

Transcriptome and metabolome analyses of *Rosa chinensis* identify heat stress response genes and metabolite pathways

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Abstract

Background

Global warming has made high-temperature stress one of the most important factors causing crop yield reduction and death. In the rose flower industry, high-temperature stress leads to bud dormancy or even death, reducing ornamental value and incurring in economic loss. Understanding the molecular mechanisms underlying the response and resistance of roses to high-temperature stress can serve as an important reference for the cultivation of high-temperature-stress-resistant roses.

Results

To evaluate the impact of high temperature on rose plants, we initially measured physiological indices in rose leaves after heat stress. We observed a significant decrease in protein and chlorophyll content, while proline and malondialdehyde (MDA) levels, as well as peroxidase (POD) activity, increased. Subsequently, transcriptomics and metabolomics analyses were conducted to detect changes in gene expression and metabolite content after high-temperature stress. Compared to the untreated control (T0), the number of differentially expressed genes (DEGs) and differentially abundant metabolites (DAMs) in rose plants subjected to heat peaked at time points T6-T9. This trend closely aligned with the observed physiological changes. Enrichment analysis showed that most DEGs and DAMs primarily involved in the mitogen-activated protein kinases (MAPK) signaling pathway, plant hormone signal transduction, alpha-linolenic acid metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, etc.

After heat stress, the DEGs and DAMs combined analysis revealed a predominant downregulation of genes and metabolites related to the flavonoid biosynthesis pathway. Similarly, genes involved in the jasmonic acid pathway within the MAPK signaling pathway exhibited decreased expression, but genes associated with the ethylene pathway were mostly upregulated, suggesting a role in roses' heat stress responses. Furthermore, heterologous overexpression of the heat stress-responsive gene *RcHP70* in *Arabidopsis thaliana* increased resistance against heat stress.

Conclusion

The present study provides new insights on the genes and metabolites induced in roses in response to high temperature; the present results provide a reference for analyzing the molecular mechanism underlying resistance to heat stress in roses. The obtained candidate genes and metabolites could be valuable resources for breeding of heat stress resistant roses.

Background

Heat stress due to global warming poses a significant threat to food security and agricultural sustainability [1]. The elevated temperatures associated with heat stress can decrease water content, disrupting cellular homeostasis and compromising vital biological processes [2]. These physiological disturbances can have devastating effects, leading to reduced crop yields and, in extreme cases, plant

death [1]. *Rosa chinensis* is an important ornamental plant, with an optimum temperature to grow and bloom of 22–26°C. Roses are sensitive to high temperatures, which often decreases the number or size of flowers, makes them enter dormancy, and not blossom [3, 4].

Plants have evolved complex and diverse systems to cope with environmental heat stress [1, 2, 5]. At the molecular level, plants rely on transcriptional regulatory networks to orchestrate their stress response. Heat shock proteins (HSPs) and reactive oxygen species (ROS) are major makers used for detecting plant responses to heat stress [6–9]. The transcriptional regulatory network activated by heat stress is a highly dynamic and coordinated system. For instance, tomato and *Arabidopsis* heat shock transcription factor A1s (HfA1s) play critical roles in the heat shock response by decreasing induction of heat stress-responsive genes and heat stress sensitive phenotypes [10, 11]. Among them, some genes encode proteins involved in protecting cellular components from damage caused by heat stress, while others encode proteins involved in repairing damage or restoring cellular homeostasis [12–15].

In addition to transcriptional regulation, post-translational modifications play a pivotal role in the plant's heat stress response. These modifications, which include phosphorylation [16], ubiquitination [17], and SUMOylation [18–20], can alter the activity, localization, or stability of proteins involved in stress signaling and responses. For instance, Ca^{2+} and ROS may be involved in heat stress sensing through MAPK signaling [21–23]. Moreover, plant hormones such as jasmonic acid play a key role in abiotic stress responses [24] and early signaling enhances heat tolerance in *Arabidopsis* [25]. In roses, Li et al. found Ca^{2+} signaling pathways and transcription factors associated with rapid sensing and signal transduction in heat stress responses [4].

Despite these advances in understanding the molecular mechanisms underlying plant heat stress responses, the molecular mechanism underlying the response to heat stress in roses remains unknown. In this study, the “red apple” rose variety was used and subjected to RNA-seq and metabolomic analysis to identify candidate genes and metabolites. The results indicated that the genes and metabolites related to flavonoid biosynthesis and MAPK signaling pathway may involve in heat stress response. The results provide valuable information for molecular breeding of resistance rose variety.

Materials and Methods

Plant materials and heat stress treatment

Two-year-old “red apple” rose cuttings (rootstock, white-flowered roses) were used as experimental materials. After being purchased from a nursery, they were pre-cultured in an artificial climatic chamber for 10 days under the following conditions: temperature of 24°C, humidity of 55%, light duration of 12 h (day) / 12 h (night), and a light intensity of 100%. Subsequently, high-temperature treatments at 42°C were conducted for 0 h (Treatment 0 h, T0), 3 h (T3), 6 h (T6), 9 h (T9), and 12 h (T12), respectively. After each treatment, functional leaves were collected, immediately frozen in liquid nitrogen, and stored in a

-80°C freezer for subsequent transcriptome and metabolome sequencing. The transcriptome analysis included three biological replicates and the metabolome analysis included six biological replicates.

Photosynthetic and antioxidant enzyme analysis

Plant leaves were collected to determine the concentration of photosynthetic pigments and proline. Samples (0.5 g fresh weight per treatment) were extracted in 80% (v/v) methanol and thoroughly ground. The concentration in the supernatant of this mixture was determined spectrophotometrically as described by Wang and Huang [26]. Malondialdehyde (MDA), peroxidase (POD), and protein levels were analyzed using specific assay kits obtained from Nanjing Mo Fan Biotechnology Co., Ltd (Nanjing, China).

RNA extraction and sequencing

The total RNA of leaves was extracted according to the protocol for extraction of plant RNA (<https://www.yuque.com/yangyulan-ayaeq/oupzan>). After measuring the RNA level and quality, RNAs were used to construct libraries according to the protocol for mRNA library preparation (BGI, China). Then, the libraries were sequenced by DNBSEQ (BGI, China). The SOAPnuke (v1.5.6) software was used for quality control of the raw data [27, 28]. The reference *R. chinensis* genome and annotation files were downloaded from the NCBI database (accession no. GCF_002994745.2) [29]. The clean data was mapped to the reference genome by HISAT (v2.1.0) [30]. Bowtie2 was applied to align the clean reads to the gene set, where known and novel as well as coding and noncoding transcripts were included [31]. Gene expression levels were calculated by RSEM (v1.3.1) [32]. Differentially expressed gene (DEG) analysis was performed using DESeq2 (v1.4.5) with a q -value ≤ 0.05 [33]. The time series analysis was performed using Mfuzz (v2.34.0) [34], and gene co-expression network analysis was performed with WGCNA (v1.48).

Metabolites extraction and analysis

A total of 50 mg tissues were extracted by directly adding 800 μ L of precooled extraction reagent (MeOH: H₂O) (70:30, v/v, precooled at -20°C); then, 20 μ L of internal standards mix was added for quality control of the sample preparation. Two small steel balls were added to the Eppendorf tube. After homogenizing at 50 Hz for 5 min by using TissueLyser (JXFSTPRP, China), samples were sonicated for 30 min at 4°C and incubated at -20°C for 1 h. Then, samples were further centrifuged for 15 min at 14,000 rpm and 4°C. Next, 600 μ L of the supernatants were filtered through 0.22 μ m microfilters and transferred to autosampler vials for liquid chromatography mass spectrometry (LC-MS) analysis. To evaluate the reproducibility and stability of the whole LC-MS analysis, a quality control (QC) sample was prepared by pooling 20 μ L of the supernatant from each sample.

