadyPure Plant RNA Extraction kit (Accurate Biotechnology, Hunan, China). Extracted RNA was quantified and qualified using a NanoDrop2000 Spectrophotometer (Thermo Scientific, Waltham, United States) and

an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California), respectively. Poly (A) mRNA was purified using oligo (dT) magnetic beads and then digested into short fragments (approximately 200 bp). The RNA-Seq library preparation method was performed according to our previous method [40]. The amplified fragments were sequenced while using an Illumina HiSeq™ 2000. The produced reads were paired-end 2×150 bp. All the data about the present study has been included in the tables/figures of the manuscript. The raw RNA sequencing (RNA-Seq) data will be deposited in NCBI's Sequence Read Archive (SRA) database until the manuscript is accepted.

### Analysis of sequencing data

RNA-Seq bioinformatics analysis was used to analyze the transcriptome raw sequences. The original images were converted into sequence data by base calling to provide the raw reads. Subsequently, dirty reads were removed before data analysis to acquire the clean reads. The clean reads were mapped to the potato reference genome (*Solanum tuberosum* group *Phureja* DM1-3) PGSC v4.03 [41]. Gene expression levels were quantified using the Expectation Maximization (RSEM) software package [42]. Gene identification (gene ID), length, log2 ratio, and false discovery rate (FDR) were obtained. An FDR value  $\leq 0.01$  and an absolute value of log2 ratio  $\geq 2$  were used as thresholds to judge the significant differences in gene expression. The differently expressed genes (DEGs) at different time points were screened with the Noiseq software package [43]. The most enriched 10 'Bins' with a level equal to '2' from each treatment were extracted. Heatmaps were constructed using a Multiple Array Viewer software MeV 4.9.0 (<http://www.tm4.org/mev.html>). The GO classification of DEGs and the distribution analysis of gene function in species at the macro level was conducted using WEGO software [44], and pathway enrichment analysis was obtained from KEGG [45]. GO categories with a level equal to "5" and "FDR<0.05" in each sample were screened, while the ten most enriched pathways were selected for further investigation.

### Quantitative real-time PCR validation

To verify RNA-seq results, qRT-PCR was performed for eight randomly selected DEGs. The samples used for RNA-seq and qRT-PCR analysis were the same ones. Primer design was performed using the online software program Primer 3 (<http://primer3.ut.ee/>). The primer sequences are listed in Supplementary file 1. The qRT-PCR protocol used is the same as our previous study [40]. Relative gene expression was calculated by the  $2^{-\Delta\Delta C_t}$  method as described by Livak and Schmittgen (2013) [46]. Data from qRT-PCR analysis were expressed as

means  $\pm$  SD of three independent biological replicates. The significance of differences between means was statistically analyzed using a *t*-test ( $P < 0.05$ ).

### Potato transient expression assays

The full-length CDS of *StHPI1*, *StMYB2*, *StHSP3* and *StNAC5* were isolated and then inserted into the pFGC5941 vector under the control of the CaMV 35S promoter. The sequencing-confirmed vectors were transformed into *Agrobacterium tumefaciens* strain GV3101 according to Yao et al. (2017) [47]. After overnight culture, bacterial pellets were precipitated, washed, and resuspended in infiltration buffer (10 mM MgCl<sub>2</sub>, 10 mM MES, pH 5.6, 200 mM acetosyringone), with absorbance (OD 600 nm) at 0.2. Approximately, 10  $\mu$ l of the above agrobacterium mixture was injected into *Atl* potato leaves. 3 d later, 20  $\mu$ l spore suspension ( $7 \times 10^4$  spores/ml) was placed on the above leaves, then stored on the moist tissue and sealed in a box. The samples were inoculated for 5 d and then photographed. The primers used for cloning are listed in Supplementary File 1.

### Trypan blue staining

In plant science, trypan blue, an azo dye, has been used to visualize disease lesion areas and wounded leaves [48]. The detached potato leaves were placed in a Petri plate with trypan blue solution, boiled for 5 min, and placed overnight on a rotary shaker. On the second day, the solution was replaced with a destaining solution (chloral hydrate or ethanol) and placed on the shaker. Lastly, the leaves were replaced with 50% glycerol and then photographed.

### Statistical analysis

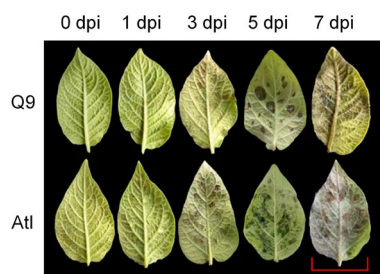
The enzyme activity and the expression of DEGs were counted using Microsoft Excel 2016 and analyzed using Origin 2018 software. Data presented were means of at least three biological replicates with error bars indicating standard deviation.  $P < 0.05$  was considered to be statistically significant.

## Results

### Changes of physiological indexes at different time points after *P. infestans* inoculation

Time-dependent experiments were conducted to investigate the change of leaves after *P. infestans* inoculation. The phenotypic observation revealed that the leaf spots became more severe than the control at 3 dpi. Noticeably, the *Atl* lesion was more pronounced than Q9. The mycelium attached to the leaf surface, and the leaf decay was serious at 7 dpi. These results indicated that the *Atl* leaves were more severely damaged by *P. infestans* infection (Fig. 1).



