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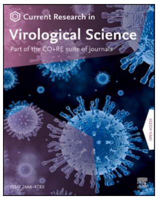

Transcriptome analysis of Crimson seedless grapevine (*Vitis vinifera* L.) infected by grapevine berry inner necrosis virus

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ABSTRACT

Grapevine fruits produced in China are often infected by grapevine berry inner necrosis virus (GINV). GINV disease is the most economically important viral disease of the grapevine, characterized by discoloration on the fruit surface and necrosis of the flesh. In this study, the transcriptome sequencing technology revealed 1700 differently expressed genes (DEGs), with 846 up-regulated and 854 down-regulated genes in Crimson seedless grapevine during the GINV infection. Both Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to classify the DEGs. The top 10 GO terms were enriched in GINV versus mock, and these enriched GO terms were mainly classified into three categories, namely, 324 cellular components, 2062 biological processes, and 940 molecular functions. Based on the *P*-values, the KEGG pathway analysis indicated that 96 major pathways were identified in GINV versus mock, and 20 major enrichment pathways were significantly enriched. Using quantitative real-time polymerase chain reaction (qRT-PCR), we validated the differential expression of 12 genes. The expression patterns of 11 genes were consistent with the RNA-sequencing (RNA-seq) results, except for one gene. Thus, our study provides comprehensive transcriptome information on the GINV–grapevine interaction, thereby improving our understanding of virus–host interactions.

1. Introduction

GINV was first identified in grapevine in 1984 in Japan (Yoshikawa et al., 1997). After being infected with GINV, the grapevine leaves were characterized by the development of the mosaic symptom and thus the disease was initially named grapevine mosaic disease in Japan (Yoshikawa et al., 1997). According to the latest International Committee on Taxonomy of Viruses (ICTV) Master Species List 2020 (https://talk.ictvonline.org/files/master-species-lists/), GINV belongs to the genus *Trichovirus* and the family Betaflexiviridae. GINV is a positive single-stranded RNA virus; its genome encodes three open-reading frames (RNA-dependent RNA polymerase, coat protein, and movement protein) and two untranslated regions. GINV is the most economically important viral disease of the grapevine in Japan. The infected grapevine berries show discoloration on the fruit surface and necrosis of the flesh (Kunugi et al., 2000). GINV disease has been known to be related to grape cultivars, the environment, susceptible cultivars, and insect vector transmission. In the field, GINV can be transmitted by grapevine erineum mites and lack of standardization farm work (Kunugi et al., 2000). GINV infection eventually leads to reduced nutritional quality and commodity values, with growers often suffering heavy losses.

GINV was first reported in 2016 in China by Fan et al., who identified it by sequencing small RNA extracted from the “Beta” and Thompson seedless grapevines (Fan et al., 2016). Next year, Fan et al. investigated the prevalence and genetic diversity of GINV in China, with 35.9% of samples testing positive for GINV (Fan et al., 2017). GINV disease is one of the most important diseases of grapevines and causes severe economic losses in China (Fan et al., 2016). Grapevines of susceptible cultivars grow less vigorously and sprout late in spring, and the vines exhibit inner necrosis of the berries, shoots, shortened internodes, and various patterns of yellow leaf discoloration (Yoshikawa et al., 1997; Fan et al., 2016, 2017). GINV is widely present in major grapevine rootstocks and cultivars in China, and it can be associated with chlorotic mottling and ringspot in grapevine berries (Fan et al., 2016, 2017).

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Viral infection is a complicated procedure, involving complex interactions between viruses and host plants. The suppression of host defenses by invading pathogens represents a major step in establishing a successful infection. Hosts have evolved multiple defense strategies, including antiviral RNA silencing and salicylic acid (SA)- and jasmonic acid (JA)-mediated defense responses, to protect themselves against viruses. As a counter-defense, plant viruses often manipulate plant responses for their benefit. The majority of plant viruses encode one or more RNA-silencing suppressors (RSSs) to counter antiviral RNA silencing (Ding and Voinnet, 2007). RNA-sequencing (RNA-seq) is a recently developed technique using deep-sequencing technology; it is similar to transcriptome profiling. It precisely measures the levels of transcripts and their isoforms than other methods (Wang et al., 2009). Rajarapu et al. identified functionally important genes in the salivary glands of the black-faced leafhopper using RNA-seq methods for transcriptome profiling analysis (Rajarapu et al., 2020). The RNA-seq results showed the significance of the interaction of the pathogen with maize and suggested targets of interest for further functional studies and improving pest control and disease transmission (Rajarapu et al., 2020). Similarly, Li et al. (2018) and Sun et al. (2017) used RNA-seq approaches to analyze host genes involved in the plant defense system against tobacco curly shoot virus and cucurbit chlorotic yellow virus infections. These results provided comprehensive transcriptomic information about countering counter-defense and will enhance our understanding of host-virus interactions. Therefore, this technique was widely applied to decipher the interactions between viruses and host plants.