Sample analysis was performed on a Waters ACQUITY UPLC 2D (Waters, USA), coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, USA) with a heated electrospray ionization source. Chromatographic separation was performed on a Hypersil GOLD aQ column (2.1 $\times$ 100 mm, 1.9 μ m, Thermo Fisher Scientific, USA), with mobile phase A consisting 0.1% formic acid in water and mobile

phase B consisting 0.1 formic acid in acetonitrile. The column temperature was maintained at 40°C. The gradient conditions were as follows: 5% B over 0.0–2.0 min, 5–95% B over 2.0–22.0 min, held constant at 95% B over 22.0–27.0 min and washed with 95% B over 27.1–30 min. The flow rate was 0.3 mL/min and the injection volume was 5 µL.

The mass spectrometric settings for positive/negative ionization modes were as follows: spray voltage, 3.8/–3.2 kV; sheath gas flow rate, 40 arbitrary units (arb); aux gas flow rate, 10 arb; aux gas heater temperature, 350°C; and capillary temperature, 320°C. The full scan range was 100–1,500 m/z with a resolution of 70,000, and the automatic gain control (AGC) target for MS acquisitions was set to 1e6 with a maximum ion injection time of 100 ms. Top three precursors were selected for subsequent mass spectrometry fragmentation with a maximum ion injection time of 50 ms and a resolution of 30,000, the AGC was 2e5. The stepped normalized collision energy was set to 20, 40, and 60 eV.

Metabolome data preprocessing

The raw data collected by LC-MS/MS was imported into Compound Discoverer 3.1 (Thermo Fisher Scientific, USA) for data processing. The molecular weight, retention time, peak area and identification were derived from this analysis. Metabolites were identified using the BGI self-built standard library and mzCloud database. Data preprocessing was performed using metaX [35]. The DAMs between groups were screened by multivariate statistical analysis using principal component analysis (PCA) and discriminant analysis, partial least squares method-discriminant analysis (PLS-DA) [36, 37], and univariate analysis using fold-change analysis and T test (Student's T test). DAM screening thresholds were as follows: the variable importance in projection (VIP) values of the first two principal components of the PLS-DA model ≥ 1 , Fold-Change ≥ 1.2 or ≤ 0.83 , and q -value < 0.05 .

Function enrichment analysis

Gene ontology (GO) (<http://www.geneontology.org/>) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (<https://www.kegg.jp/>) enrichment analysis were performed by Phyper based on a hypergeometric test. The significant levels of terms and pathways were corrected by a q -value with a rigorous threshold (q -value ≤ 0.05).

Correlation analysis of RNA-seq and metabolomic data

The correlation between DEGs and DAMs was analyzed based on regularized canonical correlation analysis (rCCA). Sparse partial least squares discriminant analysis (SPLSDA) was performed using mixOmics package in R [38].

Quantitative real-time PCR analysis

Total RNA was extracted and reverse transcribed into cDNA using the abovementioned method. Real-time qRT-PCR was performed using 20 µL of cDNA using the TB Green™ Premix Ex Taq™ II reagent (Takara; Tli RNaseH Plus); 16S RNA was used as internal reference gene. The primers of all detected genes are listed in Supplementary Table 1. Three biological replicates (each with three technical

replicates) were subjected to the QuantStudio™ 6 Flex System (Applied Biosystems, USA) with the following amplification parameters: activation at 50°C for 2 min, predenaturation at 95°C for 2 min, denaturation at 95°C for 15 s, and annealing at 60°C for 1 min for 40 cycles. The relative gene expression level was calculated using the $2^{-\Delta\Delta C_t}$ method.

RcHP70 overexpression in Arabidopsis and heat stress treatment

The vector *35S::RcHP70* was constructed and transformed into *A. tumefaciens* GV3101. Transformation of *Arabidopsis* plants was performed using the floral dip method. For selection, seeds were planted in aseptic conditions on MS agar containing 25 mg L⁻¹ hygromycin. T3 lines displaying 100% hygromycin resistance were considered homozygous and used for further experiments. Young seedling under high-temperature treatment at 42°C was conducted for 0 h, 0.5 h, 1 h, 2 h, and 3 h, respectively.

Results

Heat stress affects photosynthetic and antioxidant enzyme activities in *Rosa chinensis*

The physiological function of *R. chinensis* was affected by heat stress. Analysis of physiological and biochemical indices at various periods after heat stress indicated that the content of chlorophyll and total protein decreased significantly over time, reaching a minimum at 9 h before increasing/recovering (Fig. 1AB). Similarly, the proline content increased significantly after heat stress, reaching a maximum at 9 h, before slightly decreasing (Fig. 1C). Moreover, the POD activity level increased significantly and reached a maximum at 6 h, followed by a rapid decrease (Fig. 1D). MDA content showed a similar trend to that of POD, but the increase was relatively weak (Fig. 1E).

RNA-sequencing and screening for DEGs

After the five groups of samples were sequenced and processed, clean reads were mapped to the rose genome and the fragment per kilobase of transcript per million mapped value of each gene was calculated. PCA showed that the control group (T0) was significantly distinct from the treatment groups (T3–T12), with some treatment groups being relatively close to each other, such as T3 and T9. The samples within each group could also be effectively clustered into multiple replicates, indicating high reproducibility (Fig. 2A). Since multiple treatment groups were not clustered with the control group, indicating that significant changes occurred in multiple treatment groups, comparing multiple treatment group time points with the control group showed 11,233 DEGs (5,855 upregulated and 5,378 downregulated genes) at T3-vs-T0, and 13,403 DEGs at T6-vs-T0 (6,738 upregulated and 6,665 downregulated genes), T9-vs-T0 had 13,050 DEGs (6,448 upregulated and 6,602 downregulated genes), and T12-vs-T0 had 11,676 DEGs (5,693 upregulated and 5,983 downregulated genes; Fig. 2B).

Functional enrichment analysis of DEGs in the four compared groups showed that the common enriched pathways including: alpha-linolenic acid metabolism, sulfur metabolism, pentose phosphate pathway, glycerophospholipid metabolism, porphyrin and chlorophyll metabolism, etc. (Fig. 2C–F). In addition, some pathways were enriched only in the T3-vs-T0 comparison group, such as homologous recombination, nucleotide excision repair, terpenoid backbone biosynthesis, RNA degradation, spliceosome, plant hormone signal transduction, ether lipid metabolism (Fig. 2C); and the glyoxylate and dicarboxylate metabolism, glycosphingolipid biosynthesis, propanoate metabolism, nicotinate and nicotinamide metabolism, citrate cycle, and SNARE interactions in vesicular transport pathways were enriched only in the T6-vs-T0 comparison group (Fig. 2D). Further, limonene and pinene degradation was only enriched in the T9-vs-T0 comparison group (Fig. 2E) and the fructose and mannose metabolism, phosphonate and phosphinate metabolism, beta-amylase metabolism, alanine metabolism, beta-amylase metabolism, and pyrimidine metabolism were only enriched in the T12-vs-T0 group (Fig. 2F).

Function enrichment of DEGs in response to heat stress

Further analysis of the four comparison groups showed 4,652 common DEGs (Fig. 3A); the corresponding enriched metabolic pathways were plant-pathogen interaction, MAPK signaling pathway, spliceosome, mismatch repair, pentose phosphate pathway, homologous recombination, DNA replication, etc. (Fig. 3B). According to the pattern of gene expression changes during heat stress, the expression of genes in cluster 6 gradually increased after heat stress treatment, reaching the highest point at 6–9 h. The expression of genes in cluster 8 gradually decreased after heat stress treatment reaching the lowest point at 6–12 h (Fig. 3C). The genes in clusters 6 and 8 were basically consistent with the trends of changes detected in some physiological indices and antioxidant activities. Functional analysis of the genes in cluster 6 showed that they were mainly related to spliceosome, mismatch repair, DNA replication, sulfur metabolism, etc (Fig. 3D). The pathways significantly enriched of cluster 8 genes included plant-pathogen interaction, phenylpropanoid biosynthesis, flavonoid biosynthesis, MAPK signaling pathway, etc. (Fig. 3E).