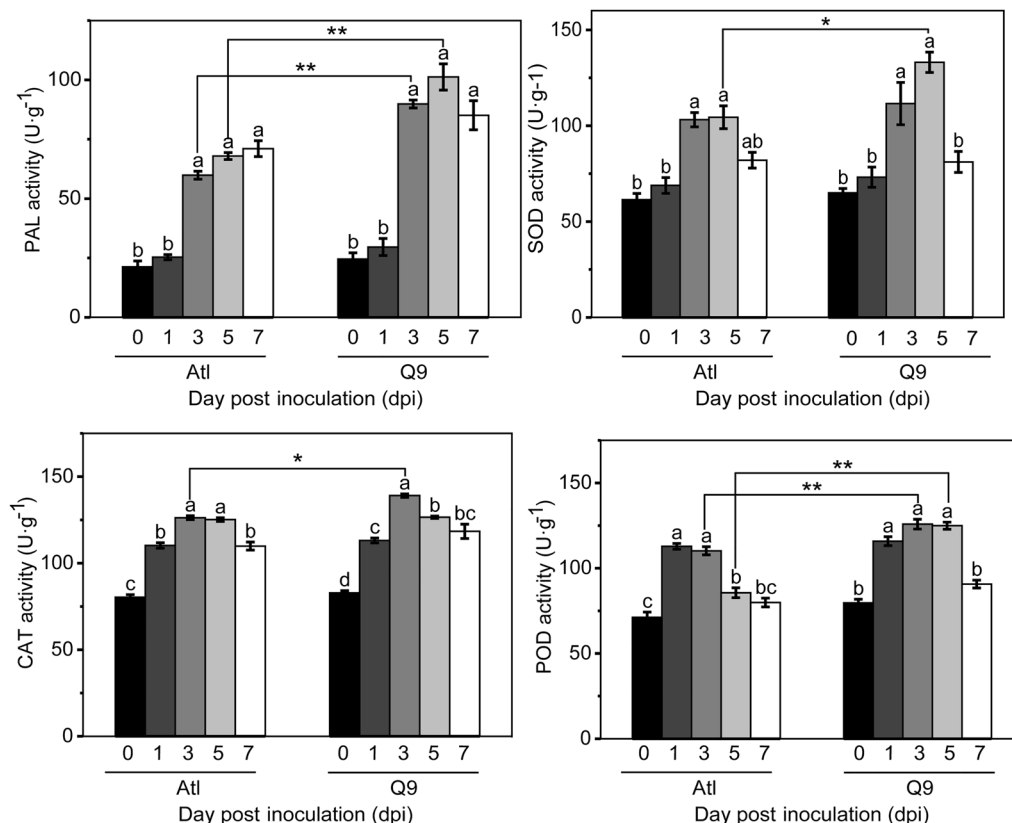


**Fig. 1** Lesion development were photographed at different time points (0, 1, 3, 5 and 7 dpi, respectively) in leaves (Q9 and Atl) after *P. infestans* inoculation. The experiments were repeated thrice with similar results. In the picture, the scale bar is 4 cm

### Changes in the activity of antioxidant enzymes at different time points

As the plant antioxidant, CAT, SOD, POD and PAL are essential parts of the plant antioxidant system. After *P. infestans* inoculation, the activity of antioxidant

enzymes in leaves of Q9 and Atl began to increase immediately. With the extension of infection time, they displayed the same pattern of change, showing the law of initially growing and then declining and reaching their maximum at 3 or 5 dpi. CAT activity increased by 57.23% and 67.86% at 3 dpi, and SOD activity increased by 67.91% and 76.61% at 5 dpi in Atl and Q9, respectively. It is worth noting that there were specific differences between Atl and Q9. For example, the activity of CAT and SOD in Q9 was generally higher than in Atl, with the most significant differences occurring at 3 dpi or 5 dpi, respectively ( $P < 0.01$ ) (Fig. 2). There were also significant differences in PAL and POD in Q9, reaching 1.50 and 1.49-fold, compared to Atl at 3 dpi and 5 dpi, respectively. The above results indicated that the response of the antioxidant system of the two cultivars was different after inoculation with *P. infestans*. Q9 may enhance the pathogen defense response by increasing the antioxidant accumulation and scavenging the ROS. Based on the above results, 0, 1, 3 and 5 dpi were selected for an in-depth analysis.

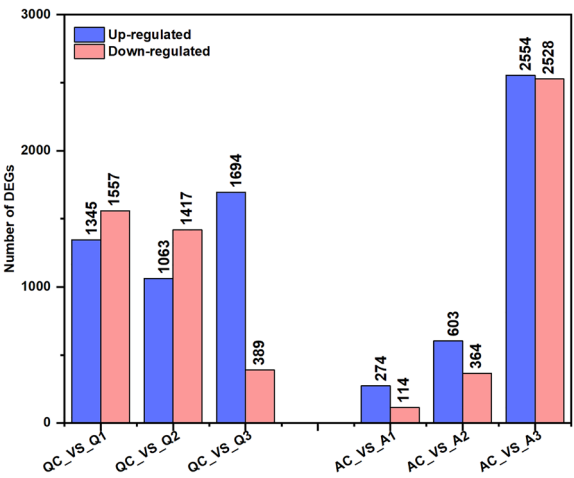


**Fig. 2** The antioxidant system of Q9 and Atl was affected when *P. infestans* was inoculated for different time points (0, 1, 3, 5 and 7 dpi). Values followed by different lowercase letters were significantly different at  $P \leq 0.05$ . Error bars represent the SD, and different letters indicate differences among treatments at the statistical (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ). Experiments were repeated thrice with a similar result

Transcriptome analyses of two potato cultivars in response to *P. infestans*

To explore the gene networks involved in different stages of infection with *P. infestans*, we collected leaf samples from 4 time points (0, 1, 3 and 5 dpi) for RNA-Seq. An average of 38.42–48.38 million and 32.77–42.80 million clean data were produced for Q9 and Atl after filtering, respectively (To explore the gene networks Table 1). Above 82% of clean reads of each sample were mapped to the potato reference genomes ([http://spudadb.uga.edu/pgsc\\_download.shtml](http://spudadb.uga.edu/pgsc_download.shtml)), suggesting a good RNA-seq quality of the transcriptome in the present study.

To analyze the different responses between the two cultivars, differentially expressed genes (DEGs) in response to *P. infestans* were determined. The up-regulated DEGs were 1345, 1063 and 1694 in Q9 and 272, 603 and 2554 in Atl, while the down-regulated DEGs were 1557, 1417 and 389 in Q9 and 114, 364 and 2528 in Atl, respectively (Fig. 3). Compared with the control, the up-regulated and down-regulated genes of Atl were nearly identical at 5 dpi (A3). Interestingly, the



**Fig. 3** The up-regulated and down-regulated DEGs between Q9 and Atl. Blue represents up-regulated DEGs, and pink represents down-regulated DEGs. QC, Q1, Q2 and Q3 denote the Q9 samples inoculated with *P. infestans* for 0, 1, 3 and 5 dpi, and AC, A1, A2 and A3 denote the Atl samples inoculated with *P. infestans* for the same time points, respectively

