Integrating transcriptome and proteome analysis to determine the lignin synthesis pathway involved in Panax notoginseng in fungal stress

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Research

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Abstract

Background Plants are constantly threatened by various pathogens in a challenging environment. *Altemaria panax* Whetzel is a destructive pathogen that affects many plants, including *Panax notoginseng*, and significantly reduces the yield and product quality of *Panax notoginseng*. It is not clear how *Panax notoginseng* responds to pathogen infection.

Methods Using the advanced advantages of transcriptome and proteomics technology, we studied the response of *Panax notoginseng* to *Altemaria panax* stress.

Results Compared with the control, fungal infection caused significant changes in the *Panax notoginseng* transcriptome and proteome. Specifically, a total of 136,100 transcripts and 4,468 proteins were identified. The integration of transcriptome and proteome profiles revealed many candidate transcripts/proteins, which may be involved in lignin synthesis during the activation of defense responses by *Panax notoginseng*. Many genes and proteins are induced or inhibited by fungi. Among them, the expression levels of genes *PAL*, *4CL*, *COMT*, *CAD* and *POX* in the lignin synthesis pathway are significantly increased, which indicates that the fungus activates the defense response of *Panax notoginseng*.

Conclusions As far as we know, this is the first time that transcriptome and proteome analysis have been combined to study the response of *Panax notoginseng* to disease. This study provides a wide range of new information about the transcriptome, proteome and their correlation of *Panax notoginseng* in response to fungal stress. The analysis of this resource allows us to examine the mechanisms of transcription and protein diversification, which expands the knowledge of the complexities of the transcriptome and proteome in traditional Chinese medicines.

1. Introduction

*Panax notoginseng* (Bruk.) FH Chen is an herbaceous perennial plant belonging to the genus *Panax* and family Araliaceae (Park et al. 2012). It is a valuable traditional Chinese herbal medicine with a long history and can be used to treat a variety of diseases (China 2000; Chan et al. 2002). However, *P. notoginseng* is easily infected by various diseases and insect pests during its growth because of its special growth environment and long growth years. At present, the infection of plant pathogens is the main factor affecting the yield of *P. notoginseng*. Among them, *P. notoginseng* black spot disease caused by *Altemaria panax* Whetzel is one of the fungal diseases seriously harming *P. notoginseng* (CHEN et al. 2005). Black spot disease occurs quickly and spreads rapidly, and *P. notoginseng* has suffered heavy losses due to this disease all year round (Jiang et al. 2011). Therefore, the breeding of new varieties of anti-black spot disease has become an urgent problem in the production of *P. notoginseng*.

Phenylpropane compounds act as inducible antibacterial compounds and signaling molecules in plant-pathogen reactions (Dixon et al. 2002; Naoumkina et al. 2010). In addition, phenylpropane metabolism is the most important metabolic pathway in plant defense against biological stress (La Camera et al. 2004;
Lignin biosynthesis is a downstream branch of the phenylpropane pathway (Li et al. 2019). Prior to inoculation, lignin can enhance the mechanical strength of plants and thicken cell walls to form physical barriers and inhibit pathogen invasion and colonization (Li et al. 2019; Hu et al. 2018). Meanwhile, the accumulation of lignin in infected cells not only inhibits the spread of toxins and enzymes produced by pathogens (Li et al. 2019), but also prevents pathogens from extracting water and nutrients from host plants (Mottiar et al. 2016).

In recent years, despite the development of high-throughput sequencing technology, screening high-throughput transcriptome analysis based on the screening of functional genes related to good traits of crops has been relatively mature, and the use of molecular markers to guide breeding has been an effective way for crop breeding, such as Gossypium hirsutum L. (Yao et al. 2011), Macrotyloma uniflorum (George et al. 2018), Ocimum americanum var. pilosum (Zhan et al. 2016) and Digitalis purpurea (Wu et al. 2012). However, for P. notoginseng, due to its weak genetic research foundation, the research on functional genes related to P. notoginseng disease started late, it is difficult to adopt molecular methods to guide the breeding of P. notoginseng resistant varieties. Current related research report, Xu et al. (Xu et al. 2016) used high-throughput sequencing technology to perform transcriptome analysis on P. notoginseng during the fungus infection and found a highly expressed chitinase gene (PnCHI1), at the same time, after inoculation of A. pax Whetzel with P. notoginseng leaves, the expression of PnCHI1 increased rapidly, showing a fluctuating upward trend (Xu et al. 2016).

