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Transcriptome Sequence Analysis of Defense Response of Resistant and Susceptible Bottle Gourd to Powdery Mildew

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Abstract: Powdery mildew (PM) is the main disease that afflicts bottle gourd. Previous studies on PM mainly focused on its effects on pumpkin, melon, and other crops; however, the exact molecular mechanism of bottle gourd resistance to PM remains unclear. RNA sequencing (RNA-Seq) technology was used to investigate the dynamic changes in leaf transcriptome profiles between resistant and susceptible gourd at 12, 24, 48, and 72 h post-inoculation with powdery mildew. Compared with a susceptible variety (G3), the expression levels of the differentially expressed genes of phenylpropanoid biosynthesis, starch, and sucrose metabolism, and plant–pathogen interaction pathways in disease-resistant plants were upregulated. We propose that disease resistance and tolerance in bottle gourd are enhanced via several pathways, including the antioxidant system, phenylalanine biosynthesis, and cell wall cellulose synthesis. Our research will provide an important basis for further screening and breeding PM resistance in bottle gourd.

Keywords: powdery mildew resistance; bottle gourd; RNA-seq

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1. Introduction

Bottle gourd (*Lagenaria siceraria* (Mol.) Stand) is an annual vine herb (2n = 2x = 22) of the gourd family, which originates from the tropical lowlands of southern equatorial Africa and has been independently domesticated in Africa and Asia [1]. Furthermore, bottle gourd has been cultivated in China for more than 7000 years for edible, medicinal, ornamental, and processed products [2]. In addition, because of its excellent resistance and close relationship with watermelon, it is often used as rootstock material for watermelon and other Cucurbitaceae vegetable crops [3,4].

However, bottle gourd is susceptible to powdery mildew (PM) at all stages of development, resulting in a serious loss of its yield. Therefore, PM has become the main disease endangering bottle gourd production in recent years. When the disease is serious, the whole plant's leaves are covered with a white powdery mildew layer, causing the diseased leaves to wither, yellow, brittle, or curl, losing the ability to photosynthesize, affecting the growth and development of fruit, and eventually leading to premature senescence and death of the whole plant. Moreover, PM not only affects the yield but also the quality of fruit [5]. Therefore, it is urgent to study the mechanism of PM resistance in bottle gourd production.

Currently, compared with pumpkin, cucumber, melon, and other melon vegetable crops, there are few studies on the resistance of bottle gourd to PM, and the mechanism

of its resistance to PM is still unclear. At present, the research on the resistance of bottle gourd to PM mainly focuses on the symptoms of harm, defense measures, physiological characteristics, and genomics [6]. One study showed that the resistance of bottle gourd to PM was controlled by a pair of recessive genes [7], and the resistance was positively correlated with the number of favorable alleles carried [8]. PM-related genes in melon mainly include the PM [9] and MLO families [10]. In the bottle gourd, Wang [11] developed the world's first bottle gourd PM marker GPDSATG/CTC75, with a linkage distance of 9.6 cM with a PM resistance gene, which can be used for the auxiliary breeding of PM-resistant bottle gourd varieties. Wu et al. [8] selected the candidate gene cysteine receptor protein kinase 3 (CRK3) to develop PM-resistant bottle gourd using GWAS. Wang et al. [12] analyzed the resistance of the NBS-LRR gene family and found the potential candidate gene *Lsi04g015960* for breeding PM tolerance.

RNA-seq analysis is a powerful tool to deeply understand the molecular response of plants to biotic stress. In recent years, with the development of histological technology, transcriptome sequencing technology has been widely used in the research of melon powdery mildew. For example, using comparative transcriptome analysis, Zhao et al. [13] analyzed the comparative transcriptome of susceptible melon varieties TG-5 and resistant TG-1 and determined that the xylan metabolism process, hydrolase activity, and oxidative stress reaction were involved in melon resistance to powdery mildew. Cao [14,15] and others used F-1-isolated populations from resistant and susceptible melon varieties (wm-2 and 6D-12, respectively) to identify new QTLs and candidate genes combined with transcriptome sequencing. However, a transcriptome study of bottle gourd powdery mildew has not been reported yet. Therefore, in this study, based on the germplasm resources of the bottle gourd collected by the research team, we selected susceptible samples and analyzed their gene expression levels 12, 24, 48, and 72 h after fungal infection by transcriptome technology, identified the genes related to disease resistance during *Podosphaera xanthii* infection, and identified the potential related regulatory genes by using expression correlation. The expression regulation network was constructed to provide a scientific basis for the breeding of new varieties of powdery-mildew-resistant bottle gourd.

2. Materials and Methods

2.1. Planting Material and Pathogen Infection

G3 (powdery mildew susceptible) and G6 (powdery mildew resistant) were used for inoculation with powdery mildew in this study, both of which are from a high-generation inbred line, and the original germplasm resources are all from the Institute of Vegetable and Flower Research of the Chinese Academy of Agricultural Sciences (Crop Germplasm Resources Platform–Vegetable Germplasm Resources Sub platform). We soaked G3 and G6 seeds in 55–60 °C warm water for 2 h and put them in a 28 °C incubator to accelerate germination until 50% of the seeds were exposed. G3 and G6 were sowed in a plastic flowerpot (18 cm × 20 cm) under a controlled temperature of ~28 °C/20 °C (day/night) and an illumination of 16 h/8 h (day/night).