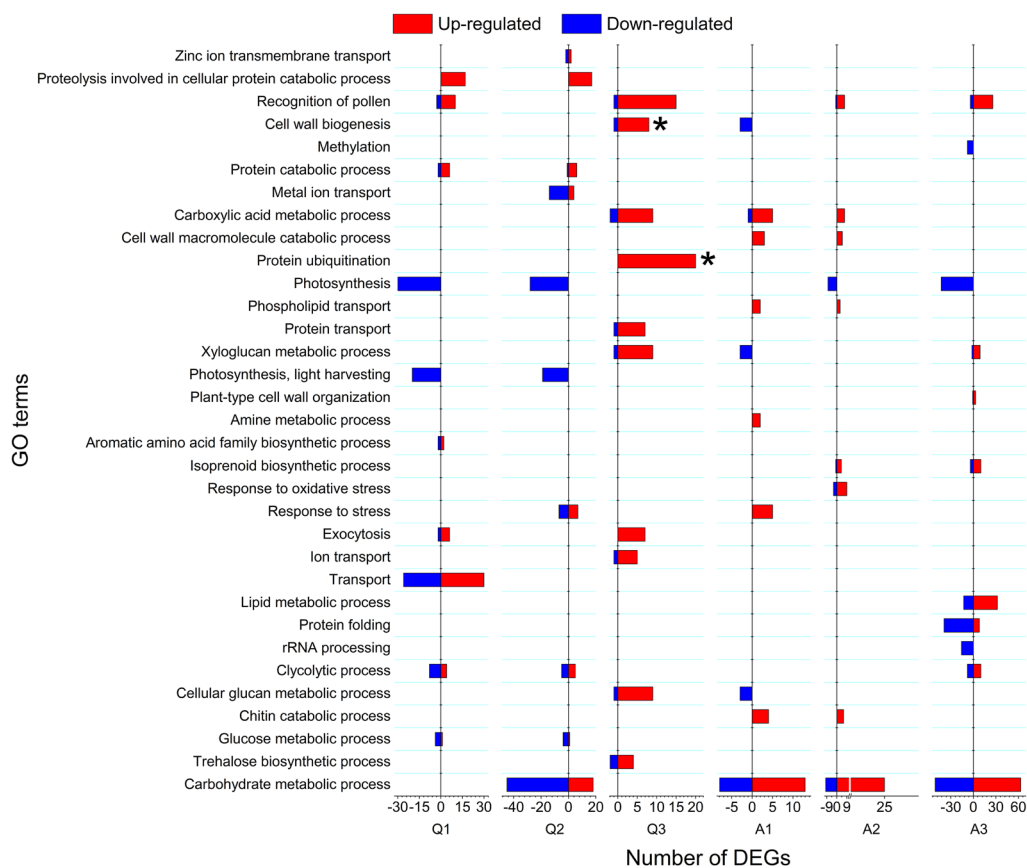
**Table 1** Sequencing statistics for the 24-transcriptome data of Atl and Q9 potato at four time points when infected with *P. infestans*

Sample	Clean Data	Clean Data Ratio (%)	Clean Data Q30 (%)	Mapped reads (%)	Uniquely mapped reads (%)
QC-1	54,952,282	96.16	93.23	82.92	71.63
QC-2	37,123,122	96.08	93.43	86.14	76.21
QC-3	46,037,702	96.13	93.33	84.54	73.92
Q1-1	37,800,336	96.69	93.59	88.78	75.89
Q1-2	43,299,476	96.69	93.95	90.49	72.98
Q1-3	40,549,906	96.69	93.76	89.64	74.46
Q2-1	36,029,832	96.08	93.84	87.97	78.57
Q2-2	38,688,838	96.9	93.71	89.92	80.91
Q2-3	40,543,594	96.83	93.41	90.4	81.39
Q3-1	49,063,786	95.6	93.12	89.69	24.24
Q3-2	47,625,928	96.45	93.8	91.17	42.75
Q3-3	48,463,774	96.43	93.38	69.51	32.18
AC-1	36,757,412	96.43	93.53	87.67	76.98
AC-2	45,366,794	96.21	93.18	86.18	76.84
AC-3	34,646,712	96.27	93.32	87.63	77.35
A1-1	38,829,654	95.5	93.29	85.49	71.46
A1-2	46,774,418	96.23	92.98	88.15	77.46
A1-3	42,802,036	95.87	93.15	86.82	74.46
A2-1	40,839,744	95.55	92.88	85.84	74.29
A2-2	41,672,806	96.18	93.81	88.59	77.8
A2-3	40,167,408	96.2	93.59	83.7	73.05
A3-1	34,750,778	95.92	93.72	79.52	67.49
A3-2	30,793,670	96.38	93.83	85.53	75.6
A3-3	32,772,224	96.15	93.78	82.55	71.57

down-regulated genes decreased in resistant cultivar Q9. In contrast, the up-regulated and down-regulated DEGs in Atl increased, and there were many more DEGs in Atl than in Q9 in the third period. The DEGs identified were further used for functional classification and annotation (Fig. 3).

GO enrichment of DEGs

To reveal the function of DEGs, the top 10 Biological Processes (BP) of GO terms in Q9 and Atl were performed. At the early stage, the overrepresented GO terms in Q9 (Q1) included “proteolysis involved in cellular protein catabolic process”, “recognition of pollen”, “protein catabolic process”, “photosynthesis”, “photosynthesis, light harvesting” and “transport”, while the high GO terms were “carboxylic acid metabolic process”, “response to stress”, “carbohydrate metabolic process” in Atl (A1) (Fig. 4). At the later stage, the categories “recognition of pollen”, “cell wall biogenesis”, “protein ubiquitination”, “Protein transport”, “Xyloglucan metabolic process”, “Exocytosis”, “Ion transport”, “cellular glucan metabolic process” were enriched in Q9 (Q3), whereas multiple categories such as “carbohydrate metabolic process”, “protein folding” “lipid metabolic process” and “photosynthesis” were significantly enriched in Atl (A3) (Fig. 4). The enriched terms “cell wall biogenesis” and “protein ubiquitination” in Q3 played a positive role in forming protective tissues, which were associated with disease resistance in plants.



**Fig. 4** Gene Ontology classification analysis of DEGs subjected to biological process. Q1, Q2 and Q3 mean the treatment of Q9 with *P. infestans* for 1, 3 and 5 dpi, and A1, A2 and A3 mean the treatment of Atl with *P. infestans* for 1, 3 and 5 dpi. The \* in the figure means that the genes in this annotation differed significantly between Q9 and Atl