Transcription levels only predict protein expression because they do not consider post-transcriptional processes, such as post-transcriptional regulation or protein stability (Liu et al. 2019). Although substantial progress has been made in understanding the transcriptome dynamics of plants in response to stress, changes at the protein level or biochemical regulation remain largely undetected (Zhang et al. 2017). Proteomics has been successfully applied at tomato (Ahsan et al. 2007), maize (Qi et al. 2016) and wheat (Zhang et al. 2016) to study the changes of protein components during stress. Currently, the proteomics of P. notoginseng has been studied in relation to ginsenosides. Lin et al. (Lin Yangjie) performed proteomic analysis on two groups of P. notoginseng with different contents of saponins and identified 248 differentially expressed proteins.

However, to our knowledge, no proteomic analysis related to P. notoginseng response to fungal infection has been reported. Therefore, in this study, we used RNA-Seq and label-free quantification (LFQ) proteomics methods to comprehensively study the P. notoginseng transcriptome and proteomics profiles before and after inoculation with pathogens, and to determined in lignin biosynthesis pathway, the expression of key synthetic genes, this study provides a detailed framework for the proteome dynamics of P. notoginseng in response to fungal infections, and lays a theoretical foundation for the breeding of P. notoginseng disease-resistant molecular markers.

2. Materials And Methods

2.1 Plant, pathogen and plant treatment
Three-year-old *P. notoginseng* plants were used throughout the experiments. The plants were cultured in a greenhouse located at Kunming University of Science and Technology (E 102.87°, N 24.85°, altitude 1895 m). *Alternaria panax* Whetzel, which isolated from our own laboratory, was propagated in solid potato dextrose agar (PDA) medium (200 g of potatoes, 20 g of glucose, and 15 g of agar) at 28°C in the dark for 2 weeks. An acupuncture method was used to inoculate plant leaves with the fungi the leaves were sprayed with spore suspension (1x10^6 spores per milliliter, Inf) as previously described (Delventhal et al. 2016). Following treatment, plants were bagged to increase humidity. These plants were grown in the greenhouse in a 16 h light/8 h dark photoperiod at temperatures of 25°C (day) and 20°C (night). After 120 hours of treatment, the physiological indexes of *P. notoginseng* leaves were detected, as well as the analysis of transcriptome and proteome. At this time, *P. notoginseng* leaves were collected and quickly frozen, and stored at -80 °C until use. Three independent biological replicates were used for each treatment, and each biological replica consisted of leaf tissue pooled from multiple plants.

### 2.2 Detection of physiological indexes of *P. notoginseng* leaves after fungal stress

The activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and superoxide anion, and the contents of chlorophyll, \( \text{H}_2\text{O}_2 \) content in the leaves of *P. notoginseng* during the infection process of pathogens were assayed using kits (Bioengineering, Nanjing, China) according to the manufacturer’s recommended protocol. The protein content of *P. notoginseng* leaves was detected using BCA protein assay kit (Solarbio, China). Each sample was subjected to three biological replicates, and Prism 8.0.1 software (GraphPad Software) was used to analyze the data.

### 2.3 Transcriptome analysis

Methods S1 provides details on RNA extraction, library preparation and sequence analysis, and bioinformatics analyses.

### 2.4 Proteome analysis

Methods S2 provides details on lable-free proteomics sample preparation, peptide digestion, LC-MS / MS data processing and analysis and GO annotation and KEGG pathway annotation.

### 2.5 Correlation Analyses of Transcriptome and Proteome Profiles

If both genes and proteins are expressed after fungal stress, the genes and their corresponding proteins are considered relevant. Then, the significance of expression between related transcripts and proteins can be determined. If the expression levels of genes and their corresponding proteins show significant differences after fungal stress, they are defined as differentially expressed related transcripts (DECT) and proteins (DECP), respectively. Next, DECT and DECP were annotated and enriched by KEGG pathway, and transcripts/proteins related to lignin synthesis was screened for subsequent verification.