In this study, we used the RNA-seq technology along with a next-generation sequencing (NGS) approach to identify differentially expressed genes (DEGs) in Crimson seedless grapevine following GINV infection. Our results showed that 1700 genes were differentially expressed after GINV infection, with 846 up-regulated and 854 down-regulated genes. Based on the *P*-values, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis identified 96 major pathways in GINV versus mock, with 20 major enrichment pathways as significantly enriched. Using quantitative real-time polymerase chain reaction (qRT-PCR), we validated the differential expression of 12 genes. We believe that the results of this study will help reveal the molecular mechanisms of GINV-host interactions and could identify new resistance genes. In addition, the findings contribute to a better knowledge of the molecular mechanisms of GINV-host interactions and provide a basis for devising new strategies to control the GINV disease complex.

2. Materials and methods

2.1. Plant materials, virus sources, and inoculation

Vitis vinifera cultivar "Crimson seedless" was selected for this study. Grapevine fruits were obtained from 10-year-old trees grown at a grapevine orchard at Xiaodian village grapevine orchard, Hongmen Town, Xinxiang City, Henan Province, China, on single-tree replicates for all measurements on October 26, 2020. All grapevine cv. Crimson seedless plants were detected for viruses twice annually and were tested negative for GINV. A GINV infectious clone was constructed to infiltrate Crimson seedless grapevine berries (Fan et al., 2020). The infectious clones of GINV isolate LN_Beta_RS were provided by Professor Yafeng Dong at the Chinese Academy of Agricultural Sciences.

The GINV infectious clone plasmid was transformed into the *Agrobacterium* strain EHA105. *Agrobacterium* cells were cultured in a tube at 28 °C, with shaking at 180 rpm in the Luria–Bertani (LB) medium for 8 h. When the *Agrobacterium* cells reached an optical density (OD) of 1.0 at 600 nm, cells were harvested by centrifugation and resuspended in 10 mL of MMA buffer (10 mM MES/NaOH, pH 5.6, 10 mM $MgCl_2$, 150 μM acetosyringone) in a centrifugal tube. The cells were cultured in the tube at 28 °C with shaking at 180 rpm for 1 h. Crimson seedless grapevine fruits with consistent growth status were selected for *Agrobacterium*-mediated transformation. The bacterial suspensions of GINV infectious clones and mock were injected individually into grapevine fruits with a 1-mL syringe when the fruits became hygrophilous. After 7 days, injected fruits were harvested, immediately frozen in liquid nitrogen, and stored at −80 °C until total RNA extraction.

2.2. RNA extraction, PCR detection, and qRT-PCR validation

The grapevine fruit samples were ground individually in liquid nitrogen, and total RNA was independently isolated from samples using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The complementary DNA (cDNA) synthesis and PCR were performed according to a previously described method (Wang et al., 2020). Coat protein (*CP*) gene-specific primer pairs were used to detect GINV. qRT-PCR was performed using the SYBR PrimeScript RT-PCR kit (Takara Shuzo, Kyoto, Japan) according to the manufacturer's instructions.

2.3. RNA-seq library construction and sequencing

A total amount of 3 μg of high-quality/non-degraded RNA per sample was used as input for RNA sample preparation. Oligo-dT-attached magnetic beads were used to purify the poly-A-containing mRNA molecules. The mRNA was broken into approximately 300 bp small molecular fragments.

First-strand cDNA was synthesized using the random hexamer-primed reverse transcription. Second-strand cDNA was generated using RNase H and DNA polymerase I, with the first-strand cDNA as the template. The final cDNA fragments were purified and washed for end-repair and ligated to sequencing adapters. The cDNA library was sequenced using the HiSeq 2500 equipment (Illumina, San Diego, CA, USA) (Sun et al., 2017). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using the Illumina PCR Primer Cocktail in a 15-cycle PCR reaction. Products were purified (AMPure XP system) and quantified using the Agilent high-sensitivity DNA assay on a Bioanalyzer 2100 system (Agilent). Next, the sequencing library was sequenced on a NovaSeq 6000 platform (Illumina) by Shanghai Personal Biotechnology Co., Ltd.