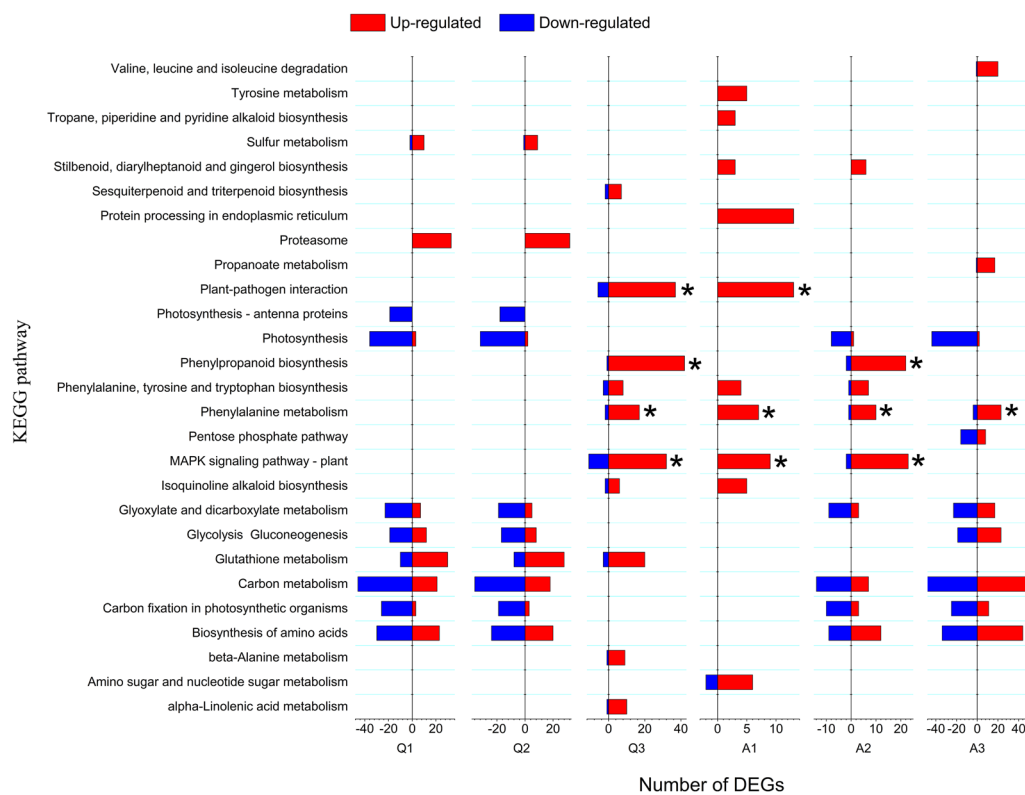
### Pathway enrichment analysis of DEGs

To reveal how the resistant and susceptible cultivars respond to *P. infestans* and to identify pathways in which DEGs might be involved, the 10 most enriched pathways of KEGG enrichment analysis were selected (Fig. 5). The expression of DEGs involved in Carbon metabolism, Carbon fixation in photosynthetic organisms, Biosynthesis of amino acids was significantly down-regulated at least two time points. The pathways relating to Plant-pathogen interaction, Phenylpropanoid biosynthesis and MAPK signaling pathway-plant were significantly upregulated and enriched in both Q9 and Atl. Moreover, the three signaling pathways were all significantly expressed at the third treatment time of the Q9 cultivar (Q3). The above results showed that the different pathways were involved in the response of two cultivars to *P. infestans*.

### DEGs participated in plant-pathogen interactions and lignin synthesis during *P. infestans* infection

The expressions of most DEGs involved in plant-pathogen interactions and lignin synthesis were considerably activated

after *P. infestans* inoculation (Fig. 6A and B). The DEGs involved in  $\text{Ca}^{2+}$  signals, such as “Calcium-binding protein (CaM)”, “cyclic nucleotide gated channels” (CNGCs, “ $\text{Ca}^{2+}$ -dependent protein kinases” (CDPKs) and “respiratory burst oxidase homologue” (Rboh) were detected in Q9 and Atl (Fig. 6A). Ten CaM genes were upregulated in Q3, while seven genes were highly expressed in Atl. In addition, three CDPK genes (StCDPK16 (PGSC0003DMG400021338), StCDPK18 (PGSC0003DMG40000890) and StCDPK24 (PGSC0003DMG400033335)), two Rboh genes (PGSC0003DMG400024754, PGSC0003DMG400014168), as well as four WRKY (PGSC0003DMG400016769, PGSC0003DMG400011633, PGSC0003DMG400012318, PGSC0003DMG400000064) genes were all significantly up-regulated both in Q9 and Atl. The expression of FLS2 genes (PGSC0003DMG400003790, PGSC0003DMG400017864 and PGSC0003DMG400016015) was much higher in Q9 than those in Atl except PGSC0003DMG400027586. Moreover, after *P. infestans* inoculation, the defense-related genes of three PR (PGSC0003DMG400005111, PGSC0003DMG400005113 and



**Fig. 5** The 10 most enriched DEG pathways in each sample. Q1, Q2 and Q3 mean the treatment of Q9 with *P. infestans* for 1, 3 and 5 dpi, and A1, A2 and A3 mean the treatment of Atl with *P. infestans* for 1, 3 and 5 dpi. The \* in the figure means that the genes in the pathway are more different in Q9 and Atl

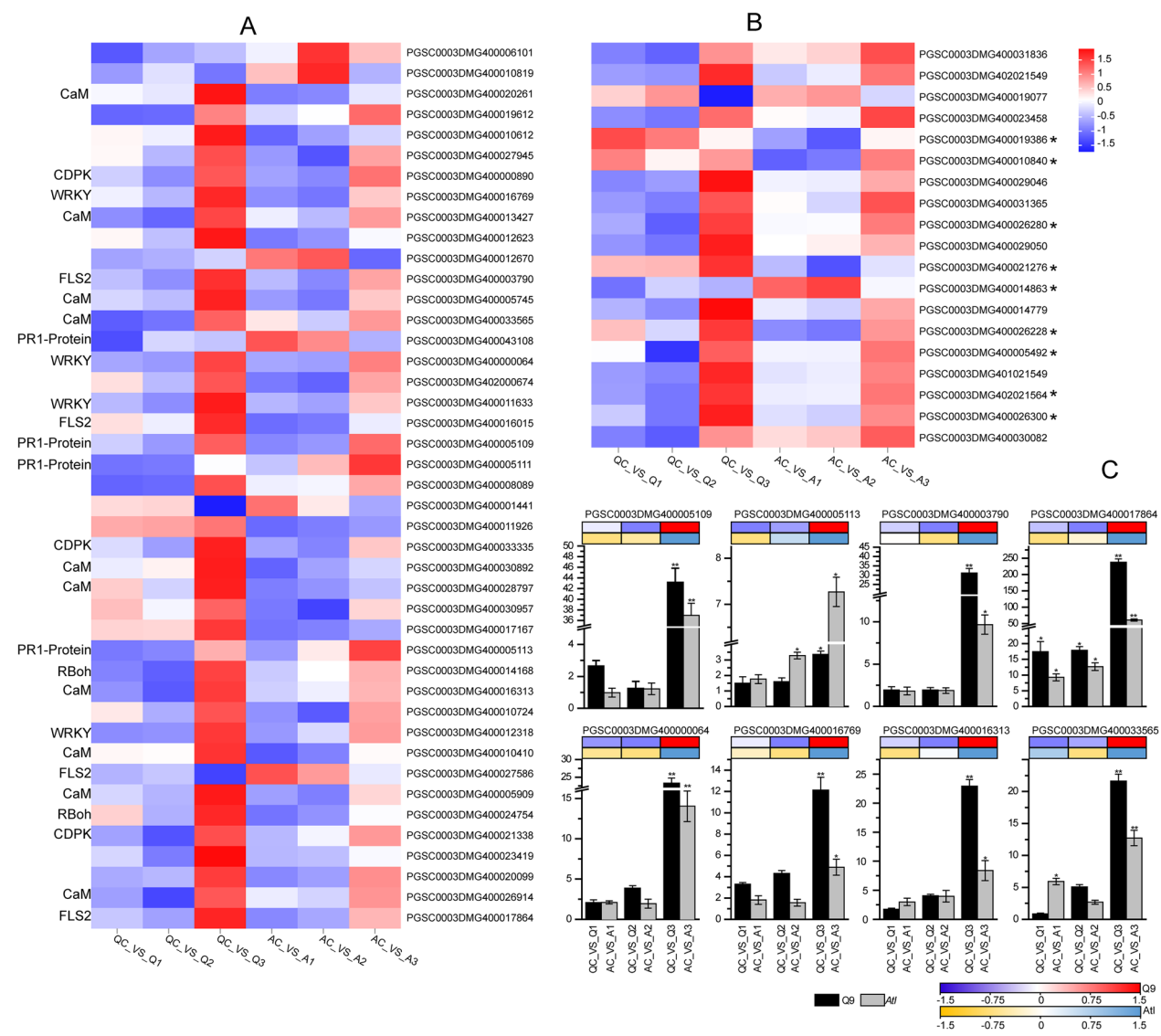
PGSC0003DMG400005109) were induced. As observed, PGSC0003DMG40000510 expression was 5.43- and 5.21-fold up-regulated in Q3 and A3 compared with QC and AC. However, PGSC0003DMG400043108 was down-regulated in the whole process. Moreover, three 3-ketoacyl-CoA synthase (KCS) genes showed down-regulation in the detected process in Q9, whereas mainly up-regulated in A2 and down-regulated in A3. Most of the expression of DEGs that participated in plant-pathogen interactions showed greater expression in Q9 than in Atl, especially in the later stage. However, the genes PGSC0003DMG400028797, PGSC0003DMG400020261, PGSC0003DMG400030892, PGSC0003DMG400023419 and PGSC0003DMG400011926 showed the opposite trend in the two cultivars, which will be used for subsequent functional validation.