### 2.6 Quantitative Real Time PCR analysis
Total RNA was extracted from *P. notoginseng* leaves of the Inf and Con treated with Trizol reagent (TaKaRa), and 5 ng of total RNA was reversely transcribed into cDNA using GoScript™ Reverse Transcription System (Promega). Prime 5.0 software was used to design gene-specific primers for qRT-PCR (Table S1). The PCR reaction was performed in a 20 μL volume containing 10 μL 2×qPCR Master Mix reagent (Promega), 1 μL template cDNA and 0.4 μL of each primer. The reaction conditions are as follows: 95°C for 120 seconds; then 40 cycles of 95°C for 15 seconds and 60 ºC for 60 seconds; 95°C for 10 seconds, 65°C for 60 seconds, and 97°C for 1 second. *P. notoginseng* PnACT2 gene was used as a reference gene for internal control. The relative gene expression level was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

### 2.7 Targeted Protein Quantification by Parallel Reaction Monitoring (PRM)

PRM analysis was performed as described by Kim et al (Kim et al. 2016). Each sample was analyzed separately by LC-PRM / MS. In short, peptides were prepared according to the label free method described above, and PRTC-stabilized isotope peptides were incorporated into each sample as an internal standard reference. Before chromatographic separation by HPLC system, trypsin digestion, and desalting the peptide. Using a 1-hour liquid chromatography gradient, acetonitrile is 5-35% in 45 minutes. PRM analysis was performed on a Q-Exactive HF mass spectrometer (Thermo Scientific). The mass spectrometer operates in positive ion detection mode and has the following parameters: MS1 scanning range: 300-1800 m/z, resolution 60,000 (200 m/z), automatic gain control (AGC) target value $3.0\times10^6$, and the maximum ion implantation time (Maximum IT) is 200 ms. After each full MS scan, 20 PRM scans (MS2 scan) were performed with a resolution of 30,000 (m/z 200), AGC $3.0\times10^6$, and Maximum IT 120 ms. The target peptide was isolated with a 1.6Th window. The MS2 activation type is HCD, and the normalized collision energy is 27. The raw data was analyzed using Skyline 3.5.0 (MacCoss Lab, University of Washington).

### 3. Results

#### 3.1 *A. panax* infection induces chlorophyll degradation and oxidative stress in *P. notoginseng* leaves

We first analyzed the physiological changes of *P. notoginseng* leaves upon *Alternaria panax* infection. With the prolongation of fungal infection time, the leaves of *P. notoginseng* appeared obvious lesions at 120h (Fig. 1A, B), and the *P. notoginseng* plants withered after 120h treatment, and its leaves fell. *Alternaria panax* infection caused a significant decrease in the content of chlorophyll a, chlorophyll b and total chlorophyll (Fig. 1C), reaching the minimum value at 120h; the content of H$_2$O$_2$ (Fig. 1D) gradually increased, anti-superoxide anion (Fig. 1E), SOD (Fig. 1F), POD (Fig. 1G), and CAT activity (Fig. 1H) also gradually increased, and reached the maximum value at 120 hours of infection. Because the phenotype of *P. notoginseng* was the most obvious at 120h after *Alternaria panax* infection, and its related physiological indexes reached the maximum value at 120h, so we chose this time point to study the transcriptome and proteome.
3.2 Transcriptome analysis of *P. notoginseng* responding to *Alternaria panax* infection

In order to study the defense pathways of *P. notoginseng* in response to the *Alternaria panax* infection, we first analyzed the transcriptome levels of *P. notoginseng* before and after the fungal infection. A total of 136,100 genes were detected in all sample combinations (Table S2). The genes with absolute value of log2Foldchange greater than 1 and statistically significant (padj<0.05) compared with Con were selected as the significant difference genes. A total of 9045 genes were found to be differentially expressed in all samples (Table S3), there are 4003 down-regulated genes and 5042 up-regulated genes. We further screened the top 40 significantly DEGs (20 most up-regulated genes and 20 most down-regulated genes) (Fig. 2A), the up-regulated significant DEGs mainly include: plastid ATP/ADP transporter, cytochrome P450, polyphenol Oxidase is mainly related to plant energy transportation, plant defense, and response to stress, while the down-regulated significant difference genes are mainly related to plant growth and development such as chlorophyll synthesis and photosynthesis.

On this basis, in order to determine the major biochemical metabolic pathways and signal transduction pathways involved in the significantly DEGs, we enriched the detected differential genes by the KEGG pathway (Fig. 2B) and found that in the most significant enrichment 20 pathways, pathways related to plant defense include phenylalanine metabolism, calcium signaling pathway, plant MAPK signaling pathway, and phenylalanine, tyrosine, and tryptophan biosynthesis.

3.3 Proteomic analysis of *P. notoginseng* responding to *Alternaria panax* infection

In order to obtain the global changes of the proteins of *P. notoginseng* in response to fungal infection, we used label free technology to detect *P. notoginseng* proteome on the based of the transcriptome analysis of *P. notoginseng*. In total, 4468 proteins were identified (Table S4), and among these, 632 proteins showed altered levels between treatment groups and Con (Table S5).