The diseased leaves were collected from the plots with serious powdery mildew at the Zhuang Hang Comprehensive Test Station of Shanghai Academy of Agricultural Sciences, and the spores on the leaves were washed with distilled water to make a spore suspension, which was diluted to a 1.0× inoculate with a 105 spores/mL concentration and then evenly sprayed on the entire leaf (most plants were inoculated at the stage of 1–2 leaves). We moisturized the plants in a small arched shed for 12 h, kept the temperature at 23–26 °C during the day and 18–20 °C at night, and maintained the humidity above 95%. Subsequently, we restored the original temperature and humidity and, 7 days later, the levels of powdery mildew disease was recorded according to the method [16]. To detect the changes in the G3 and G6 gene expression levels during the first stages of their interaction with PM, leaf samples were taken before infection (0 days and control) and after 12, 24, 48, and 72 h. Three biological replicates were prepared at each time point. In total, 30 samples

were immediately frozen in liquid nitrogen and stored at −80 °C for total RNA isolation and further analysis.

2.2. mRNA Extraction and Sequencing Data

The RNA-Seq was performed by OE Biotech Co., Ltd. (Shanghai, China). Total RNA was extracted from two bottle gourds using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) following the manufacturer's protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with an RNA integrity number (RIN) $\geq$ 7 were subjected to the subsequent analysis. The libraries were constructed using the TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then, these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeq X Ten), and 125 bp/150 bp paired-end reads were generated.

Raw data (raw reads) were processed using Trimmomatic [16]. The reads containing ploy-N and the low-quality reads were removed to obtain the clean reads. Then, by using hisat2, the clean reads were mapped to the bottle gourd reference genome (http://cucurbitgenomics.org/organism/13, accessed on 22 March 2022). The FPKM value of each gene was calculated using cufflinks [17], and the read counts of each gene were obtained by htseq-count [18]. Differentially expressed genes (DEGs) were identified using the DESeq [19] (2012) R package functions estimateSizeFactors and nbinomTest. We set p-value < 0.05 and foldChange > 2 or foldChange < 0.5 as the threshold for significantly differential expression.

2.3. GO and KEGG Analysis

GO enrichment and KEGG pathway enrichment analyses of DEGs were performed using R based on the hypergeometric distribution.

2.4. Quantitative RT-PCR Assay

We use Primer Premier 5.0 software to design the primers (Table 1). RNA was reversely transcribed into cDNA using a reverse transcription kit. We used the *LsGAPDH* gene as an internal reference for the real-time fluorescence quantification of selected core genes.

Table 1. The primers of differential expression genes.

Gene	Primer Sequence
LsGAPDH	F: CCCAGGGGATATCTGCAGGG R: CATGGTGTTTTCAATGGAACCA
Lsi02G027070	F: GAGACTCAACGACACAGGCA R: GTGAGGAAGAAGGTGGTCCG
Lsi05G003210	F: CGGTATCCCACTAAAAGCAAAGC R: ACTGAGCCTTTGGTTCCACC
Lsi05G003220	F: AGAGGTAAAAGAGAGGCTGGC R: ACTGAGCTGCCATTGCTACC
Lsi05G009870	F: TTGCCACCAGGTGAAACCAT R: TCAGCCAATGGAAGGGATTGA
Lsi05G013660	F: TGCTTGAGCTTTGTCACGGT R: TCATGCCTTAGGAGCTTCAACA
Lsi11G001370	F: TGGAGGTGATTGGGACAACC R: AACACATGTCCATCCCGTCC
Lsi04G011480	F: ATGTGGAAATTGAGGCTCCAG R: CCCACTTTAGCCTTTCCATAGC
Lsi05G013650	F: GGGAAGTGTGGTTCACTGGT R: GGTTCATCACGACCTCCTGT
Ls11G001420	F: AGTCGTGCCAAAGGGTCAAA R: TCGATATCTTTGGTCACACCGA

Table 1. *Cont.*

Gene	Primer Sequence
Ls04G003530	F: GAGCACCAAACCCCATCTCC R: CAGCCTTCTTGTTTGGACCG
Lsi04G011510	F: GGGAAAGGGAAGTGTAGCTC R: ATCAAGGTCTCTGCCGACTTC

2.5. Regulatory Network Construction

We used 44 identified disease-resistance genes to construct a regulatory network and used the R language to calculate the co-expression correlations between the differential genes in the module.

3. Results

3.1. Changes in the Disease-Resistance Phenotype

Seven days after inoculation, some leaves from the G6 plant showed a small amount of white powder, with the disease spot area accounting for less than 1/3 of the entire leaf, which is a highly resistant material (Figure 1A). A large number of leaves from the G3 plants showed a thick and continuous layer of powdery mildew, with disease grade 4 spots covering more than 2/3 of the whole leaf area (Figure 1B), which is a highly susceptible material. This result confirmed that G6 was more resistant to *Podosphaera xanthii* than G3. This result laid the foundation for the subsequent research.

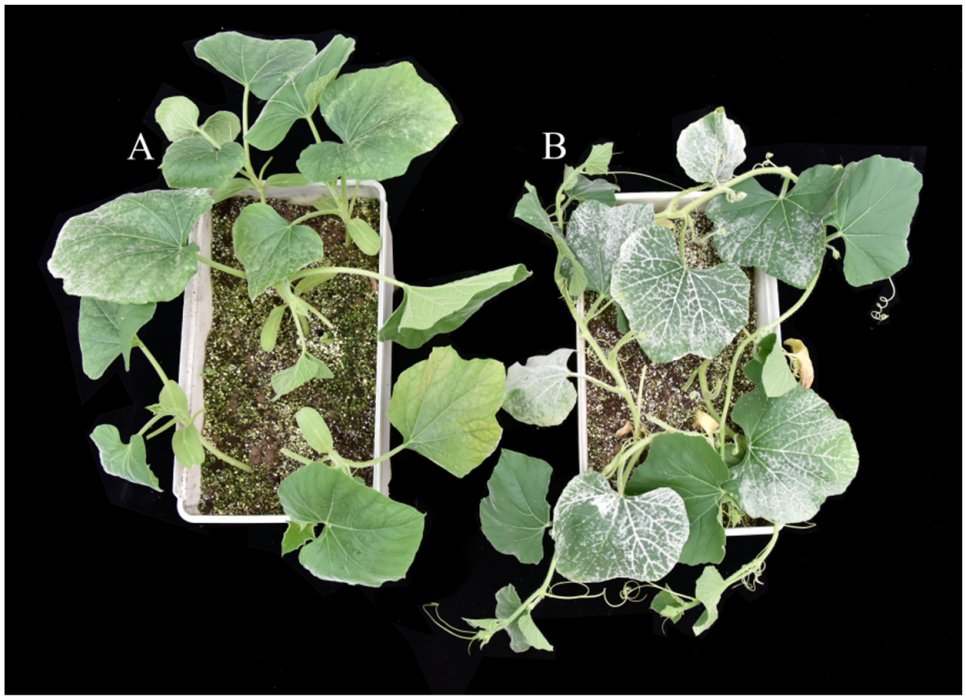

Figure 1. The disease symptoms of G6 (**A**) and G3 (**B**) after 7 days of PM.