2.4. Mapping reads to the reference genome

The draft sequence of the *V. vinifera* reference genome was downloaded from the HTTPS site directly (https://plants.ensembl.org/Vitis_vinifera/Info/Index). Index of the reference genome was built using Bowtie v2.2.3 (Broad Institute, Cambridge, MA, USA) (Langmead and Salzberg, 2012), and single-end clean reads were aligned to the reference genome using TopHat v2.0.12 (Broad Institute, Cambridge, MA, USA) (mismatch = 2) (Kim et al., 2013; Li et al., 2018) (http://ccb.jhu.edu/software/hisat2/index.shtml).

2.5. Evaluation of differentially expressed genes

After annotation, the gene expression levels were compared between pairs of samples. The false discovery rate (FDR) was used to calculate the *P*-value in multiple tests. For the analysis, we used FDR ≤0.001 and a log2 ratio ≥1 to evaluate the significance of differences in gene expression.

We used HTSeq (0.9.1) statistics to compare the Read Count values on each gene as the original expression of the gene, and then used Fragments Per Kilobase of transcript per Million (FPKM) to standardize the expression. Afterward, the difference in the expression of genes was analyzed by DESeq (1.30.0) using the following screened conditions: expression difference multiple $|\text{log2FoldChange}| > 1$, significant *P*-value < 0.05. Simultaneously, we used the R package Pheatmap (1.0.8) to perform a bi-directional clustering analysis of all DEGs of samples. We obtained a heatmap according to the expression of the same gene in different samples and the expression patterns of DEGs in the same sample. The Euclidean method was used to calculate the distance and the complete linkage method for clustering.

The Plant Transcription Factor Database (PlantTFDB) was used to predict the transcription factors (TFs) and the family information to which the TFs belong.

2.6. GO term and KEGG pathway enrichment analyses

Gene Ontology (GO, http://www.geneontology.org/) enrichment analysis of DEGs was performed using the GOseq R package (corrected $P < 0.05$), in which the gene length bias was corrected (Young et al., 2010). We mapped all genes to terms in the GO database and calculated the numbers of differentially enriched genes in each term. The topGO package was used to perform GO enrichment analysis on DEGs, and the *P*-value was calculated using the hypergeometric distribution method (the standard of significant enrichment was $P < 0.05$), and the GO term with significantly enriched DEGs was identified to determine the main biological functions performed by DEGs. KEGG (http://www.genome.jp/kegg) is a database resource for understanding high-level functions and utilities of the biological system at the molecular level [17](Mao et al., 2005). ClusterProfiler (3.4.4) software was used to perform the enrichment analysis of the KEGG pathway of DEGs, focusing on the significant enrichment pathway with $P < 0.05$ (Mao et al., 2005).

3. Results

3.1. GINV elicits necrosis in Crimson seedless grapevine fruits

Previous research found that agroinoculated GINV full-length infectious cDNA clones can infect the "Beta" grapevine plants. The infection caused severe leaf chlorotic mottling and ringspot symptoms on the whole plant (Fan et al., 2020). To investigate whether the GINV infectious clone can infect grapevine fruits as well, we agroinoculated the Crimson seedless grapevine fruits with the GINV full-length infectious cDNA clone. Three independent experiments were conducted, and symptom development on the agroinfiltrated fruits was monitored daily. As shown in Fig. 1A, Crimson seedless berries infiltrated with GINV showed necrotic symptoms 7 days post-inoculation (dpi), whereas the mock-inoculated plants showed only mild mechanical injury on the surface of fruits. We used qRT-PCR to detect the GINV *CP* gene in the Crimson seedless fruit; the *CP* gene was detected in infiltrated GINV infectious clone fruits but not in mock fruits (Fig. 1B). Symptom observation and virus detection results showed that these fruits were reliable for follow-up RNA-seq.