Phenylalanine metabolism and phenylpropanoid biosynthesis, including the lignin synthesis-related pathways, were significantly enriched (Fig. 6B). Seven PAL genes (PGSC0003DMG402021549, PGSC0003DMG400023458, PGSC0003DMG400019386, PGSC0003DMG400031365, PGSC0003DMG400005492, PGSC0003DMG401021549 and PGSC0003DMG401021564) showed different expressed pattern in Q9 and Atl. Among them,

PGSC0003DMG400005492 and PGSC0003DMG401021564 were down-regulated in Q2 and then up-regulated in Q3, while in Atl, the expression of the two genes was dramatically up-regulated in the treatment time. PGSC0003DMG400019386 showed down-regulated trend in Q9. However, its expression declined at 3 dpi and increased at 5 dpi in Atl. Interestingly, the phenylacetaldehyde synthase gene PGSC0003DMG400014863 showed downregulation in both Q9 and Atl except at the A2 time. The above results indicated that two cultivars enhanced the cell wall by activating lignin synthesis, thus improving the tolerance to pathogens.

Moreover, the reliability of transcriptome data was validated by qRT-PCR technology. Eight representative genes including plant disease resistance PR1 (PGSC0003DMG400005109 and PGSC0003DMG400005113), WRKY transcription factor (PGSC0003DMG400000064 and PGSC0003DMG400016769), FLS2 (PGSC0003DMG400003790 and PGSC0003DMG400017864) and CaM (PGSC0003DMG400016313 and PGSC0003DMG400033565) were selected (Fig. 6C). As





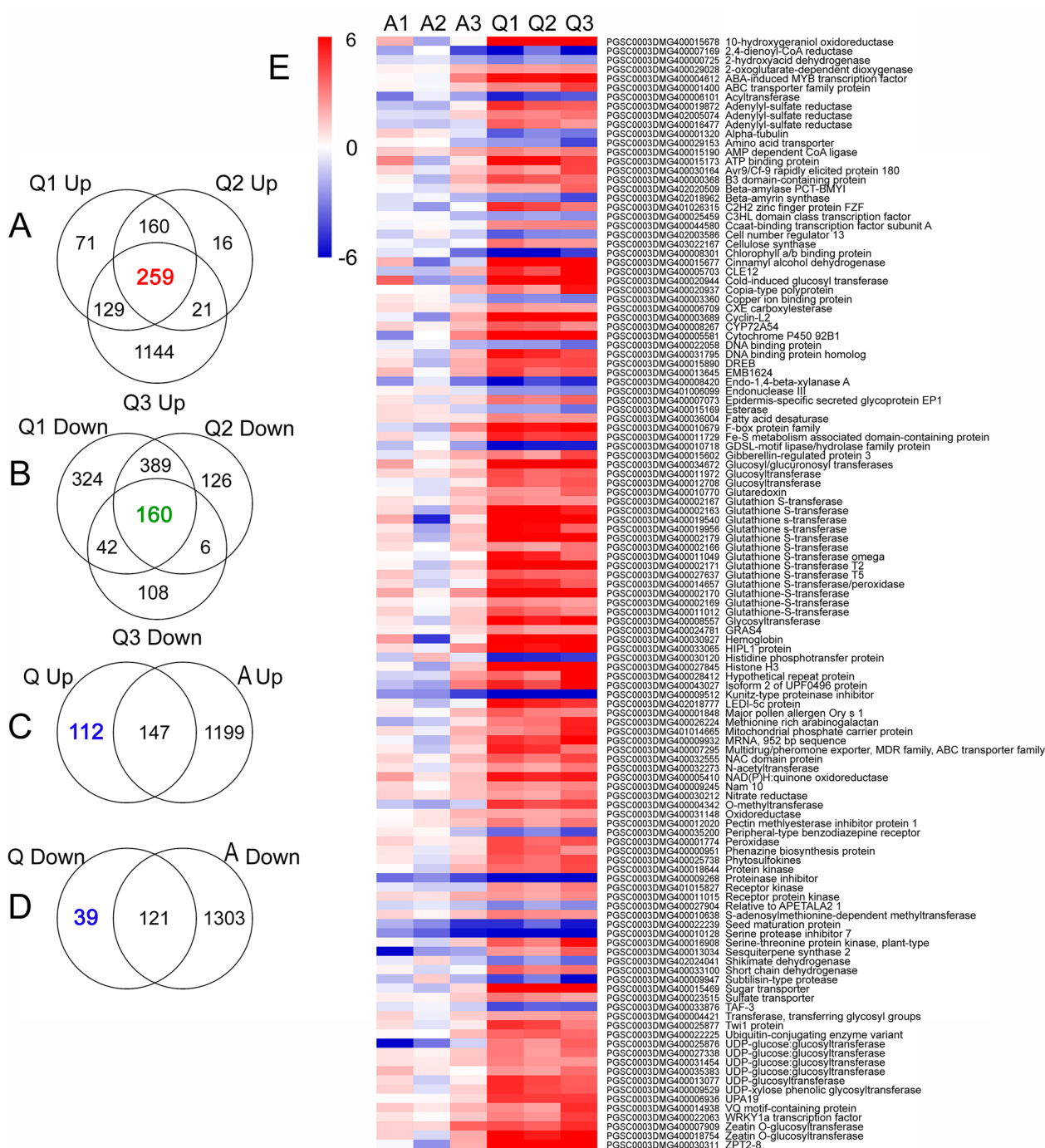
**Fig. 6** Expression patterns of DEGs involved in plant-pathogen interactions (A) and phenylalanine metabolism (B) and the expression was validated by qRT-PCR analysis (C). A and B: Different colored squares represent the expression level of genes compared with the control, expressed in terms of log<sub>2</sub>(fold change), and blue squares indicate that genes were not expressed under this time point. C: Relative expression of eight DEGs during different time points of *P. infestans* inoculation. The error bars represent the standard error of three biological replicates. The heatmap at the top of the bar chart represents the FPKM value of transcriptome sequencing