3.2. Overview of Sequencing Data and Comparison Results

To investigate the transcriptome of the PW-infected G6 and G3, we prepared RNA samples of the leaves obtained at 4 infection stages (12, 24, 48, and 72 h after infection), as well as the RNA samples of the non-inoculated leaves. Approximately 200.67 G clean reads were generated for the 30 samples. The ratio of the Q30 sequencing value was 94.77–98.74%, which indicated that the sequencing sufficiently captured most of the expressed genes. Supplementary Table S1 shows the quality assessments of the sample sequencing data.

3.3. Identification and Expression Pattern of mRNA

We detected 20,978 genes in the 30 samples and found the highest and average expression levels of 68,949.86 and 34.68, respectively, in the new transcripts. The number of genes identified from the 30 samples ranged from 19,063 to 19,333.

Combining the PCA plots (Figure 2A) with the number of DEGs for G3 and G6 at different periods compared with the untreated G3 and G6, respectively, we found that the differences between the G3 and G6 samples at 12 h after inoculation correlated least with the untreated leaf group, while the number of differentially expressed genes was highest compared with the others, indicating that gourd leaves respond to powdery mildew at 12 h.

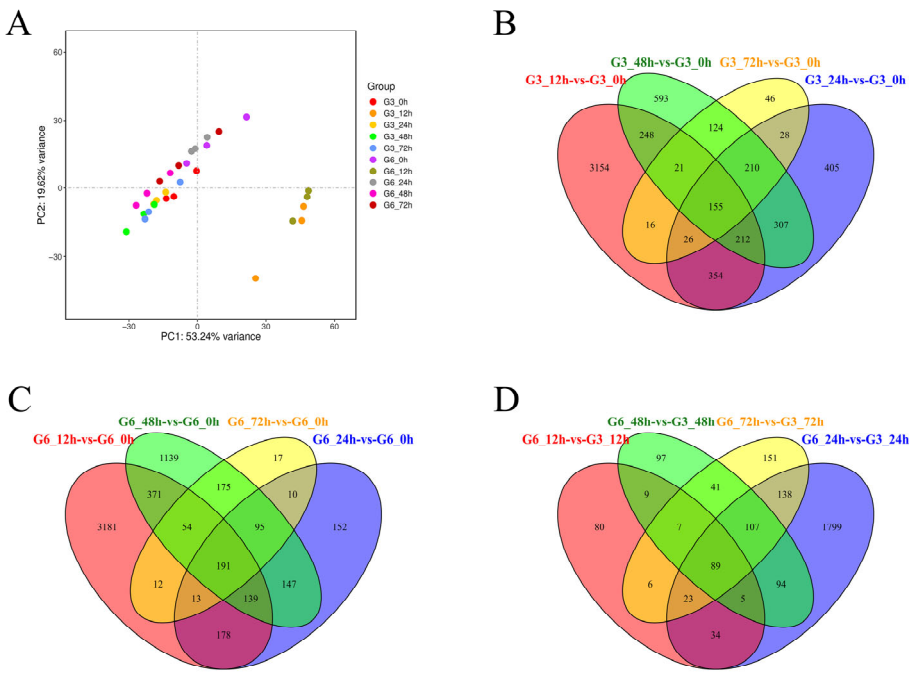

Figure 2. (**A**) Principal component analysis (PCA) plots of transcripts identified by RNA-seq of bottle gourd leaves of G6 and G3 at 0, 12, 24, 48, and 72 h after PM treatment. (**B**) Venn diagram illustrating DEGs of 0 h, 12 h, 24 h, 48 h, and 72 h of G3 line. (**C**) Venn diagram illustrating DEGs of 0 h, 12 h, 24 h, 48 h, and 72 h of G6 line. (**D**) Venn diagram illustrating DEGs of G3 and G6 lines at the same time of 0 h, 12 h, 24 h, 48 h, and 72 h.

DEGs were identified by five pairwise comparisons of transcriptome datasets (G6_0 h-vs.-G3_0 h, G6_12 h-vs.-G3_12 h, G6_24 h-vs.-G3_24 h, G6_48 h-vs.-G3_48 h, and G6_72 h-vs.-G3_72 h). In total, 422 DEGs (220 upregulated and 202 downregulated) were identified in the G6_0 h-vs.-G3_0 h comparison, 253 (138 upregulated and 115 downregulated) in the G6_12 h-vs.-G3_12 h comparison, 2289 (1552 upregulated and 737 downregulated) in the G6_24 h-vs.-G3_24 h comparison, 449 (235 upregulated and 214 downregulated) in the G6_48 h-vs.-G3_48 h comparison, and 562 (283 upregulated and 279 downregulated) in the G6_72 h-vs.-G3_72 h comparison.