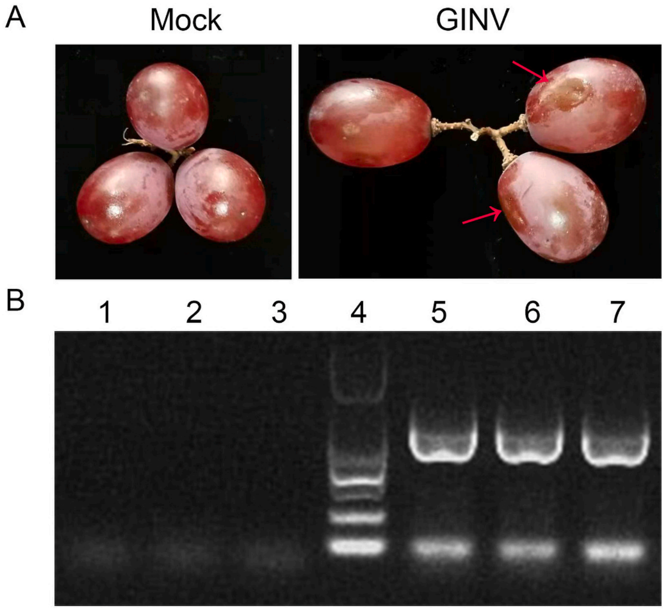

Fig. 1. Comparison of symptoms of virus-infected Crimson seedless grapevine fruits with mock at 7 dpi. (A) Crimson seedless grapevine fruits, mock infiltrated (left) and infiltrated with GINV (right). (B) Specific detection of GINV in Crimson seedless grapevine fruits for sequencing by qRT-PCR. Lanes 1–3 are infiltrated mock in Crimson seedless grapevine fruits. Lane 4 is the DNA marker. Lanes 4–6 are Crimson seedless grapevine fruits injected with GINV. Arrows indicate necrotic spots and infiltrated pinholes.

3.2. Overview of transcriptome sequencing

To profile differential gene expression during GINV infection, transcriptomic sequence libraries of mock- and GINV-inoculated Crimson seedless fruits were constructed. There were 6 GB of clean base data, and 17,958 unigenes were obtained from six samples. In total, 39,594,574 to 45,284,056 clean reads of GINV-infected grapevine fruits, and 37,712,490 to 39,976,358 clean reads of mock-inoculated grapevine berries were obtained (Table 1). More than 95.15% of the clean reads showed quality scores at the Q30 (an error probability for base calling of 0.1%) level (Li et al., 2018) (Table 1).

3.3. Analysis of DEGs after GINV infection

An FDR ≤0.001 and log2 ratio ≥1 were used to identify DEGs. In total, 1700 DEGs were identified, of which 846 were up-regulated and 854 were down-regulated in response to the GINV infection (Fig. 2). The fold change in the gene expression was mainly between 1 and 8 (Table S2).

3.4. GO enrichment analysis

GO, an internationally standardized gene function classification system, was used to classify the DEGs. Our results showed that DEGs contained 324 cellular components, 940 molecular functions, and 2062 biological process genes (Table S3). Further, the top 10 GO functional annotation terms were listed; the main categories identified corresponded to the thylakoid membrane, photosynthetic membrane, calcium transmembrane transporter activity, xyloglucan metabolism, cell wall organization or biogenesis, and cellular glucan metabolic process (Fig. 3).

3.5. KEGG pathway enrichment analysis

To further investigate the molecular and biological functions of DEGs, the genes were mapped to the KEGG database. Our results showed that 96 major pathways were identified in GINV versus mock (based on the *P*-values), and 20 major enrichment pathways were significantly enriched (Fig. 4). In addition, the KEGG pathway included 192 up- and 285 down-regulated genes, with major pathways involved in plant–pathogen interactions, photosynthesis, amino sugar and nucleotide sugar metabolism, starch and sucrose metabolism, and glyoxylate and dicarboxylate metabolism (Table S4).

3.6. Validation of selected genes by qRT-PCR analysis

As shown in Fig. 5, 12 genes were selected randomly for qRT-PCR analysis to confirm the RNA-seq expression patterns, including genes encoding *V. vinifera* basic helix-loop-helix 112 (*VvbHLH112*), *V. vinifera* heat shock protein 83 (*VvHSP83*), *VvWRKY57*, *V. vinifera* calmodulin-like protein 5 (*VvCaM5*), *V. vinifera* disease resistance protein (*RPM1*), *VvWRKY75*, *V. vinifera* pathogenesis-related protein 1 (*VvPR1*), *V. vinifera* allene oxide cyclase gene (*VvAOC*), *V. vinifera* allene oxide synthase 1 (*VvAOS1*), *VvbHLH111*, *VvWRKY20*, and *VvbHLH36*. These genes were selected based on significantly different expression patterns following GINV infection and a high degree of homology with genes that

Table 1
Summary of RNA-seq data.