illustrated in Fig. 6C, the expression patterns were consistent with the results of RNA-Seq analysis, which confirmed the reliability of RNA-Seq data.

### Key DEGs distinguished between Q9 and Atl

To further understand the resistance mechanism between Q9 and Atl, the up-regulated and down-regulated DEGs in the three treated time points were identified in Q9 (Fig. 7). There were 259 up-regulated DEGs and 160 down-regulated DEGs in all the treated time

points of Q9 (Fig. 7A and B), while 112 DEGs were only up-regulated and 39 DEGs were down-regulated in Q9 compared with Atl (Fig. 7C and D). The expression of DEGs was analyzed, showing the significant difference between the two cultivars (Fig. 7E). Although these DEGs were up-regulated or down-regulated in Q9, they displayed no significant difference in Atl, which may have contributed to the resistance of Q9. Furthermore, we found that many transcription factors, such as MYB, NAC, and ERF, were included, which can be used for further functional research.



**Fig. 7** The DEGs expressed significantly in Q9 during *P. infestans* inoculation. **A** and **B** the number of the DEGs up-regulated or down-regulated in Q9 in all treated time points; **C** and **D** the number of the DEGs up-regulated or down-regulated significantly only in Q9 compared with Atl. **E** The expression fold-change of DEGs in Q9 and Atl. Values are average gene expression levels measured in three biological replicates

**Transient expression indicated the positive regulation of the four DEGs**

To further investigate the function of the above DEGs, we performed transient expression analysis (Fig. 8). So far,

the functions of four genes only upregulated in Q9 have been confirmed, including hypothetical protein 1 (StHP1, PGSC0003DMG400028412), MYB transcription factor 2 (StMYB2, PGSC0003DMG400004612), Histone

superfamily protein 3 (StHSP3, PGSC0003DMG400027845) and NAC transcription factor 5 (StNAC5, PGSC0003DMG400032555). In the potato leaves with transient gene overexpression, the lesion area was smaller (Fig. 8A). Especially when StMYB2 was overexpressed, the lesion area was 22.17% of the control (Fig. 8D). Trypan blue staining, which can visualize leaf disease and wound responses [49], was applied in our study. Detached leaf assays infected with *P. infestans* showed smaller lesions in overexpressed leaves than in controls (Fig. 8B and D). Thus, we suggested that these four DEGs only expressed in Q9 worked as positive regulators of plant immunity.

## Discussion

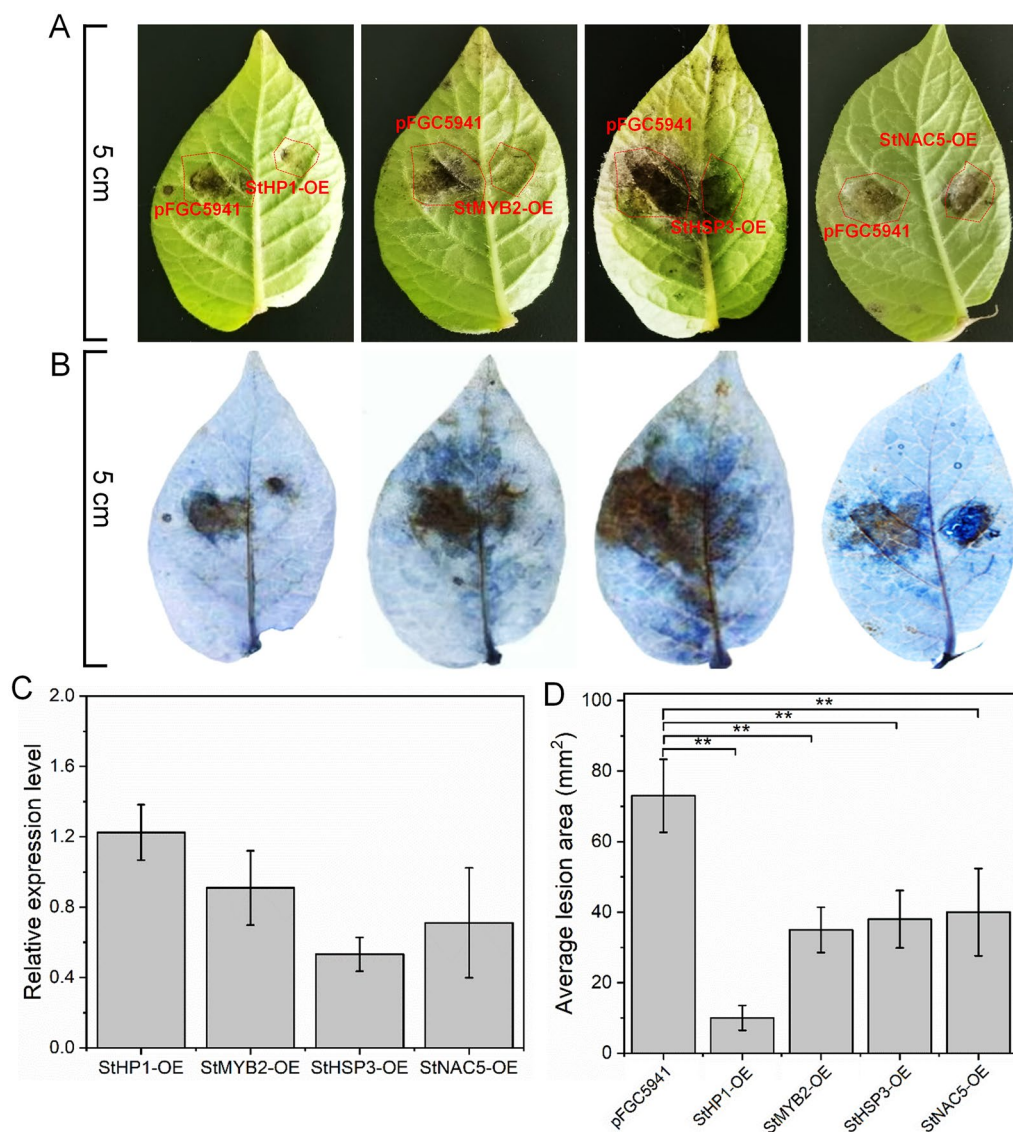
Potato late blight is one of the most devastating oomycosis diseases in the potato industry in China, leading to substantial economic losses worldwide. Farmers have controlled late blight primarily with chemicals, but they are expensive and result in environmental pollution. In Gansu province, Q9 are widely planted because of high yield and strong resistance, while Atl are popular with local farmers because of their short ripening period and good eating quality. However, the two varieties differ in their resistance to late blight [50]. In the present study, the dynamic transcriptome analysis and functional verification were carried out to reveal the different responses to late blight infection of the two potato cultivars, which can provide helpful information for resistance breeding.