We screened the top 40 significantly DEPs (20 most up-regulated proteins and 20 most down-regulated proteins) (Fig. 3A), Chitinase, cytochrome P450, peroxidase, disease-related proteins, etc., most of these are related to plant defense and plant response to stress; while the down-regulated significant difference proteins are mostly related to plant growth and development.

The KEGG pathway enrichment analysis of the detected DEPs revealed (Fig. 3B) that plant defense-related pathways included phenylpropane biosynthesis, keratinine, berberine and wax biosynthesis, and stilbene, diarylheptyl and gingerol biosynthesis. In addition, photosynthesis-related pathways have also been enriched.

3.4 Correlation between transcriptome and proteome

Among the 9045 regulated genes, 7788 showed no corresponding proteins in the proteome data, and the protein products of 979 regulated genes showed no changes (Fig. 4), possibly because of the relatively low sensitivity of proteome. In the proteome data, it was found that 2297 proteins did not change, and these proteins consistently always had unaltered transcription levels (Fig. 4). Among the differentially
632 proteins, most (327 proteins) had no change in transcription level; transcripts of 278 regulated proteins were also regulated, and 27 proteins did not have corresponding transcripts in the transcriptome (Fig. 4).

Cluster analysis of 278 genes/proteins associated at both the transcriptome and proteome levels (Fig. S6). There were 156 genes that were significantly up-regulated at both the transcriptional and protein levels, there were 94 genes that were significantly down-regulated at both the transcriptional and protein levels, 19 genes that were up-regulated at the transcriptional but protein levels were down-regulated, and 9 genes were down-regulated at the transcriptional but protein levels were up-regulated.

In order to observe the global pathway changes at the two levels of transcriptome and proteome, we correlated and enriched all the significantly DECTs and significantly DECPs, so as to find that both omics are significantly enriched KEGG pathway (Table 1), a total of three pathways were enriched, namely photosynthesis-antenna protein, phenylpropane biosynthesis and stilbene, diarylheptane, gingerol biosynthesis. There are 13 genes related to the phenylpropane biosynthetic pathway, of which there are 5 genes related to lignin synthesis, namely Ppse_ynau_080241 (named PnPAL), Ppse_ynau_027778 (named PnPOX), Ppse_ynau_130961 (named Pn4CL), Ppse_ynau_082953 (named PnCOMT), Ppse_ynau_117438 (named PnCAD).

3.5 Real-time PCR and parallel reaction monitoring (PRM) verification

In this study, qRT-PCR experiments were performed on 5 selected genes with gene-specific primers. The transcriptional abundance before and after the pathogen infection was calculated (Fig. 5). At the transcription level, Alternaria panax induced significantly up-regulated genes in the lignin synthesis pathway of P. notoginseng leaves (Fig. 5A,B). The proteins of these 5 genes were also selected for PRM quantitative verification of protein abundance. In the lignin synthesis pathway, Pn4CL, PnCOMT, PnPOX and PnCAD proteins have a significant increase in abundance (Fig. 5A, C), which is consistent with the abundance of label-free quantitative analysis, but PnPAL protein has not been quantitatively verified by PRM.

4. Discussion

In China, P. notoginseng is a valuable traditional Chinese herbal medicine, but black spot disease has become an important factor affecting the output and product quality of P. notoginseng. As far as we known, this study reported for the first time the transcriptome and proteomics analysis related to P. notoginseng’s response to fungal infections, and identified the expression of key synthetic genes in P. notoginseng lignin biosynthesis pathway. Five days after inoculation with Alternaria panax, obvious disease spots and wilt appeared on the leaves of P. notoginseng (Fig. 1 A, B). These symptoms may be due to oxidative stress (Fig. 1 C-H). The decrease of chlorophyll content after pathogen invasion (Fig. 1 C) induced changes in the related physiological indicators of P. notoginseng leaves. The obvious changes in H₂O₂, anti-superoxide anion, SOD, POD and CAT activities proved this. Compared with the control, the
leaves of *P. notoginseng* after fungal stress changed significantly at the transcriptome and proteomics levels (Fig. 2, Fig. 3). After the correlation analysis between transcriptome and proteomics, a significantly enriched phenylpropane biosynthetic pathway was screened (Fig. 4), which indicated that fungal infection stimulated and activated the defense response of *P. notoginseng*.