Metabolomic changes in *R. chinensis* in response to heat stress

Further metabolomic examination of the heat treated samples showed high reproducibility of both positive ion mode (pos) metabolites and negative ion mode (neg) metabolites in samples from multiple time points (Fig. 4AB, Supplementary Table 2). A total of 723 pos metabolites and 432 neg metabolites were identified (Fig. 4C), mainly containing flavonoids (60 positive metabolites and 52 negative metabolites), terpenoids (39 positive metabolites and 33 negative metabolites), phenylpropanols (33 positive metabolites and 23 negative metabolites), phenols (15 positive metabolites and 15 negative metabolites), phenolic acids (16 positive metabolites and 11 negative metabolites), and others (Fig. 4D). Comparison of DAMs between the four treatment groups and the control group showed 243 DAMs (including 144 positive and 89 negative metabolites) in the T3-vs-T0 comparison group, 254 DAMs (including 157 positive and 97 negative) in the T6-vs-T0 comparison group, 246 DAMs in the T9-vs-T0

comparison group (including 165 positives and 81 negatives), and 265 DAMs in the T12-vs-T0 comparison group (including 174 positives and 91 negatives; Fig. 4E).

Enrichment analysis of the DAMs in four comparison groups showed that the enriched pos DAMs in KEGG metabolic pathways include alpha-linolenic acid metabolism and arginine biosynthesis, while the neg DAMs were enriched in pathways such as plant hormone signal transduction, cyanoamino acid metabolism, tropane, piperidine and pyridine alkaloid biosynthesis, aminoacyl-tRNA biosynthesis, phenylpropanoid biosynthesis, and glucosinolate biosynthesis (Fig. 4F–I). In addition, pos DAMs were significantly enriched in ABC transporters in the T6/9/12-vs-T0 comparison group except at the early stage of heat stress treatment (T3-vs-T0), related to the transmembrane transport of metabolites at the later stage of the treatment (Fig. 4F–I).

DAMs in response to heat stress

Further analysis showed that 57 common DAMs in the four comparison groups (Fig. 5A). These 57 DAMs were significantly enriched in the pathway of aminoacyl-tRNA biosynthesis, glucosinolate biosynthesis, and cyanoamino acid metabolism, and associated with two major metabolites, L-Isoleucine and L-Phenylalanine (Fig. 5B). Analysis of the changes in the content of these DAMs showed three clusters, cluster I showed the lowest content at T0, increasing significantly from T3 to T12; the changes in these metabolites were consistent with the trend in the changes in POD activities, proline, and MDA content. In cluster III, the trend was almost the opposite, with the highest content at T0, decreasing significantly thereafter; the trend was also consistent with the trend of changes to chlorophyll and total protein contents. In addition, the contents of cluster II metabolites decreased at T3 followed by a rapid increase at T6 and a subsequent rapid decrease (Fig. 5C).

Combined RNA-seq and metabolomic analysis

The Spearman correlation coefficient was calculated for DEGs and DAMs. Network diagrams were plotted for DEGs and DAMs with absolute correlation coefficient values > 0.9 and p-value < 0.05 (Fig. 6A). The significant nodes in the four comparison groups were L-Phenylalanine, jasmonic acid, 5-Fluoro, biocytin may play key roles in the plant's response to heat stress (Figs. 6B–E). The patterns in content changes divided the metabolites into three subgroups: cluster 1 upregulated after heat shock (T3–T12); cluster 2 downregulated after heat shock (T3–T12); cluster 3 increased at T6 and later decreased (Fig. 6F). This trend was consistent with the results shown in Fig. 5C. In addition, DAMs and DEGs in the four comparison groups were mainly enriched in alpha-linolenic acid metabolism, plant hormone signal transduction, phenylpropanoid biosynthesis, etc. (Fig. 6G).

Heat stress response pathways

After heat stress, phenylpropanoid biosynthesis and downstream flavonoid biosynthesis were enriched in roses. Multiple hormones are involved in the regulation of the heat stress process. In the flavonoid biosynthesis pathway, the expression level of *CHI*, *HCT* (excluding 112197593 and 112165685), *FLS*, and

DFR genes was downregulated after heat stress. Four metabolites in this pathway showed significant changes, pinocembrin and dihydroquercetin decreased after heat treatment, and eriodictyol and dihydromyricetin showed an increasing trend after heat stress (Fig. 7A).

The significant enrichment of plant hormone signal transduction and MAPK signaling indicates a close relationship between these two pathways. We analyzed jasmonic acid and ethylene related pathway in the MAPK signaling pathway. The expression levels of *MKK3* and *MPK6* were significantly downregulated after heat stress, which play an inhibitor of *MYC2* and caused upregulated of *MYC2* homologues (except 112200522). In addition, *ERF1* repression (whose expression levels increased after heat stress) also play a role in regulated *VSP2* expression (Fig. 7B). In the ethylene signaling pathway, *RAN1*, *ETR*, and *CTR1* (excluding 112178901) significant increased their expression levels while *MPK3/6* expression was inhibited. In addition, *MYC2*, involved in the ethylene pathway, and which coordinates the regulation of *ChiB* expression levels, upregulated the expression of a ChiB homologous after heat stress (Fig. 7B).

RNA-seq expression validation by qRT-PCR

qRT-PCR was used to confirm the reliability of our RNA-seq data. The results of this analysis revealed that the expression patterns of 16 selected DEGs were consistent with the RNA-seq dataset (Fig. 8). Among these genes, *HSP70*, a typical marker gene in response to heat stress, showed significant up-regulation after heat stress. WRKY transcription factors (four homologues), RBOP, and PR1, crucial genes in the MAPK signaling pathway, were downregulated after heat stress, which suggested that heat stress may regulate the resistance of rose to high temperatures (Fig. 8).

RcHSP70 overexpression decreases rose's timely responses to high temperature

To determine whether *RcHSP70* has a function in resistance to heat stress, *RcHSP70* was introduced into *Arabidopsis* wild-type (WT). *RcHSP70* overexpression (OE) plants showed obviously stronger than WT (Fig. 9A, 0h). After high-temperature treatment, *RcHSP70* OE plants showed higher resistance to high temperature, as well as slowed water loss and wilting rate than WT plants (Fig. 9A). High *RcHSP70* expression was also detected in *Arabidopsis* OE plants by qRT-PCR (Fig. 9B, 0h). After high-temperature treatment, *RcHSP70* expression significantly increased in both OE and WT plants, indicating that heat stress induces *RcHSP70* expression. In OE plants, *RcHSP70* expression was significantly lower than that in WT. *RcHSP70* expression was approximately ≥ 20 -fold than that of untreated plants, but OE2 and OE3 plants increased approximately 4- to 14-fold (OE5 increasing similarly to WT; Fig. 9B).

Discussion

As an important ornamental plant, rose holds a high market share in flower industry and has important economic value. Breeding varieties resistant to high temperatures is crucial for increasing the production of rose flowers. Although key genes involved in heat stress responses have been reported in other

species, whether the regulatory and responsive mechanisms in roses are consistent with those species remains unclear. Li et al. conducted a preliminary exploration of heat stress-responsive genes in roses through transcriptome analysis, but there is still a lack of systematic research on the relationship between gene and metabolite changes after heat stress [4]. This study further explored the characteristics of gene and metabolite changes in roses induced by high temperature using transcriptomic and metabolomic methods.