Sample	Read no.	Bases (bp)	Clean read no.	Clean reads (%)	Q30 (bp)	Q30 (%)
Mock1	42837136	6425570400	37712490	88.03	6152992666	95.75
Mock2	45170442	6775566300	39976358	88.5	6449251229	95.18
Mock3	43476544	6521481600	38560488	88.69	6209128047	95.21
GINV1	44518176	6677726400	39594574	88.94	6354122948	95.15
GINV2	50685288	7602793200	45284056	89.34	7249454544	95.35
GINV3	46830398	7024559700	42089992	89.87	6693415606	95.28

Note: Read no.: read number. Bases: total bases. Clean read no.: reads from sequencing after filtering out low-quality reads. Q30 (bp): total bases with a Phred value > 30. N: Percentage of bases with fuzzy bases. Q30 (%): Percentage of bases with a Phred value > 30.

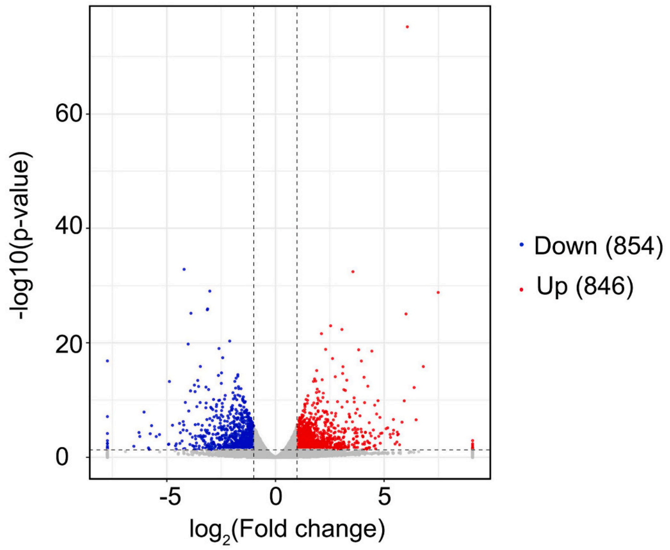

Fig. 2. Volcano plots of DEGs. The abscissa shows the fold change difference in the expression of genes in different groups. The vertical coordinates indicate the adjusted *P*-values for the differences in the expression. Genes without significant differences are indicated by gray dots. The up-regulated genes are represented by red dots, and the down-regulated genes are represented by blue dots. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

play essential roles in several metabolic pathways.

Our results indicated that the gene expression patterns revealed by qRT-PCR were consistent with those obtained by RNA-seq, except for one gene, *VvWRKY57* (Fig. 5). This discrepancy in the expression patterns for one gene between qRT-PCR and RNA-seq analysis was caused by a complicated gene family, which is allowed in the RNA-seq technique.

3.7. Analysis of JA and SA pathways

Plant hormones such as SA and JA are signal molecules that move through plants to stimulate responses to different environmental stresses. In addition, they are known for their roles in tuning plant defense responses to pathogen attacks (Alazem and Lin, 2015; Wang et al., 2021). The RNA-seq data and qRT-PCR showed that the expression of *VvAOC* and *VvAOS1*, the key JA synthesis genes, was significantly increased following GINV infection (Fig. 5, Table 2). We speculate that the expression of JA increases with GINV infection. In addition, the expression of JA and SA signaling pathways was altered partially; *V. vinifera putative methylesterase 11*, *V. vinifera protein TIFY 5A*, and *V. vinifera outer envelope pore protein 16* were up-regulated, and *V. vinifera protein SAR deficient 1* was down-regulated following GINV infection.