In the early stages of plants being attacked by a specific pathogen, ROS are usually produced. Antioxidant enzymes, which can help prevent damage caused by ROS to the cell membrane system, play a crucial role in enhancing the host's resistance to disease stress [51, 52]. In this study, SOD and POD activities were positively correlated with the intensity of plant disease resistance (Fig. 2). In tomatoes, SOD activity increases during the initial stage of *Botrytis cinerea* infection but decreases with the appearance of necrosis [8], which is consistent with our results in potatoes. Moreover, the increases in the activities of SOD and CAT in partially resistant wheat (BRS 229) are more significant compared to susceptible wheat (BR 18) [53]. Similarly, the SOD and CAT activities in the *P. infestans*-resistant cultivar (Q9) were higher than in the *P. infestans*-susceptible cultivar (Atl) in our current study (Fig. 2), which may contribute to the lower levels of  $O_2^-$  and  $H_2O_2$ . Moreover, after potato plants were infected with *P. infestans*, the earliest activated genes were *PALs*, indicating that phenylpropane metabolism may play a central role in the defense response. The activities of PAL in resistant varieties are much higher than in susceptible varieties [54]. In our study, the activity of PAL in Q9 was about 1.5 times that of Atl (Fig. 2). The above results further confirmed that the variation in

antioxidant enzyme activity can enhance Q9 resistance to *P. infestans*.

In the later stage of infection, plants limit pathogen spread by transcribing pathogenesis-related (PR) proteins and developing the hypersensitive response (HR) [55]. However, the effects of gene expression regulation on gene functions and the molecular mechanisms in Q9 and Atl were unclear. In our study, gene expression in potatoes with varying levels of disease resistance was detected using RNA-Seq. The up-regulated DEGs in Q9 were more numerous than those in Atl at 1 dpi and 3 dpi, indicating that *P. infestans* could stimulate rapid gene expression in Q9 potato leaves (Fig. 3). It was interesting that the up-regulated DEGs in Q9 gradually increased and the down-regulated DEGs gradually decreased, while the number of up-regulated and down-regulated DEGs in Atl gradually increased along the treatment time. We also found that in the early stage (1 dpi and 3 dpi), the number of DEGs in Q9 was more than that in Atl, potentially reflecting the different responses of the different resistant plants to *P. infestans* inoculation. The upregulation of gene expression may be involved in the interaction between plants and pathogens. Seven PAL-related genes were upregulated in both cultivars, but the levels were not similar, which may contribute to the differences in resistance between Q9 and Atl. WT tubers exhibited mainly up-regulation of genes at later stages of *P. infestans* infection (24 hpi–48 hpi), while that susceptible variety 'Russet Burbank' line displayed approximately equal numbers of up-regulated and down-regulated genes [30]. He et al. (2021) showed that 24 hpi was the critical period of interaction between Q9 and *P. infestans*, while 48 hpi was critical to the immune response [35]. The results have some differences with ours, which suggested that transcriptome dynamics depend not only on the time of genes activated but also on the category of pathogen and host plant.

Phenylpropanoids are inducible antimicrobial compounds and essential in plant-pathogen responses [56]. When plants are subjected to biotic stress, phenylpropanoid biosynthesis pathway is activated to enhance the resistance [57, 58]. The phenylpropanoid biosynthesis pathway induced by plant pathogens has been reported in cotton, potato, pepper, soybean, black pepper, *Ricinus communis* and flax plants [39, 59–64]. The phenylpropanoid biosynthesis pathway was one of the 10 most enriched KEGG pathways in Q9 and Atl (Fig. 5), indicating that phenylpropanoid biosynthesis was the critical metabolic pathway in potatoes in response to *P. infestans*. Previous studies have demonstrated that the secondary metabolite phenylpropanoid biosynthesis participates in the potatoes' resistance defense [65], which was consistent with our results. As the first and most important



**Fig. 8** The four selected DEGs positively regulated plant immunity. **A:** Pictures show lesion development on *StHP1*-, *StMYB2*-, *StHSP3*- and *StNAC5*-overexpressed *AtI* leaves upon *P. infestans* inoculation. Pictures are taken at 5 d after infection (**A**) and after Trypan blue staining (**B**). **C:** Relative expression of the DEGs in potato leaves. Total RNAs were extracted at 3 dpi after transient expression. **D:** Bar graphs show the average lesion areas when DEGs are transiently overexpressed and then inoculated with *P. infestans* for 5 dpi

enzyme of phenylpropanoid biosynthesis pathway, PAL can convert phenylalanine to cinnamic acid. In the present study, PAL activity was enhanced in the detected time, and seven PAL-related genes were all up-regulated at 5 dpi compared with the control, which indicated that *P. infestans* infection activated the genes encoding PAL, thus turned on the master switch of the phenylpropanoid biosynthesis pathway, and further synthesized phenolic compounds to enhance resistance. Previous studies showed that phenolic compounds are essential in defense against pathogens. There is a significant variation in

the levels according to the different resistances [66, 67], which is similar to our results. Similarly, we have previously reported on potato tubers inoculated with *F. sulphureum* [39]. In resistant cotton, genes involved in the phenylpropanoid pathway are significantly induced compared to susceptible cotton plants after *V. dahliae* infection [68]. However, in our research, the phenylpropanoid pathway was induced in both cultivars (Fig. 5). It was worth our attention that the expression levels of these genes were different, which may cause the level of susceptibility of potato late blight.