In this study, we found a higher number of DEGs at T6 and T9 time points than at T3 and T12, indicating that the impact of heat stress on roses peaks at T6 and T9. This finding is generally consistent with multiple physiological indicators, such as the lowest chlorophyll and protein content at T9 and the highest proline content at T9. The POD and MDA activities reached their peaks at T6. Typically, the activity of plant is often inhibited under high-temperature stress, leading to suppressed protein and chlorophyll synthesis. Both substances reached their lowest levels around 9 h after heat stress, suggesting that the impact of heat stress on roses may be less severe during early stages or within 9 h of continuous heat stress. The accumulation of proline helps plants tolerating high-temperature stress [39–41]. In roses, the proline content peaked at 9 h of high-temperature stress. POD and MDA activity are usually associated with a plant's ability to cope with stress, indicating that roses gradually accumulate substances to resist high temperatures during stress responses.

This study employed transcriptomic and metabolomic approaches. Despite the vast amount of gene data changed in roses after high-temperature treatment, the enrichment of DEGs and DAMs exhibited high similarity. Significant enrichments were observed in pathways such as alpha-linolenic acid metabolism, flavonoid biosynthesis, phenylpropanoid biosynthesis, plant hormone signal transduction, and MAPK signaling pathway, suggesting a strong correlation between DEGs and DAMs.

The accumulation of flavonoids plays a crucial role in increasing a plant's tolerance to heat stress. For instance, flavonoids have a short-term heat stress effect in *Anoectochilus roxburghii* [42], and flavonoid accumulation regulation through hormones reduced heat stress in rice [43]. In this study, flavonoid biosynthesis, a downstream pathway of phenylpropanoid biosynthesis, exhibited a decreasing trend in the expression levels of multiple genes. Similarly, the contents of several metabolites (eriodictyol, dihydroquercetin, and pinocembrin) in this pathway decreased during the early stages of heat stress, consistent with gene expression trends. However, the metabolite levels increased at T12, possibly to facilitate adaptation and recovery after heat stress.

Plant hormones and MAPK signaling are involved in various stress responses and crosstalk closely [44–48]. The MAPK pathway not only responds to pathogen infection but also plays a crucial role in responses to plant hormone regulation, cold, salt, drought, and wounding [47, 48]. In this study, plant hormones and MAPK signaling were significantly enriched after high-temperature stress, indicating that this type of stress may share response pathways with the aforementioned stresses. In particular, regulatory pathways involving jasmonic acid and ethylene showed different expression patterns. Multiple genes in the jasmonic acid pathway tended to decrease their expression levels after high-temperature

stress, while genes in the ethylene pathway did the opposite. Although both pathways are involved in defense responses, further research is needed to determine whether the gene expression changes are due to heat stress induction or resistance responses.

HSPs are crucial response proteins and markers for detecting heat stress in plants [6, 49, 50]. HSPs are diverse and possess the important function of restoring proteins denatured due to heat stress to their undenatured state [7]. Therefore, the high expression and constitutive presence of HSPs in plants provide the potential for timely restoration of denatured proteins. In this study, we heterologously overexpressed a selected HSP70 protein in *Arabidopsis thaliana*, and the results confirmed that overexpressing RcHSP70 in *Arabidopsis* plants confers a significant advantage in resisting high-temperature stress.

Conclusions

This study examined genetic and biochemical changes in rose plants at five time points after heat stress. DEGs and DAMs were enriched in pathways including phenylpropanoid biosynthesis, MAPK signaling, alpha-linolenic acid metabolism, etc. The present results suggest that flavonoids and plant hormones play crucial roles in rose plant's resistance to heat stress. Our findings provide insights into rose response to high temperature and can serve as foundation to improve rose plant resistance against heat stress.

Abbreviations

MDA Malondialdehyde

POD Peroxidase

DEG Differentially expressed gene

DAMs Differentially abundant metabolites

MAPK Mitogen-activated protein kinases

HSP Heat shock proteins

ROS Reactive oxygen species

HfA1 Heat shock transcription factor A1

LC-MS Liquid chromatography mass spectrometry

QC Quality control

AGC Automatic gain control

PCA Principal component analysis

PLS-DA Partial least squares method-discriminant analysis

VIP Variable importance in projection

GO Gene ontology

KEGG Kyoto Encyclopedia of Genes and Genomes

rCCA regularized Canonical correlation analysis

SPLSDA Sparse partial least squares discriminant analysis

pos positive ion mode

neg negative ion mode

Declarations

Acknowledgements

Not applicable.

Authors' contributions

H.W. conceived the study; W.X., X.Z. and S.Z. analyzed the data; S.Z. was involved in data interpretation; R.L. and A.R. prepared figures and tables; S. J. and W.L. collected the samples and performed the experiments; H.W. and L.W. wrote the article. All authors read and approved the final version of the manuscript.

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Data availability

All the raw data used in this study have been deposited at NCBI BioProject ID: PRJNA1090540 (<http://www.ncbi.nlm.nih.gov/bioproject/1090540>).

Ethics approval and consent to participate

The use of plant parts in the present study complies with international, national and/or institutional guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

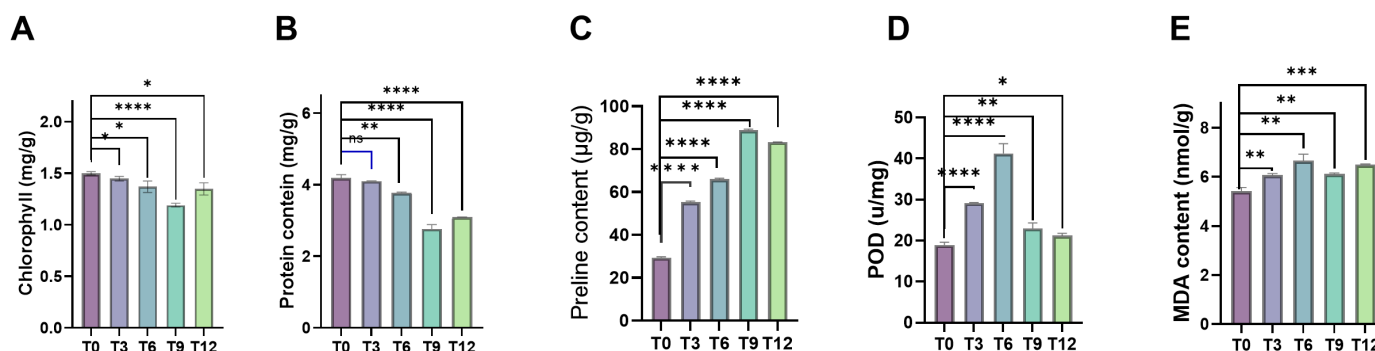


Figure 1

Photosynthetic and antioxidant enzyme activities of *Rosa chinensis* after heat stress. (A) Total chlorophyll content. (B) Total protein content. (C) Proline content. (D) POD activities. (E) MDA content. Values are means ± SD (n = 3 biological replicates); Asterisks indicate statistically significant differences as determined by Student's t-test: ns, no significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