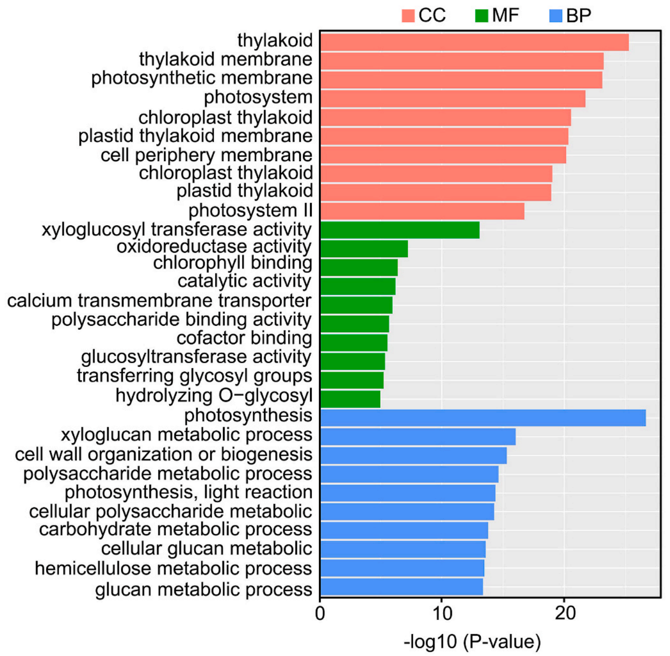

Fig. 3. GO enrichment analysis of DEGs. The vertical coordinates show most of the top 10 GO terms of three categories, and the abscissa coordinates indicate the adjusted *P*-values for the differences in the expression. CC, cellular component; MF, molecular function; BP, biological process.

3.8. Analysis of plant–pathogen interaction systems

Plants have evolved multiple defense strategies, including antiviral RNA silencing and SA- and JA-mediated defense responses, to protect themselves against viruses. As a counter-defense, plant viruses often manipulate plant responses for their benefit. The fate of the arms race between plants and viruses depends on the host–virus interaction (Zhao et al., 2021). Our RNA-seq data identified 13 up-regulated and 5 down-regulated genes in plant–pathogen interaction systems during GINV infection (Table 3). These results could provide a basis for host–virus interactions during GINV infection.

4. Discussion

GINV infection causes severe inner necrosis of berries and poor setting of fruits in grapevines, which directly affects the nutritional and commodity value of grapevines (Yoshikawa et al., 1997; Kunugi et al., 2000; Fan et al., 2020). GINV is a serious threat to grapevines in Japan and China (Yoshikawa et al., 1997; Fan et al., 2017). In this study, we used RNA-seq technology with an NGS approach to identify DEGs in Crimson seedless grapevine following GINV infection. Our results showed that 1700 genes were differentially expressed after GINV infection, with 846 up-regulated and 854 down-regulated genes (Table S2). Based on the *P*-values, the KEGG pathway analysis indicated

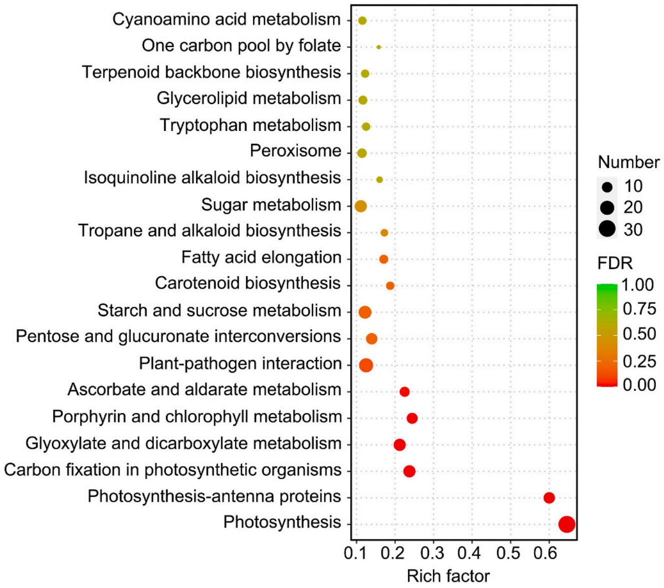

Fig. 4. KEGG pathways corresponding to the main significantly enriched DEGs. The rich factor stands for the degree of enriched DEGs in a given pathway. The number of enriched DEGs in the pathway is indicated by the circle area, and the circle color represents the range of the corrected *P*-value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that 96 major pathways were identified in GINV versus mock, and 20 major enrichment pathways were significantly enriched (Table S4).