DEGs were also analyzed between the two cultivars at the same time points after pathogen inoculation (Figure 2B,C). Venn diagrams show that both G3 and G6 have the highest DEGs within 12 h (Table S2). The highest DEGs number between G3 and G6 was 24 h after PM treatment (Figure 2D). These indicate that the response time of bottle gourd to the powdery mildew mechanism is 12 h to 24 h, and genes that determine the different powdery mildew resistances of G3 and G6 may differently express within 24 h. In addition, a total of 155 DEGs were expressed in all four comparisons of G3, and 191 DEGs were identified at all four time points in G6 (Figure 2D). The main functions of these genes are

Phenylalanine ammonia-lyase, Peroxidase, Calcium binding, Chitin-binding lectin, and Chitinase 2 (Table S3).

3.4. GO Functional Analysis of Significantly Different Genes

DEGs were identified by eight pairwise comparisons of transcriptome datasets (G3_12 h-vs.-G3_0 h, G3_24 h-vs.-G3_0 h, G3_48 h-vs.-G3_0 h, G3_72 h-vs.-G3_0 h, G6_12 h-vs.-G6_0 h, G6_24 h-vs.-G6_0 h, G6_48 h-vs.-G6_0 h and G6_72 h-vs.-G6_0 h), and GO functional enrichment analysis was performed for these genes separately. The TOP10 GO enrichment entries for G3 and G6 at 12, 24, 48, and 72 h after powdery mildew infestation was integrated, and the duplicate entries were removed to obtain a total GO enrichment entry (Table 2). Two of the GO entries, the cinnamic acid biosynthetic process and the L-phenylalanine catabolic process, were highly expressed at all time points in both materials. This is consistent with the conclusion that PAL and PPO are known to be involved in powdery mildew resistance mechanisms in other crops. In addition, a comparison of GO entries at the same time in both materials also revealed that G3 and G6 had the highest number of GO expression entries at 12 h, and there were some differences at 24 h. G6 had more cell wall macromolecule catabolic process and chitin catabolic process expressions compared with G3.

Table 2. TOP10 GO enrichment entries for G3 and G6 at 12, 24, 48, and 72 h after powdery mildew infestation.

Total	G3				G6			
	12 h	**24 h**	**48 h**	**72 h**	**12 h**	**24 h**	**48 h**	**72 h**
cinnamic acid biosynthetic process	√	√	√	√	√	√	√	√
L-phenylalanine catabolic process	√	√	√	√	√	√	√	√
alkaloid biosynthetic process	√				√	√	√	√
glutamine metabolic process	√				√			
response to auxin	√		√		√	√	√	
tyrosine metabolic process	√	√			√	√		
alanine metabolic process	√				√			
response to oxidative stress		√	√	√		√	√	√
regulation of transcription, DNA-templated		√	√	√		√	√	√
hydrogen peroxide catabolic process		√		√		√		√
cellular oxidant detoxification		√	√	√		√	√	√
serine family amino acid metabolic process		√	√				√	√
protein phosphorylation			√	√			√	√
cell wall macromolecule catabolic process				√		√		√
chitin catabolic process				√		√		√
recognition of pollen				√			√	√
chloroplast RNA processing	√				√			
chloroplast RNA modification	√				√			
starch catabolic process	√				√			
RNA secondary structure unwinding	√				√			
maturation of SSU-rRNA	√				√			
protein refolding	√				√			
microtubule-based movement		√					√	
transmembrane transport		√		√		√		√
metabolic process		√	√			√		
oxidation-reduction process		√	√	√		√		√
transport		√	√			√		
aromatic compound biosynthetic process			√			√		

We therefore carried out a further analysis of GO enrichment 24 h after infestation with G3 and G6 *Podosphaera xanthii*. The analysis top 10 showed that the 44 genes that appeared to be functionally related to disease and stress resistance were significantly up- and downregulated 24 h after infestation from the bacteria. These 44 genes associated

with disease and stress resistance (Figure 3) had 8 and 10 genes on chromosomes 5 and 11, respectively. On the remaining chromosomes, the distribution was 1–6 genes. The expression of *Lsi02G027070* for these 44 genes was significantly higher at 48 h in G6.