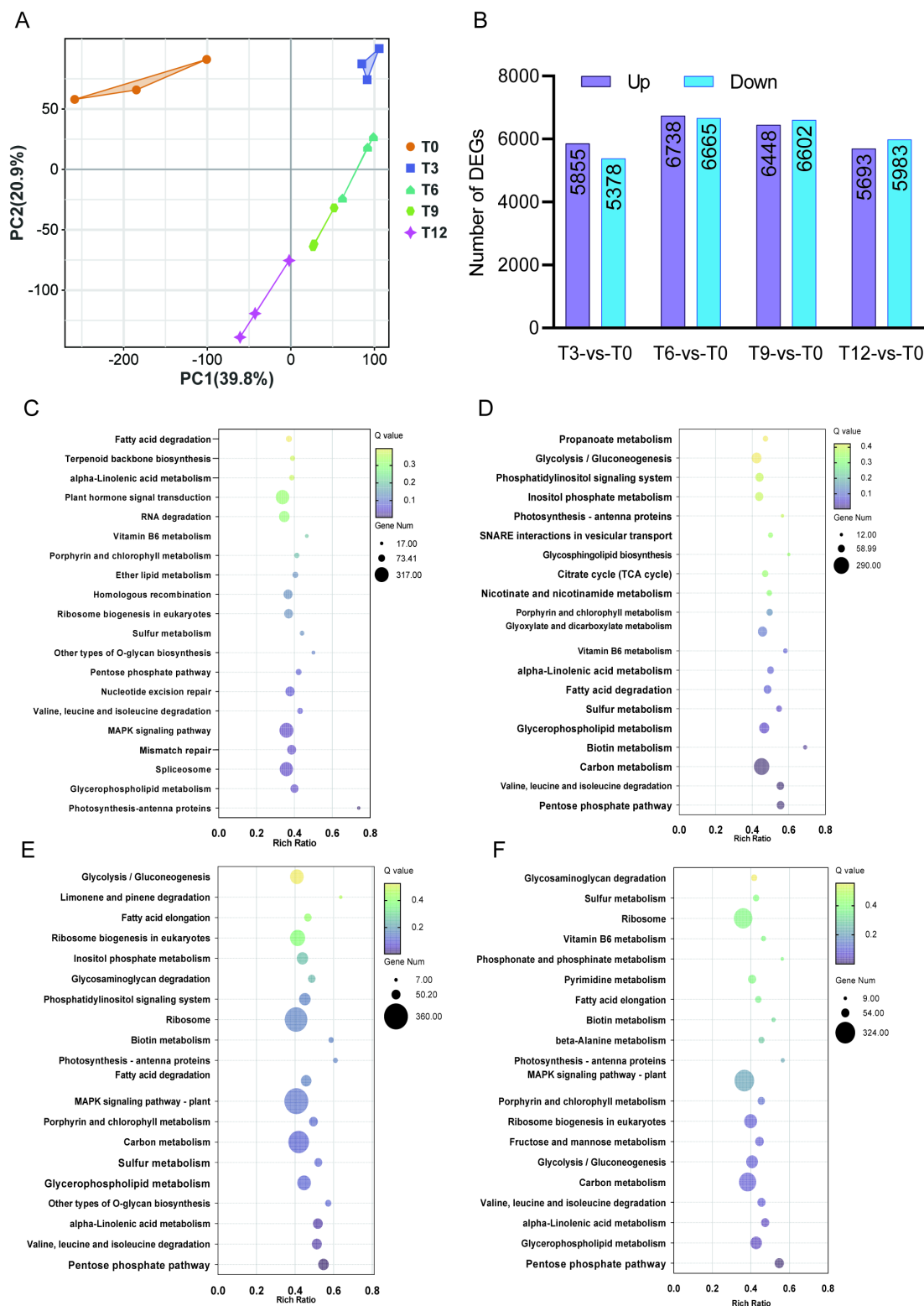


Figure 2

Analysis of DEGs and the main KEGG pathways enriched by DEGs in *Rosa chinensis* response to heatstress. (A) PCA analysis of gene expression datasets. (B) Number of DEGs among compared groups. (C) KEGG analysis of DEGs between T0 and R3h. (D) KEGG analysis of DEGs between T0 and T6. (E) KEGG analysis of DEGs between T0 and R9h. (F) KEGG analysis of DEGs between T0 and R12h. The color and size of the bubbles indicate significant enrichment and gene number, respectively.

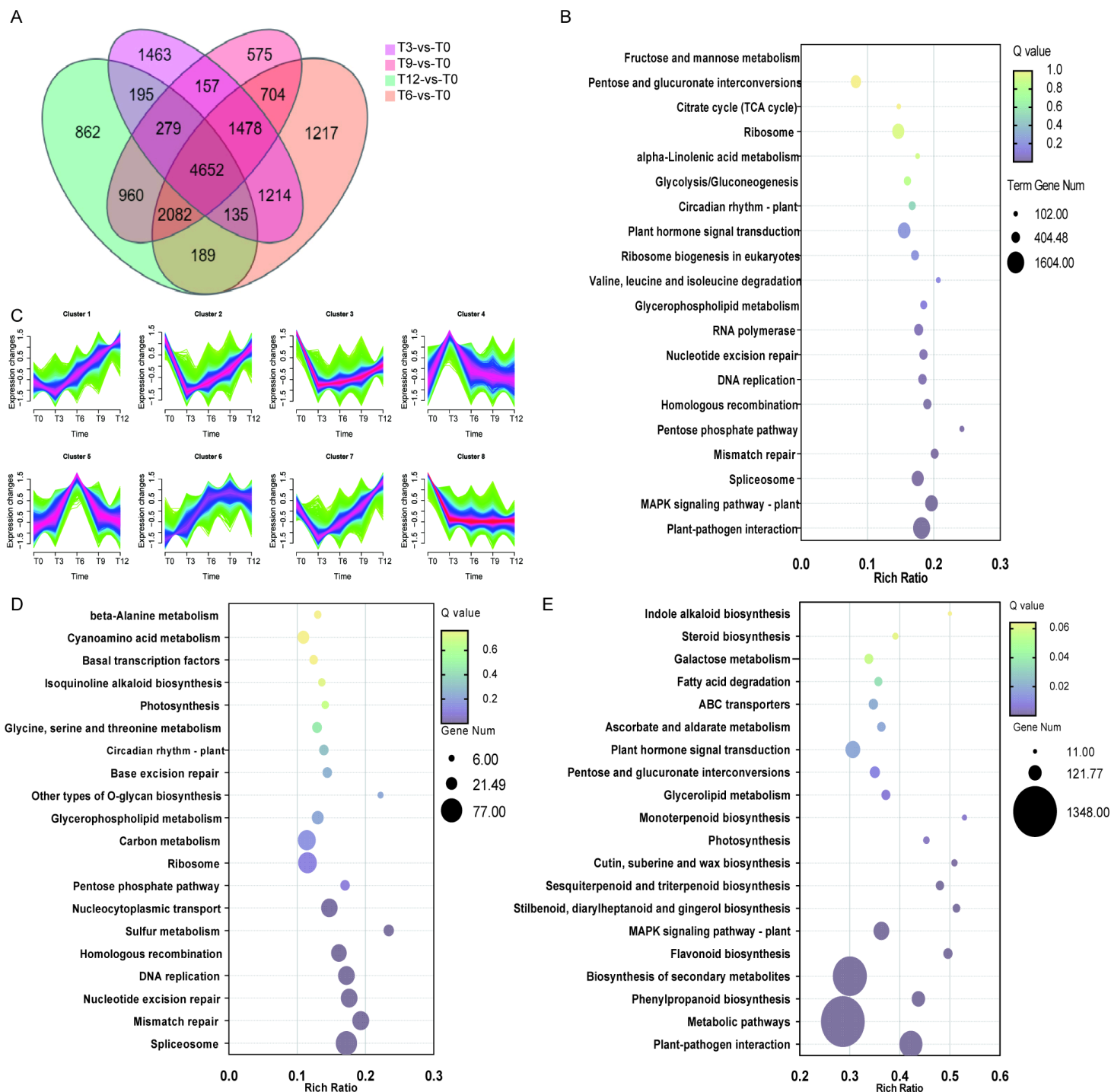


Figure 3

Venn diagram of the comparison groups and function enrichment analysis. (A) Venn diagram of the four comparison groups. (B) KEGG analysis of common DEGs in four comparison groups. (C) The trend pattern analysis of all genes expression level. (D) KEGG analysis of cluster 6 genes in Figure 3C. (E) KEGG analysis of cluster 8 genes in Figure 3C. The color and size of the bubbles indicate significant enrichment and gene number, respectively.