Previous studies have reported that certain viruses infect host plants by disrupting SA- or JA-mediated plant defense (Zhang et al., 2017). For example, cucumber mosaic virus RSS 2b interferes with SA- (Ji and Ding, 2001) and JA-signaling pathways (Lewsey et al., 2010). Similarly, βC1 of tomato yellow leaf curl China virus (TYLCCV) has been reported to attenuate the JA signaling in plants (Li et al., 2014). JA and its oxylipin derivatives, called jasmonates, enable the plants to defend themselves against biotic and abiotic stresses. Under stress-free growth conditions, JA responses are restrained by a group of nuclear proteins called jasmonate zinc-finger inflorescence meristem (ZIM)-domain (JAZ) repressors. JAZ repressors interact with both the F-box protein coronatine insensitive 1 (COI1), an integral part of the SCF complex involved in the co-reception of biologically active JA (jasmonic-L-isoleucine [JA-Ile]), as well as with several JA-responsive TFs, including MYC2, a bHLH domain-containing TF (Chini et al., 2007; Thines et al., 2007). In this case, JAZ repressor proteins suppress the transcriptional activity of MYC2. In response to pest and pathogen attacks or mechanical wounding, JA-Ile is rapidly synthesized in the plant tissue (Kazan and Manners, 2013). In addition, MYC2 is a bHLH TF and a positive regulator of JA responses (Kazan and Manners, 2013). Our RNA-seq results identified 142 bHLH TF-like genes following GINV infection (Table S2). The qRT-PCR analysis revealed three bHLH TFs (VvbHLH111, VvbHLH112, and VvbHLH36) were significantly down-regulated following GINV infection (Fig. 5). This result was consistent with the RNA-seq expression pattern result. These results indicated the expression of bHLH TF family members might be repressed to benefit the GINV infection.

Lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) in the plastids and 12-oxophytodienoate reductase in peroxisomes are involved in the biosynthesis of JA (Wasternack and Hause, 2013). We found that five genes were up-regulated and one was down-regulated, all related to SA- and JA-signaling pathways, following GINV infection (Table 2). In addition, the JA synthesis key genes, *VvAOC* and *VvAOS1*, were significantly up-regulated following GINV infection (Fig. 5), implying that the expression of JA might increase against GINV infection. It is suggested that JA and SA pathways play a role in response to GINV infection.

An important feature of the plant defense response to pathogen attack is the induction and accumulation of several defense-related

Table 2
Unigenes of JA and SA pathways with significantly different expression patterns.

Protein symbol and properties	Unigene ID	Up/down expression
JA pathway		
Vitis vinifera allene oxide cyclase (VvAOC)	VIT_01s0011g03090	Up
Vitis vinifera allene oxide synthase 1 (VvAOS1)	VIT_18s0001g11630	Up
Vitis vinifera putative methylesterase 11	VIT_01s0011g00380	Up
Vitis vinifera protein TIFY 5A	VIT_10s0003g03810	Up
Vitis vinifera outer envelope pore protein 16	VIT_06s0004g04700	Down
SA pathway		
Vitis vinifera protein SAR deficient 1	VIT_17s0000g03390	Up

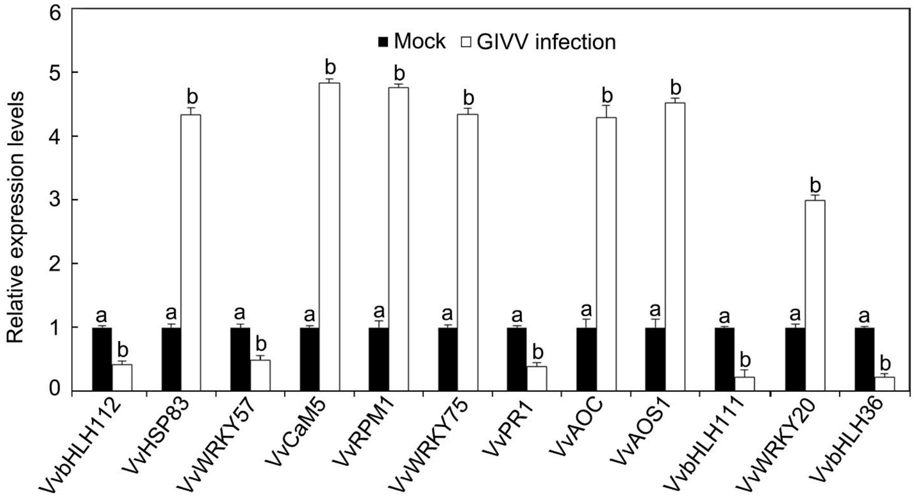

Fig. 5. Relative expression of DEGs in Crimson seedless grapevine fruits. The relative expression of genes encoding *VvbHLH112*, *VvHSP83*, *VvWRKY57*, *VvCaM5*, *VvRPM1*, *VvWRKY75*, *VvPR1*, *VvAOC*, *VvAOS1*, *VvbHLH111*, *VvWRKY20*, and *VvbHLH36*. Results are shown as the mean ± standard deviation (SD) of three independent experiments. Different letters above the bars indicate a significant difference at $P < 0.05$.