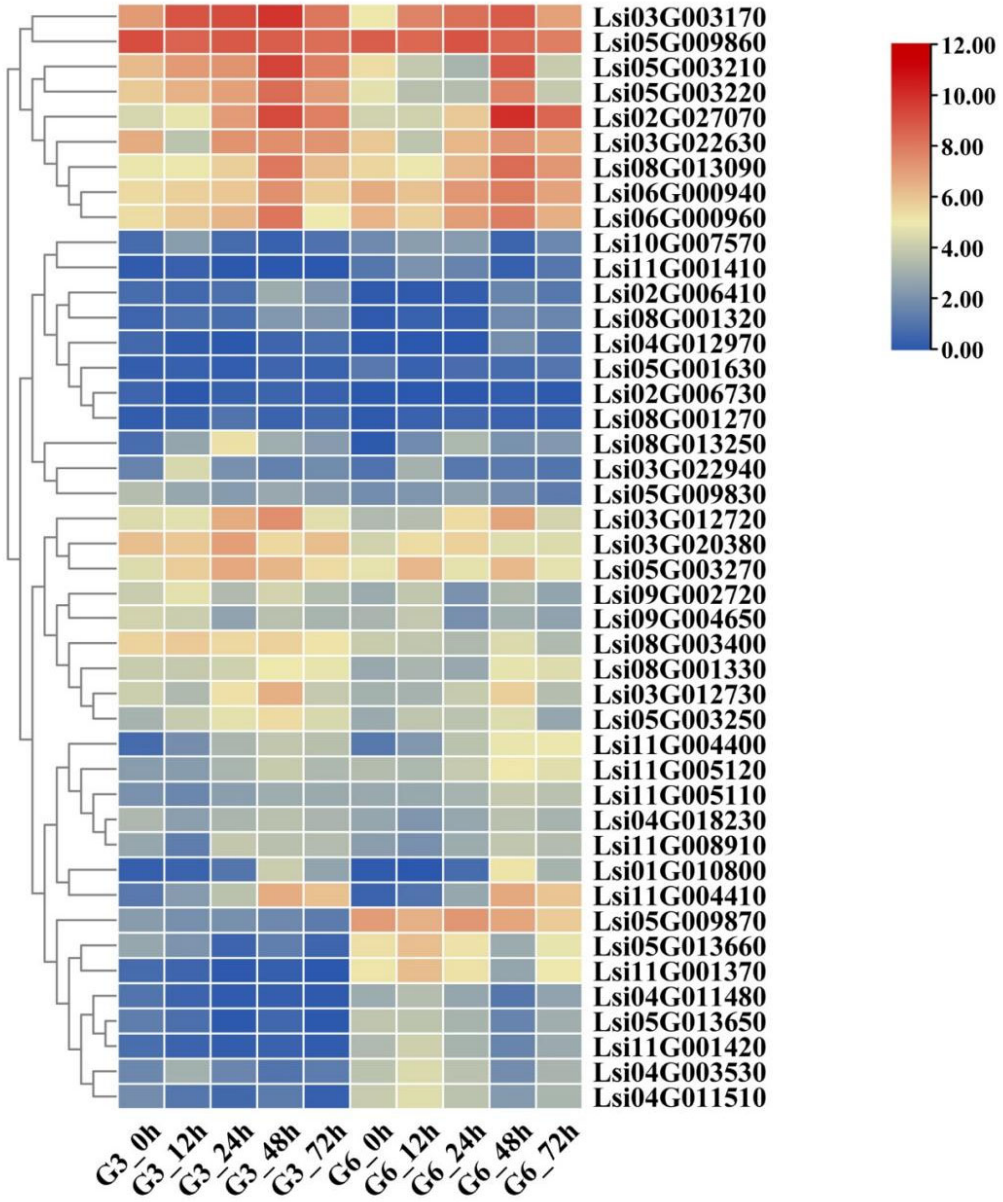

Figure 3. Expression patterns of 44 genes related to disease resistance and stress resistance.

3.5. Analysis of the KEGG Pathway for Significantly Different Genes

We therefore analyzed the KEGG pathway separately for all DEGs in the three comparison groups—G3 12 vs. 0, G6 12 vs. 0, and G3 24 vs. G6 24 h. Tables 3 and 4 show the top 10 pathways for G3 12 vs. 0 and G6 12 vs. 0. The alanine, aspartate, glutamate metabolism, phenylalanine metabolism, circadian rhythm–plant, and phenylpropanoid biosynthesis pathways were highly expressed in both groups, indicating that these pathways are involved in bottle gourd's PM-resistant mechanism, and the main response pathway was amino acid metabolism. Phenylalanine metabolism is the main response pathway in the amino acid pathway, indicating a close relationship between the bottle gourd's mechanism of resistance to powdery mildew and the biosynthesis of phenylalanine.

A KEGG enrichment analysis of G3 and G6 24-h DEGs in combination with the L2FC values of differentially expressed genes mainly involved six metabolic pathways,

namely phenylpropanoid biosynthesis, starch and sucrose metabolism (under amino sugar and nucleotide sugar metabolism), glycolysis/gluconeogenesis, cysteine and methionine metabolism, glycerophospholipid metabolism, and plant–pathogen interaction (Figure 4).

Table 3. Enrichment analysis of KEGG pathway for the DEGs from G3 bottle gourd leaves at 12 h after PM treatment.

Pathway ID	Pathway Name	ListHits	*p* Value
ko00250	Alanine, aspartate, and glutamate metabolism	25	1.53×10^{-5}
ko00360	Phenylalanine metabolism	20	0.000135356
ko04712	Circadian rhythm–plant	19	0.000224721
ko00410	Beta-alanine metabolism	16	0.001916155
ko00511	Other glycan degradation	10	0.002438963
ko00130	Ubiquinone and other terpenoid–quinone biosynthesis	17	0.00589103
ko04075	Plant hormone signal transduction	66	0.005954455
ko00062	Fatty acid elongation	11	0.006594654
ko00920	Sulfur metabolism	13	0.010577539
ko00940	Phenylpropanoid biosynthesis	51	0.013346325