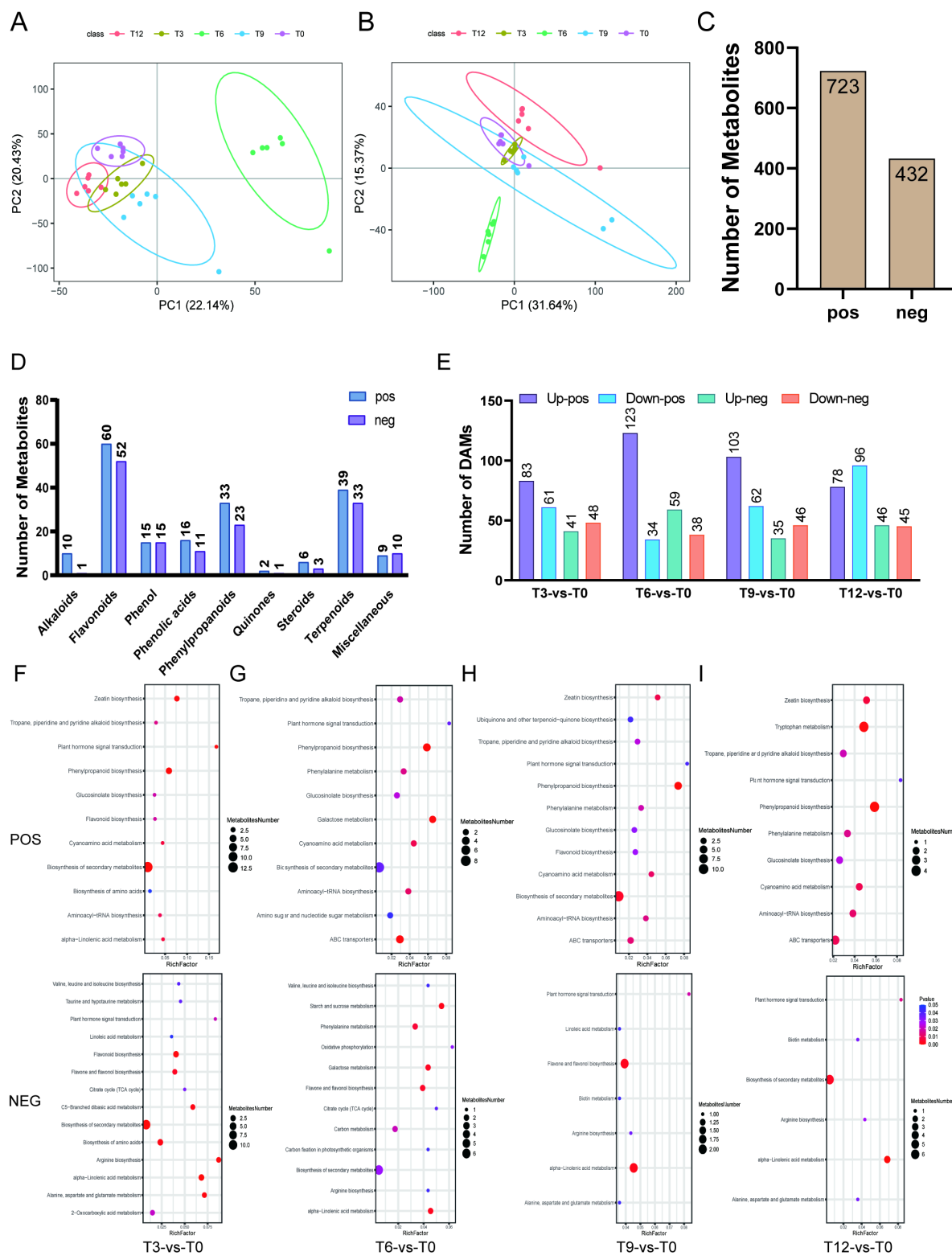


Figure 4

DAMs of *Rosa chinensis* in response to heat stress. (A) Principal component analysis of pos metabolites. (B) Principal component analysis of neg metabolites. (C) Number of identify metabolites. (D) Classified of metabolites. (E) Number of DAMs among compared groups. (F) KEGG analysis of DAMs between T3-vs-T0. (G) KEGG analysis of DAMs between T6-vs-T0. (H)KEGG analysis of DAMs between T9-vs-T0. (I) KEGG analysis of DAMs between T12-vs-T0.

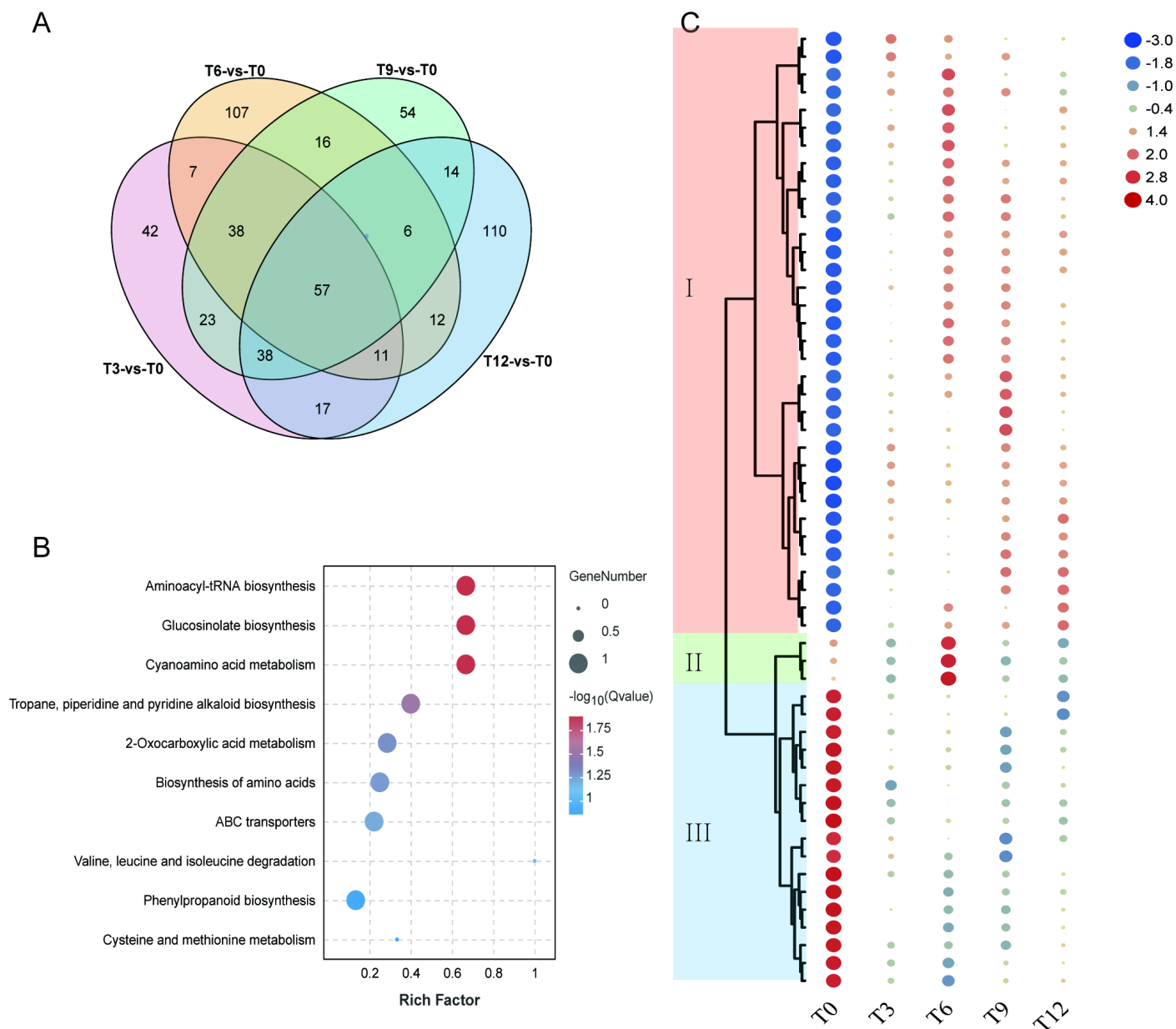


Figure 5

Venn diagram of DAMs in the comparison groups and function enrichment analysis. (A) Venn diagram of DAMs in the four comparison groups. (B) KEGG analysis of common DAMs in four comparison groups. (C) The heatmap of common DAMs in the four comparison groups. Three subgroups marked with cluster I, II, III.