Phenylpropane metabolism is an important secondary metabolic pathway in plants. Lignin is a secondary metabolite produced by secondary metabolic pathways and plays an important role in plant disease resistance (Qiao et al. 2013). In the process of plant growth and development, lignin synthesis and related metabolism play an important role. At the same time, under adversity stress conditions, plant lignin metabolism can also actively participate in the process of responding to stress (Derikvand et al. 2008; Do et al. 2007; Shadle et al. 2007). In general, the lignin synthesis reaction is a typical defense response of plants to environmental stress (Moura et al. 2010). Studies have shown that in biotic and abiotic stresses, one of the common responses of plants is the accumulation of reactive oxygen species, which is accompanied by increased synthesis and accumulation of lignin (Moura et al. 2010). Therefore, lignin metabolism has a certain correlation with plant disease resistance, insect resistance, waterlogging resistance, cold resistance, and heavy metal resistance.

At present, the most researched is the relationship between lignin metabolism and plant disease resistance. Studies have shown that in *Arabidopsis*, CAD5 is highly expressed in the roots with a strong lignification degree. At the same time, studies have shown that this gene is induced to express during the process of pathogen infection of *Arabidopsis* (Tronchet et al. 2010), It shows that they are not only related to *Arabidopsis* lignin synthesis, but may also be related to plant lignin defense pathways. The increase in lignin content can also increase the resistance of cotton to *Verticillium* (Li et al. 2019). With the increase of light intensity, the lignin content of *Phalaenopsis* orchid and the activities of PAL, CAD and POD related to lignin synthesis increase correspondingly (Ali et al. 2006). Heavy metals can also induce an increase in the content of lignin. When plants are stressed by heavy metals, the phenolic secondary metabolic synthesis pathways in their bodies will also be strengthened. The enhancement of lignin biosynthesis leads to the thickening of plant cell walls to resist and fix heavy metals and reduce their entry in plants (Bhuiyan et al. 2007).

Lignin is a phenolic polymer, an important component of the cell wall of vascular plants, and has important biological functions such as mechanical support, water transport, and resistance to pathogen invasion (Guo et al. 2001; Raes et al. 2003; Rogers et al. 2005). The biosynthetic pathway of lignin is roughly divided into two steps: firstly the synthesis of lignin monomers, and then the polymerization of lignin monomers into biologically active lignin (Haiyan and Yuxing 2011). Many enzymes are involved in the whole lignin biosynthesis process, including *PAL*, *4CL*, *COMT* and *CAD* in the synthesis pathway of lignin monomer, which are four types of key enzyme genes with landmarks, and the enzyme gene *POX* in the polymerization process of lignin monomer. It has been proved that in *Arabidopsis*, *PAL* can mediate the synthesis of lignin and salicylic acid, thereby producing disease resistance (Mauch-Mani and Slusarenko 1996). POD plays an important role in the polymerization of phenols into lignin, so its activity in plants is also related to plant disease resistance. The lignin synthesized by the metabolism of
phenylpropanes makes the cell wall lignified and enhances the mechanical strength of the plant cell wall, thereby preventing the further penetration of pathogenic bacteria. Moreover, the low-molecular-weight phenolic precursors of lignin and the free radicals produced during polymerization can inactivate fungal membranes, enzymes and toxins (Siboza et al. 2014; Singh et al. 2019). This is consistent with the results in this study that increased POD activity and increased PnPAL gene expression levels caused an increase in lignin content (Fig. 1G, Fig. 5). This may be one of the mechanisms of resistance to stress in P. notoginseng.

In addition, the qRT-PCR analysis in this study showed that the trend of transcript abundance was similar to that of RNA-Seq, which confirmed the reliability of our RNA-Seq data. The proteins of these 5 genes were also selected for PRM quantitative verification of protein abundance. However, 4 out of 5 proteins (80%) showed similar abundance trends between PRM and label free quantification. Therefore, the protein abundance from the unlabeled proteome data is reliable.

5. Conclusion

We used transcriptome sequencing and label free quantification techniques to identify 136,100 transcripts and 4,468 proteins before and after Alternaria panax infected the leaves of P. notoginseng. As far as we know, this is the first time that transcriptome and proteome analysis have been combined to study the response of P. notoginseng to disease. In this study, the integration of transcriptome and proteome profiles revealed many candidate transcripts/proteins, which may be involved in lignin synthesis during the defense response of P. notoginseng. In response to the stress of pathogenic bacteria, the synthesis of lignin is mainly regulated by the proteins 4CL, COMT, CAD and POX. This study provides a wide range of new information about the transcriptome, proteome and their correlation of P. notoginseng in response to black spot disease stress. The analysis of this resource allows us to examine the mechanisms of transcription and protein diversification, which expands the knowledge of the complexities of the transcriptome and proteome in traditional Chinese medicines.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

All data are fully available without restriction.