At the later stage infected by *P. infestans*, DEGs in plant-pathogen interaction pathways were significantly enriched in Q9 (Fig. 6A). Calcium-dependent protein kinases (CDPKs) have been found to participate in the defense against abiotic and biotic stress responses, particularly involved in plant-pathogen interaction [61, 69]. In our study, three StCDPK expressions were up-regulated in inoculated leaves during potato-*P. infestans* interaction compared to the control. Similar results have been reported by Elisa et al. [69]. Moreover, the expression levels of StCDPKs in Q9 were higher than in Atl, especially in the later stage, which may be one of the reasons for the resistance difference in Q9 and Atl. The PR genes encoding plant defense proteins were included as a marker to confirm that the defense response against *P. infestans* was triggered [70]. As observed, the PR genes were activated, and the expression of the other three StPR was upregulated at the detection time point except for PGSC0003DMG400043108 and reached the maximum at 5 dpi. The defense response induced by *P. infestans* has also been reported by Elisa et al. [69], in which the expression of *StPR-1b* reached the maximum value at 2 dpi and decreased at 4 dpi. *StPR-1* and *StPR-5* were up-regulated earlier in Kennebec (moderately resistant) than in Russet Burbank (susceptible). The above results strongly indicated that the expression of these genes determined the level of susceptibility of potato cultivars to the pathogen, and *P. infestans* genotype and the potato cultivar influenced the activation of these defense-related genes simultaneously [70]. WRKY domain-containing proteins are one of the most prominent families of transcriptional regulators in plants, and they have been proven to be involved in plant immune responses [71]. In Arabidopsis, most WRKY genes are linked to complex plant defense responses. Mutations of *AtWRKY70* enhance plant susceptibility to biotrophic and necrotrophic pathogens [72–74]. Mutations of *WRKY7*, *WRKY11*, *WRKY17*, *WRKY48*, *WRKY38* and *WRKY62*, enhanced basal plant resistance to virulent *P. syringae* strains [75–77]. A previous study showed that *StWRKY1*, which can regulate phenylpropanoid metabolites, conferred late blight resistance in the potato F06025 genotype [78]. Wang et al. [79] demonstrated that *StWRKY8* responded to the infestation of *P. infestans*, and the overexpression of *StWRKY8* could improve the potato resistance to *P. infestans*. In our study, four *StWRKY* were triggered significantly at 5 dpi in Q9, indicating that *StWRKY* was activated to participate in pathogen-induced defense responses and enhanced basal plant resistance of Q9 to *P. infestans*. Mohapatra et al. (2016) reported that the accumulation of H<sub>2</sub>O<sub>2</sub> correlated with the resistance of pea leaf tissues [80]. H<sub>2</sub>O<sub>2</sub> accumulation was more substantial and intense in the

susceptible tissues than in the resistant tissues. Overexpression of the WRKY transcription factor increased tolerance to pathogen infection in grapes and tobacco [81, 82]. In addition, many genes involved in plant-pathogen interactions were more significantly upregulated than in Atl at the later stage, enhancing the Q9's resistance to late blight. However, inconsistent with our results, in the resistant variety Kennebec, the defense-related genes showed early expression in contrast to the susceptible variety Russet Burbank [70]. The reason was that the activation time of these genes was not only affected by the potato cultivars, but also by the *P. infestans* genotype.

A large number of transcription factor (TF) families, including NAC, WRKY, AP2/ERF and MYB, were involved in defense responses [83–85]. Similar to a previous study, in our work, StNAC5 and StMYB2, which were only significantly expressed in Q9, contributed to the plant resistance. The effect of NAC-TF on biotic stress has been verified in many plants. Some NAC proteins can directly recognize virus-encoded proteins and promote or inhibit viral proliferation. In wheat, Geminivirus RepA-binding (GRAB) proteins, a novel member of the NAC domain family, can interact with the RepA protein of wheat stunting virus (WDV) to inhibit DNA replication of WDV [86, 87]. Other authors have suggested that NAC transcription factors can regulate the expression of related resistance genes, thereby improving plant resistance to biological stress. In banana fruits, MaNAC5 can regulate the expression of *PR* genes and contribute partially to pathogen resistance [88]. In the present study, StNAC5-OE leaves exhibited increased tolerance to *P. infestans*, which warranted exploration of the underlying mechanism. Moreover, MYB protein is essential for plant resistance to biotic stress such as bacterial, fungal and viral diseases. For example, *CsMYB96* enhanced *Citrus sinensis* resistance to harmful microorganisms by stimulating the biosynthesis of SA and accumulating the defense metabolites [89]. However, inhibiting *PpMYB306* expression in peach (*Prunus persica*) enhanced fruit disease resistance by regulating lignin biosynthesis [90]. Furthermore, *MdMYB73* improved apples' resistance by interacting with *MdWRKY31*, a positive regulatory factor of the *Botryosphaeria dothidea* [91]. The mechanisms of TFs in plant-pathogen interaction are diverse. In further study, we will identify the positive role of StMYB2 in regulating potato resistance to *P. infestans*. In conclusion, our study provided evidence that the resistance of Q9 to *P. infestans* was stronger than Atl, which was caused by different gene expression levels and expression patterns. Inspiringly, more work is needed to explore the underlying mechanism of the four genes transiently expressed in Atl.



## Conclusions

Dynamic transcriptome analysis uncovered DEGs and pathways responding to *P. infestans* in Q9 and Atl. The plant-pathogen interaction, phenylpropanoid biosynthesis and MAPK signaling pathway were significantly activated in Q9 at the later stage. The expression levels of related genes were significantly increased, which may be an important strategy for Q9 to cope with *P. infestans*. In addition, 112 and 39 DEGs were only up-regulated and down-regulated in Q9, and most belonged to TFs. These genes were involved in the response of Q9 to late blight. Our comprehensive gene expression data at the transcription level will help elucidate the molecular mechanisms of the potato defense response against *P. infestans*. Transient expression of four genes proved conducive to improving potatoes' resistance. This study explored more candidate genes participating in the resistance, which provided a critical value for the in-depth analysis of the resistance mechanism and laid a foundation for breeding resistance to late blight.

## Abbreviations

Dpi	Day post inoculation
PAMPs	Pathogen associated molecular patterns
PTI	PAMP-triggered immunity
RLK	Receptor-like kinases
RLP	Receptor-like proteins
ETI	Effector-triggered immunity
NBS-LRR	Nucleotide Binding Site-Leucine-Rich Repeat
SAR	Systemic acquired resistance
SA	Salicylic acid
MeSA	Methyl salicylate
ET	Ethylene
SRA	Sequence Read Archive
DEGs	Differently expressed genes
CaM	Calcium-binding protein
CNGCs	Cyclic nucleotide gated channels
CDPKs	Ca <sup>2+</sup> -dependent protein kinases
Rboh	Respiratory burst oxidase homologue
KCS	Ketoacyl-CoA synthase

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-024-00567-z>.

**Additional file 1:** Table 1 Primers used in this study.

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## Author contributions

WZ contributed to the design of experiments and manuscript writing. YM and YK reviewed data curation and writing. RZ, YW, ZC, SJ and XW was involved in revising the manuscript. XY contributed to the investigation. SQ provided fund acquisition and project administration. All authors read and approved the final manuscript.

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## Availability of data and materials

All data obtained from the current study are available from the corresponding author on a reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

### Competing interests

The authors declare there are no conflicts of interests.

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