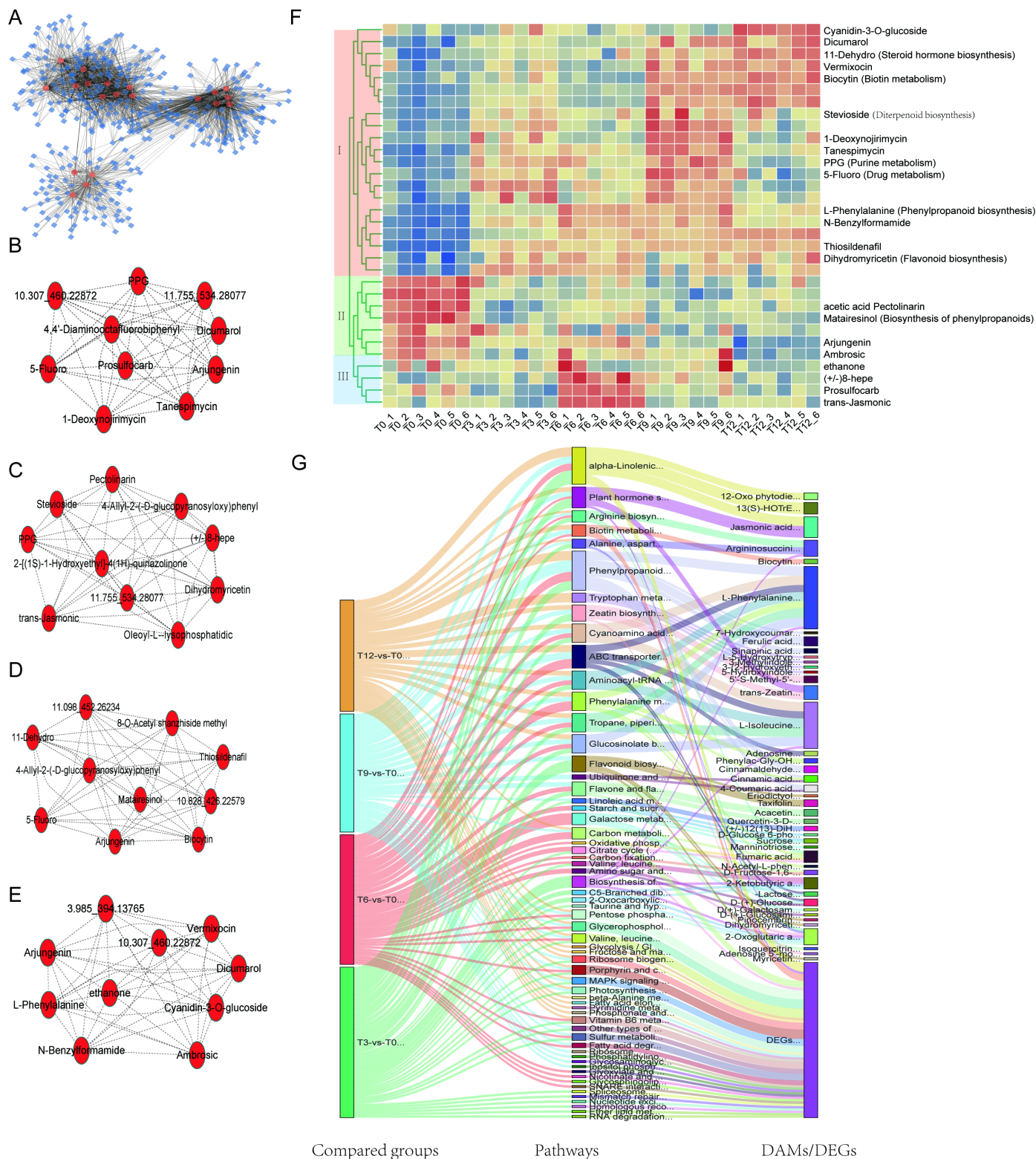


Figure 6

The connection network between DEGs and DAMs. (A) The schematic of network between DEGs and DAMs. The rhomboid and dots represent genes and metabolites, respectively. (B) The top10 core nodes DAMs in compared group T3-vs-T0. (C) The top10 core nodes DAMs in compared group T6-vs-T0. (D) The top10 core nodes DAMs in compared group T9-vs-T0. (E) The top10 core nodes DAMs in compared group T12-vs-T0. (F) The heatmap of core DAMs in four compared groups. Log2-scaled metabolites

content are shown, ranging from low (blue) to high (red). Three subgroups marked with cluster I, II, III. (G) Sankey diagram showing the relationship between pathways and DAMs/DEGs, the DEGs number were more than 4000, the gene name were not showed.

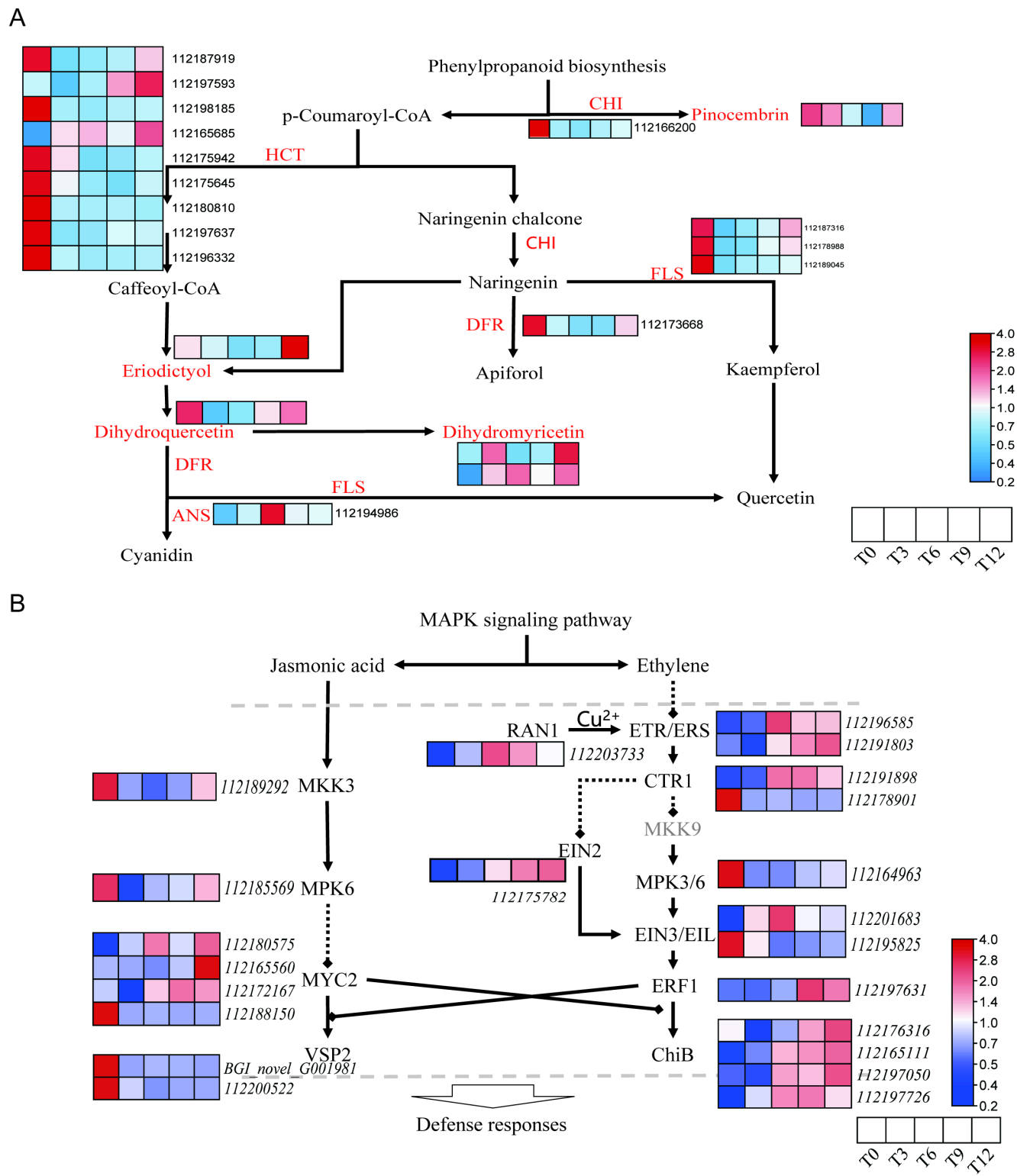


Figure 7

The pathways of flavonoid biosynthesis and MAPK signaling pathway response to heat stress. (A) The flavonoid biosynthesis pathways. HCT: shikimate O-hydroxycinnamoyltransferase; FLS: flavonol