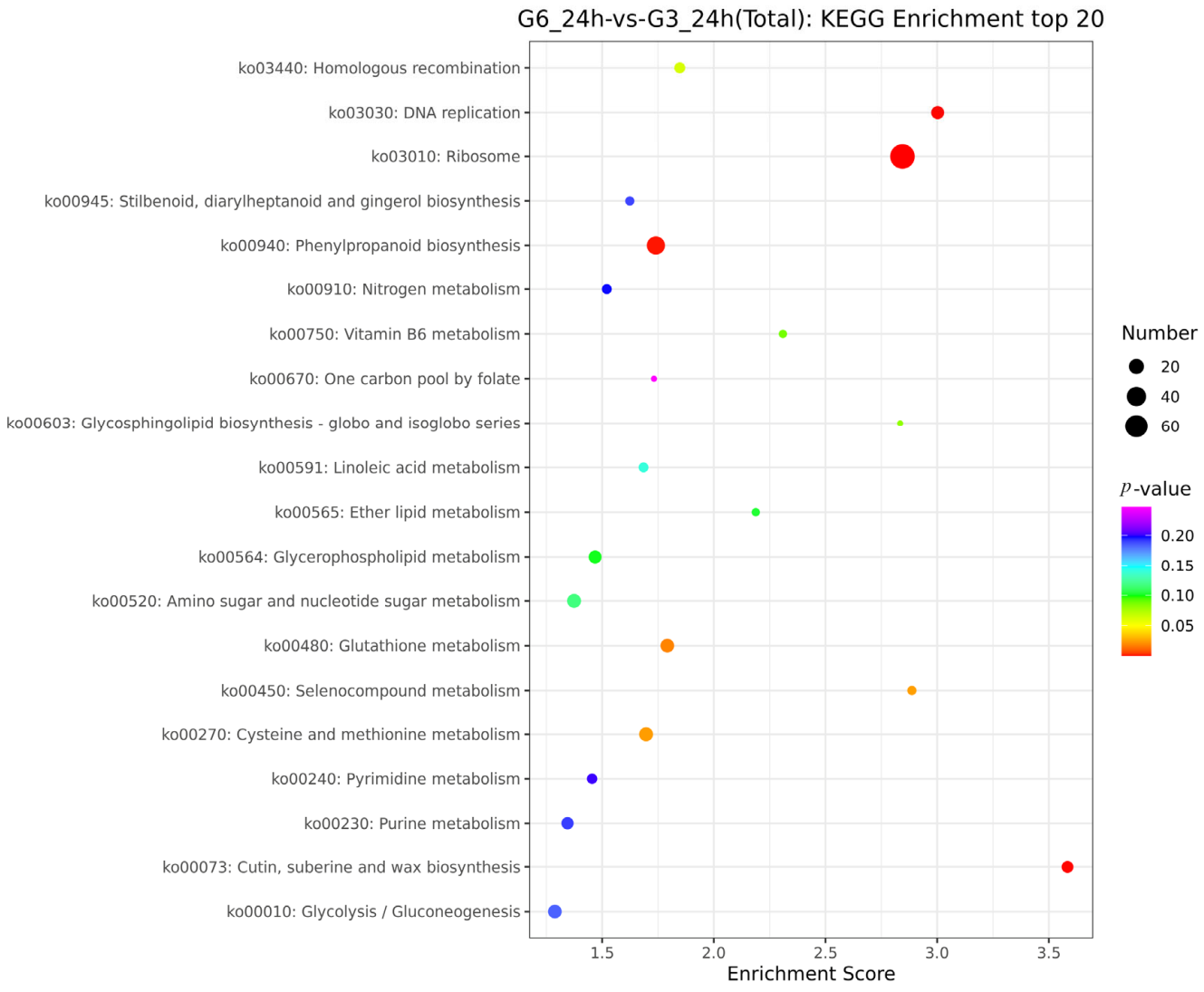

Figure 4. DEGs between G3 24 vs. G6 24 h significantly enriched the KEGG pathway.

Table 4. Enrichment analysis of KEGG pathway for the DEGs from G6 bottle gourd leaves at 12 h after PM treatment.

Pathway ID	Pathway Name	ListHits	*p* Value
ko00250	Alanine, aspartate, and glutamate metabolism	26	2.21×10^{-6}
ko03008	Ribosome biogenesis in eukaryotes	33	3.68×10^{-6}
ko00940	Phenylpropanoid biosynthesis	56	0.000457519
ko04712	Circadian rhythm–plant	18	0.000470089
ko00360	Phenylalanine metabolism	18	0.000869833
ko00196	Photosynthesis–antenna proteins	11	0.515563734
ko00906	Carotenoid biosynthesis	12	0.955349824
ko00430	Taurine and hypotaurine metabolism	5	0.363705141
ko03018	RNA degradation	24	0.369539462
ko00760	Nicotinate and nicotinamide metabolism	8	0.699410569

3.6. qRT-PCR Validation

The 11 genes were therefore validated by qRT-PCR, and the results are shown in Figure 5. The expression patterns of these differentially expressed genes were generally consistent with the results obtained by transcriptome sequencing, thus indicating that the RNA sequencing results were reliable.