Notes
RNA-Seq data generated in this study is available from the Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA706472. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (https://www.ebi.ac.uk/pride/archive) via the PRIDE partner repository with the data set identifier PXD024542.

**Competing interests**

The authors declare no conflict of interest.

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**Authors' Contributions**

Cui X. conceived and designed the study. Yang Q. performed most of the experiments, analyzed the data, wrote and revised the manuscript. Li J., Xiao L. performed or contributed to parts of some experiments. Peng J. and Sun J. analyzed the data, interpreted the results.

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Not applicable.

**Supplementary Information**

Supplementary information includes 2 methods and 6 tables.

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Table 1

Table 1 table of KEGG enrichment integration analysis of each functional item protein, gene quantity and quantity statistics
Map name, KEGG pathway name; Proteome Num, the number of proteins annotated to the pathway; Transcriptome Num, the number of genes annotated to the pathway; Correlation Num, the number of protein/gene associations annotated to the pathway.

Figures

Figure 1

Plant phonology and physiological analysis of *P. notoginseng* 5 days after inoculation with *Alternaria panax*. A, Phenotypic changes of *P. notoginseng* leaves after inoculation (0h-120h). B, Bird view of *P. notoginseng* plants in pots with (Inf, right) or without (Cont, left) *A. panax* infection. C, Content of chlorophyll a, chlorophyll b and the total chlorophyll (mg g⁻¹ fw). D-H, content of H₂O₂ (mmol g⁻¹ prot, D), activity of anti-superoxide anion (U mg⁻¹ prot, E), activity of SOD (U mg⁻¹ prot, F), activity of POD (U mg⁻¹ prot, G), and activity of CAT (U mg⁻¹ prot, H) in leaves of *P. notoginseng* with (Inf) or without (Con) infection. Bar=1 cm, values are means ± SE, Different letters indicate significant differences at P <0.05 (Student's t test).
Figure 2

The transcripts profile of *P. notoginseng* in response to *Alternaria panax* infection. A, Heatmap of the relative expression levels (fold change after log10 transformation) of the 40 significantly differentially expressed genes (20 most up-regulated genes and 20 most down-regulated genes), and high positive and negative correlations marked in red and blue, respectively. B, KEGG bubble chart of differentially expressed genes. The vertical axis represents the name of the pathway, and the horizontal axis represents the rich factor corresponding to the pathway. The size of the FDR is represented by the color of the dots. The smaller the FDR, the closer the color is to red. The number of differential genes contained in each pathway is represented by the size of the scattered dots.
Figure 3

The proteins profile of P. notoginseng in response to Alternaria panax infection. A, Heatmap of the relative expression levels (fold change after log10 transformation) of the 40 significantly differentially expressed proteins (20 most up-regulated proteins and 20 most down-regulated proteins), and high positive and negative correlations marked in red and blue, respectively. B, KEGG pathway enrichment analysis of differential proteins. The vertical axis represents the significantly enriched KEGG pathway, the horizontal axis represents the number of differentially expressed proteins contained in each KEGG pathway, and the color of the bar graph indicates the significance of the enriched KEGG pathway, which is the P value calculated based on Fisher’s exact test. The color gradient represents the size of the P value, and the color changes from orange to red. The closer to red, the smaller the P value, and the higher the significance level of the enrichment of the KEGG pathway.
Figure 4

Correlations between of proteins and transcripts. Venn diagram of the numbers of all detected genes, regulated genes, all detected proteins and regulated proteins. All-Protein, all proteins that can be quantified in the protein group; all-mRNA, all quantifiable genes derived from the transcriptome; DE-Protein, the differential protein identified by the proteome; DE-mRNA, the differential gene identified by the transcriptome.
Figure 5

Validation and expression analysis of selected genes and proteins using qRT-PCR and PRM. A, Pathway of lignin synthesis. B, the relative expression of genes. C, protein expression. Transcription and protein levels are indicated by red and blue, respectively, and changes in levels are indicated by arrows. Increased abundance indicates upward regulation (up arrow). The horizontal line represents no significant change. PAL, phenylalanine ammonia-lyase; 4CL, 4-coumarate-CoA ligase; COMT, caffeic acid-O-methyltransferase; CAD, cinnamyl alcohol dehydrogenase; POX, peroxidase.

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