synthase; DFR: bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase; ANS: anthocyanidin synthase; CHI: chalcone isomerase. (B) The MAPK signaling pathway. MKK3: mitogen-activated protein kinase kinase 3; MPK6: mitogen-activated protein kinase 6; MYC2: transcription factor MYC2; VSP2: vegetative storage protein 2; RAN1/copA, P-type Cu⁺ transporter; ETR/ERS: ethylene receptor; CTR1: serine/threonine-protein kinase; MPK3: mitogen-activated protein kinase 3; MPK6: mitogen-activated protein kinase 6; EIN2/3: ethylene-insensitive protein 2/3; ERF1: ethylene-responsive transcription factor 1; ChiB: basic endochitinase B. Log2-scaled FPKM or metabolites content are shown in different time points of leaf (here T0-T12, from left to right in each heatmap panel) are presented in the heatmap alongside the gene id. Low to high expression is indicated by a change in color from blue to red.

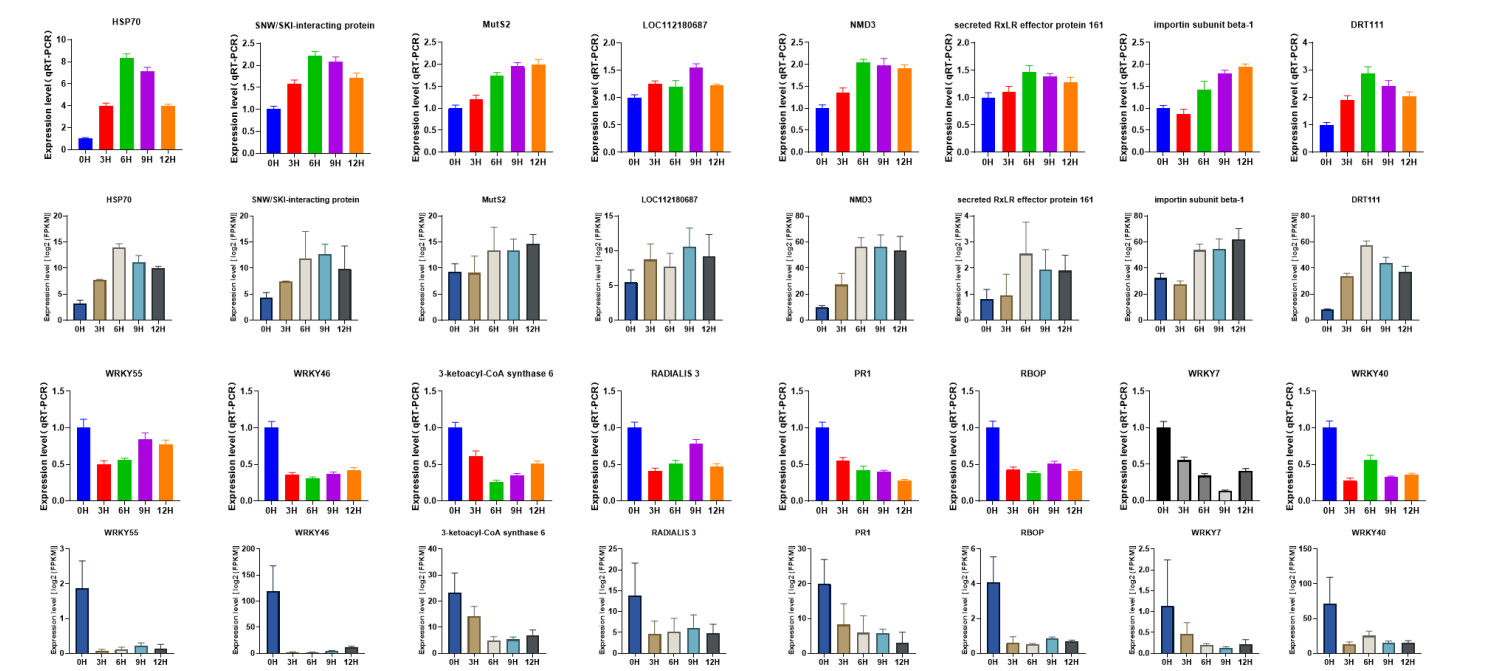


Figure 8

Expression pattern of the selected 16 genes. The first and third lines figures showed the expression level detect by qRT-PCR, and the corresponding second and fourth lines figures showed the gene expression data of RNA-seq.

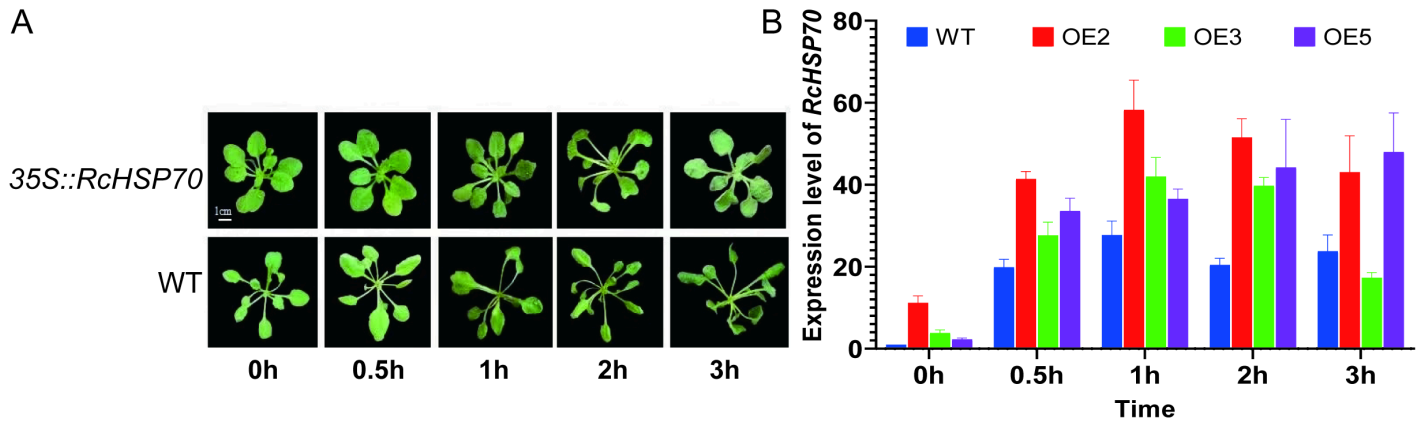


Figure 9

The *RcHSP70* gene enhance the resistance of *Arabidopsis* to heat stress. (A) Representative images showed the resistance of *Arabidopsis* to heat stress. (B) qRT-PCR analysis of *RcHSP70* expression in WT, OE2, OE3, and OE5 lines. 18S was used as the reference gene. Values are means $\pm$ SD of three technical replicates.