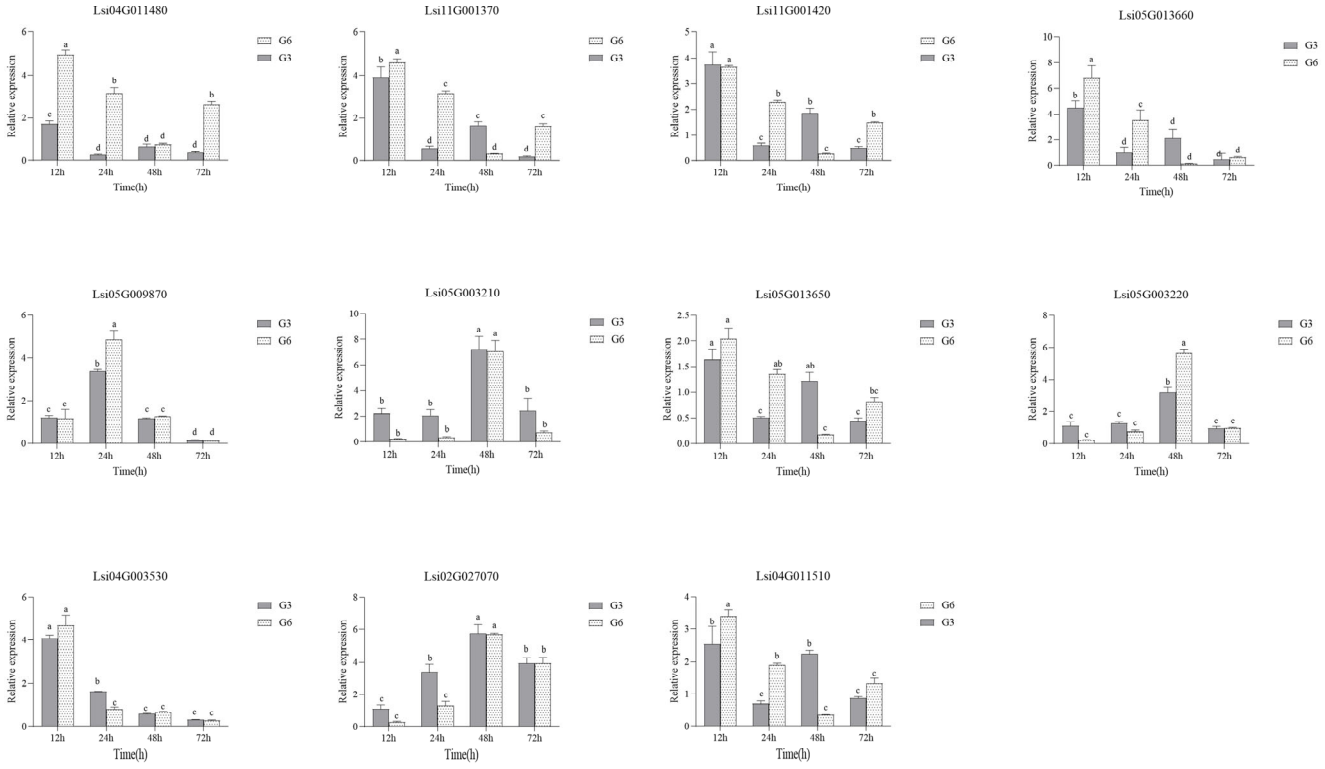

Figure 5. Quantitative real-time polymerase chain reaction analysis of the relative transcript levels of the 11 candidate DEGs at 12, 24, 48, and 72 h after PM treatment. The relative expression levels of the DEGs were calculated by the comparative threshold method ($2^{-\Delta\Delta CT}$) and were relative to those at the 0-h time point. The results are presented as the means ± standard errors of three biological replications. The different letters represent significant differences ($p < 0.05$ according to analysis of variance (ANOVA)).

3.7. Regulatory Networks of Disease-Resistance Genes

The 44 identified disease-resistance genes were used to construct a regulatory network, and the expression correlations with other genes were calculated, with genes with correlations greater than 0.99 being selected to construct the expression regulatory network

(Figure 6). The network contained 33 of the 44 disease-resistance genes, and 209 genes were detected to have potential regulatory relationships with these 33 genes. Among them, most genes were associated with *Lsi10G007570*, which belongs to the MLO gene family. Studies have shown that certain members of the MLO gene family make plants susceptible to powdery mildew, and that deletions or mutations in MLO genes result in the inability of powdery mildew spores to enter the plant's cell wall.

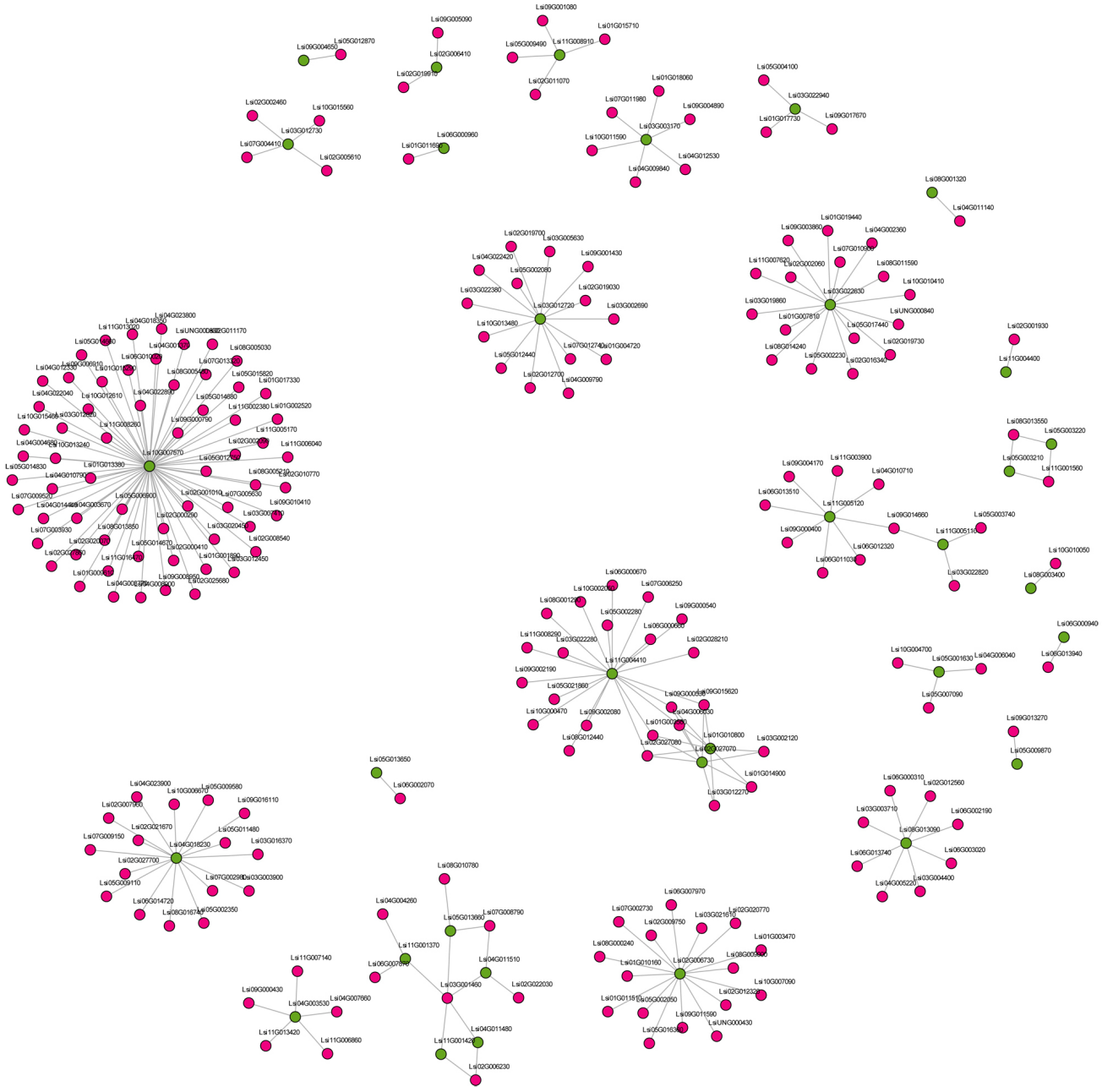

Figure 6. The regulatory network of 44 identified resistance and stress-related genes. Red represents potential regulatory genes, and green represents 44 identified resistance and stress-related genes.