Table 3
Unigenes of plant–pathogen interaction systems with significantly different expression patterns.

Protein symbol and properties	Unigene ID	Up/down expression
Vitis vinifera heat shock protein 83 (VvHSP83)	VIT_02s0025g00280	Up
Vitis vinifera calmodulin-like protein 5 (VvCaM5)	VIT_05s0102g00450	Up
Vitis vinifera calcium-binding protein CML45	VIT_14s0108g01000	Up
Vitis vinifera calcium-binding protein	VIT_01s0010g02970	Up
Vitis vinifera disease resistance protein RPM1	VIT_07s0005g06210	Up
Vitis vinifera calcium-binding like protein	VIT_14s0006g01400	Up
Vitis vinifera calcium-binding protein CML41	VIT_18s0001g11830	Up
Respiratory burst oxidase homolog protein D	VIT_01s0150g00440	Up
Vitis vinifera calcium-binding protein CML19	VIT_14s0171g00150	Up
Vitis vinifera heat shock protein	VIT_16s0050g01150	Up
Respiratory burst oxidase homolog protein B	VIT_14s0060g02320	Up
Vitis vinifera calcium-binding protein CML15	VIT_17s0000g04460	Up
Vitis vinifera calmodulin-like protein 11	VIT_05s0020g04420	Up
Vitis vinifera probable disease resistance protein	VIT_09s0002g05070	Down
Vitis vinifera pathogenesis-related protein 1	VIT_03s0088g00710	Down
Vitis vinifera cyclic nucleotide-gated ion channel	VIT_04s0069g00790	Down
Vitis vinifera pathogenesis-related genes	VIT_06s0004g08190	Down
Vitis vinifera RPM1-interacting protein 4	VIT_00s0516g00010	Down

proteins, which are also a part of systematic acquired resistance (SAR). We found that 15 up-regulated and 5 down-regulated genes were involved in the "plant–pathogen interaction" system during GINV infection. These defense-related genes included *VvHSP83*, *VvCaM5*, *VvRPM1*, *VvPR1*, and three WRKY TFs. HSPs play an important role in response to abiotic and biotic stress conditions and plant development, and their expression is upregulated in response to different pathogen infections, insect attacks, drought, and salinity (Usman et al., 2017; Ul Haq et al., 2019; Berka et al., 2022). Our RNA-seq and qRT-PCR results showed that the *VvHSP83* gene was significantly up-regulated following GINV infection (Table S4, Fig. 5). This indicated that the *VvHSP83* gene might function in response to GINV infection in Crimson seedless grapevine fruits. Previous studies have shown that the expression of several *WRKY* genes was regulated in response to different pathogen infections, including fungal, bacterial, and viral pathogens (Marchive et al., 2007; Zheng et al., 2007; Yoda et al., 2002). We found that 62 DEGs encoding the *VvWRKY* genes were induced, implying the involvement of the *VvWRKY* genes in the defense against GINV infection (Table S2). We selected three DEGs encoding the *VvWRKY* genes (*VvWRKY20*, *VvWRKY75*, and *VvWRKY57*) using qRT-PCR analysis, and the results showed that *VvWRKY20* and *VvWRKY75* were remarkably up-regulated, whereas *VvWRKY57* was significantly down-regulated during GINV infection. Therefore, the *VvWRKY* family genes encoding VvWRKY proteins could be involved in response to GINV infection.

5. Conclusion

We used the RNA-seq technology to obtain a genome-wide transcript profile of Crimson seedless grapevine fruits infected with GINV. Our results revealed that the GINV infection is a complicated process. We believe that the findings of our study will help investigate the detailed mechanisms of the GINV–host interaction and identify resistance genes.

Authors' contributions

XW carried out most of the experiments and drafted manuscript, JS participated in the design of the study. YL and LG participated the statistical analysis. HH and RZ designed and directed the study and revised the manuscript. All authors have read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crviro.2022.100024.

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