4. Discussion

Powdery mildew is one of the main diseases affecting the yield and quality of bottle gourd; however, little is known about the gourd's mechanism of resistance to powdery mildew. Currently, there are many studies on the resistance mechanism of other gourd vegetable crops, such as pumpkin, cucumber, and melon, to powdery mildew. In this experiment, we conducted transcriptome data analysis on the resistant G6 and non-resistant G3 varieties and compared their resistance mechanisms to powdery mildew with other gourd crops.

When powdery mildew occurs in melon crops, the balance of active oxygen species (ROS) in the plant itself is broken. The plant's antioxidant system is activated, and the activity of related antioxidant enzymes begins to increase while inducing the accumulation of a large amount of ROS [10,20]. Generally, the active oxygen accumulated in the metabolic process of plants will be promptly and effectively cleared by the active oxygen scavenging system to maintain the balance of active oxygen in the plant body. However, some studies have found that when plants are infected by pathogens, reactive oxygen species rapidly accumulate, which is considered to be one of the plant's early defense responses to pathogens [21]. After inoculation, the accumulation of reactive oxygen species (ROS) in disease-resistant pumpkin varieties was low. Compared with untreated pumpkin seedlings, the activity and gene expression of ROS scavenging defense enzymes (SOD, CAT, POD, and APX) in their leaves were significantly increased [22]. During the GO analysis of G3 and G6 transcriptome data in this experiment, "response to oxidative stress" was highly expressed during 24–72 h of inoculation with the powdery mildew pathogen, indicating that the antioxidant system is one of the gourd's basic resistance mechanisms against powdery mildew.

In addition, studies have shown that phenylpropanoid secondary metabolic pathways are the main metabolic pathways for melon resistance to powdery mildew, and phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) are two key enzyme activities for melon resistance to powdery mildew. Lignin, a metabolite of phenylpropanoid secondary metabolic pathways, is also involved in resistance to powdery mildew [23]. In a KEGG analysis of G3 and G6 transcriptome data, G6 specifically activated PAL in the phenylpropanoid secondary metabolic pathway compared with G3 at 24 h, and also further activated secondary products with antibacterial activity, such as flavonoids and lignin. This is consistent with the research in melon [24], cucumber [25], and pumpkin [26]; that is, PAL and PPO are enzymes related to melon PM defense.

Current research shows that powdery-mildew-resistance-related genes mainly include the Pm and MLO families. Among them, the Pm family prevents powdery mildew bacteria from invading the cell wall by regulating the accumulation of callose in the infected site. The MLO family acts as a transmembrane protein on the plasma membrane to regulate the entry of powdery mildew into plant cells [27]. Zhou cloned eight *McMLO* genes from a highly susceptible variety to powdery mildew and, based on phylogenetic analysis results, learned that two of these genes are related to the degree of susceptibility to powdery mildew [28,29]. Win et al. [30] found that the MLO gene in pumpkin plays a strong promoting role in its sensitivity to powdery mildew. During the correlation analysis of G3 and G6 transcriptome data, 44 DEGs were detected, among which the gene Lsi10G007570 in the MLO family had a strong correlation, indicating that the mechanism of resistance to powdery mildew in bottle gourd was related to the MLO family.

By analyzing transcriptome data, we also obtained eight specific genes, including Lsi05G009870, Lsi05G013660, Lsi11G001370, Lsi04G011480, Lsi05G013650, Lsi11G001420, Lsi04G003530, and Lsi04G011510. Among them, Lsi05G013660, Lsi04G011480, Lsi05G013650, and Lsi04G011510 belong to the major latex-like protein (MLP) family. MLPs generate resistance against pathogens via inducing pathogen-related protein genes [31]. MLPs expression has a strong correlation with cucumber and melon powdery mildew infection. Knocking down CsMLP1 reduced cucumber tolerance, while transient overexpression of CsMLP1 increased cucumber disease resistance [32]. The MLP-PG1 identified in zucchini plays a

crucial role in the resistance of fungal pathogens by inducing disease-related genes [33]. This suggests that MLP may also be involved in the resistance of gourd to powdery mildew.

In summary, the transcriptome data provided a global insight into the gene expression patterns in PM-infected G3 and G6, as well as elucidating the molecular mechanism of PM resistance in bottle gourd. Eleven candidate genes involved in PM resistance were identified, and characterizing their roles in bottle gourd powdery mildew resistance is of considerable significance. There are some possible reasons for the resistance and sensitivity of G3 and G6 to bottle gourd powdery mildew, respectively. Genes from the phenylalanine metabolism pathway and the antioxidant system might have affected the genome of the common bottle gourd and conferred bottle gourd G6 resistance to powdery mildew. Moreover, we speculated that MLPs may induce bottle gourd powdery mildew resistance. These results may assist breeders in utilizing the genes for future bottle gourd PM-resistance breeding.

5. Conclusions

Based on the analysis of transcriptome data, we found that the reason for the higher resistance of G6 compared with G3 may lie in (1) possible changes in leaf structure (cellulose and leaf surface wax); (2) the continuous activation of plant–pathogenic-interaction-pathway-related genes that enhance resistance (PR1, fungal pap, and calcium ion); and (3) the fact that the activation of PAL further produces secondary products with antibacterial activity, such as flavonoids and lignin, or enters the biosynthetic pathway of plant antitoxins and phytohormones. In addition, we also detected a potential powdery mildew resistance gene Lsi10G007570 and proposed that the MLP family may be involved in the resistance of gourd to powdery mildew.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051406/s1, Table S1: Quality assessments of the sample sequencing data. Table S2: Venn data; Table S3: All DEGs data.

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