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Transcriptome resequencing analysis of the responses of Ty-5-Mediated resistance to TYLCV via in resistant vs. susceptible tomato cultivars

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Tomato yellow leaf curl virus (TYLCV) is one of the most devastating viruses of cultivated tomato in both tropical and subtropical regions. Five major genes (Ty-1, Ty-2, Ty-3, Ty-4 and Ty-5) from wild tomato species have been associated with resistance to TYLCV. Researchers have recently attempted to determine the functions of these resistance genes, but molecular mechanisms underlying the observed resistance remain unclear. Here, resistant (cv. CLN3212A-23, carrying Ty-5) and susceptible (cv. Moneymaker) plants were either left untreated (R and S, respectively) or artificially inoculated with TYLCV via Agrobacterium-mediated transformation (RT and ST, respectively). The transcriptomes of the plants in the four groups were then analyzed by RNA-Seq, and the results identified 8,639 differentially expressed genes (DEGs) between the R and RT groups, 2,818 DEGs between the RT and ST groups, 8,899 DEGs between the S and ST groups, and 707 DEGs between the R and S groups. The gene expression profiles in both the resistant and susceptible tomato cultivars appeared to undergo notable changes after viral inoculation, and functional classification revealed that most DEGs were associated with 18 GO terms. Moreover, the functional classification of the response of Ty-5-carrying tomato plants to TYLCV infection identified the importance of the GO term “response to stimulus” in the BP category, which is related to disease resistance. In addition, 28 genes were significantly enriched in the “Plant hormone signal transduction”, “Carbon metabolism”, “ Carbon fixation in photosynthetic organisms ” and “ Glutathione metabolism ” pathways. The differential expression levels of 12 select genes were confirmed by quantitative real-time PCR. The present study indicates that the Ty-5 gene activates the expression of multiple genes involved in the resistance process and will aid a more in-depth understanding of the effects of the Ty-5 gene on resistance based on its molecular mechanism with the aim of improving TYLCV disease management in tomato.

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1 Transcriptome Resequencing Analysis of the Responses of Ty-5-Mediated
2 Resistance to TYLCV via in Resistant vs. Susceptible Tomato Cultivars

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8 **Abstract**

9 *Tomato yellow leaf curl virus* (TYLCV) is one of the most devastating viruses of cultivated tomato in
10 both tropical and subtropical regions. Five major genes (Ty-1, Ty-2, Ty-3, Ty-4 and Ty-5) from wild
11 tomato species have been associated with resistance to TYLCV. Researchers have recently attempted to
12 determine the functions of these resistance genes, but molecular mechanisms underlying the observed
13 resistance remain unclear. Here, resistant (cv. CLN3212A-23, carrying Ty-5) and susceptible (cv.
14 Moneymaker) plants were either left untreated (R and S, respectively) or artificially inoculated with
15 TYLCV via Agrobacterium-mediated transformation (RT and ST, respectively). The transcriptomes of
16 the plants in the four groups were then analyzed by RNA-Seq, and the results identified 8,639
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18 ST groups, 8,899 DEGs between the S and ST groups, and 707 DEGs between the R and S groups. The
19 gene expression profiles in both the resistant and susceptible tomato cultivars appeared to undergo notable
20 changes after viral inoculation, and functional classification revealed that most DEGs were associated
21 with 18 GO terms. Moreover, the functional classification of the response of Ty-5-carrying tomato plants
22 to TYLCV infection identified the importance of the GO term "response to stimulus" in the BP category,
23 which is related to disease resistance. In addition, 28 genes were significantly enriched in the "Plant
24 hormone signal transduction", "Carbon metabolism", "Carbon fixation in photosynthetic organisms" and
25 "Glutathione metabolism" pathways. The differential expression levels of 12 select genes were confirmed
26 by quantitative real-time PCR. The present study indicates that the Ty-5 gene activates the expression of
27 multiple genes involved in the resistance process and will aid a more in-depth understanding of the effects

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28 of the Ty-5 gene on resistance based on its molecular mechanism with the aim of improving TYLCV
29 disease management in tomato.
30
31 **Keywords**: Tomato, RNA-Seq, *Tomato yellow leaf curl virus*, Ty-5 gene, Differentially expressed genes.

32 **Introduction**

33 Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in the world, but in recent
34 years, *Tomato yellow leaf curl virus* (TYLCV) has caused serious losses to tomato production in the
35 United States and other countries in tropical and subtropical regions (Polston JE & Anderson PK, 1971).
36 Although this disease was first identified in the eastern Mediterranean (Cohen S & Harpaz I, 1964), it has
37 spread to reach a worldwide distribution (Czosnek H & Laterrot H, 1997; Moriones E & Navas-Castillo J,
38 2000). In addition, recent years have shown a rapid development of the disease in China, with a large area
39 spreading from south to north and from west to east, and the distribution currently covers more than 20
40 tomato production areas, such as Guangdong, Guangxi, Zhejiang, Jiangsu, Shanghai, Yunnan, Sichuan,
41 Shanxi, Shandong, Anhui, Tianjin, Hebei, Beijing, and Liaoning. In particular, this disease has resulted in
42 serious losses to the autumn protected cultivation area in the north of China and the open field cultivation
43 area in the south of China.

44 TYLCV is a begomovirus transmitted by the whitefly (*Bemisia tabaci*) in a circulative manner (Cohen S
45 & Antignus Y, 1994). Most begomoviruses possess a bipartite genome composed of two DNA
46 components of approximately 2.6 kb (M.K. Abhary et al., 2006), but TYLCV has a monopartite genome
47 composed of a single genomic DNA molecule. The genome consists of six open reading frames (ORFs)
48 encoding proteins of at least approximately 10 kDa (Antignus EY & Cohen S (1994); Noris E et al.,
49 1994). TYLCV can lead to severe cupping of apical leaves, stunting, and yellowing, resulting in
50 considerable yield losses.

51 The prevention of TYLCV is difficult because populations of its whitefly vector can reach enormous
52 numbers. Chinese farmers usually use traditional control measures such as chemical insecticides to
53 control the whitefly vector, but this measure has failed to achieve high tomato production quality under
54 severe infection conditions. In addition, yellow traps, fine screens, and intercropping with cucurbits have

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55 been used to reduce the damage caused by TYLCV-complex viruses, but these methods have also failed
56 to restore full crop productivity. Therefore, the breeding of TYLCV-resistant tomato cultivars has become
57 an attractive, environmentally sound strategy for reducing the yield losses inflicted by this virus (Lapidot
58 M & Friedmann M, 2002; Morales FJ, 2001; Polston JE & Lapidot M 2007; Lapidot M et al., 2014).
59 Because the domesticated tomato is susceptible to TYLCV, breeders have introgressed resistance traits
60 identified in wild tomato species (such as *S. chilense*, *S. peruvianum* and *S. habrochaites*) into *S.*
61 *lycopersicum* (Vidavski F et al., 2008). The resulting resistant tomato plant contains chromosomal
62 fragments from wild species in a domesticated tomato background, and these fragments can be identified
63 using polymorphic DNA markers (Ji Y et al., 2007). Five major loci (Ty-1-Ty-5) from wild tomato
64 species associated with resistance to TYLCV and related begomoviruses have been identified (Anbinder I,
65 2009).
66 Line TY172, which carries Ty-5, was derived from four different wild tomato accessions, three of *S.*
67 *peruvianum* (PI 126926, PI 126930, and PI 390681) and one of *S. arcanum* (LA0441) (Friedmann M et
68 al., 1998; Peralta IE, Knapp S & Spooner DM, 2005), through a previously described breeding protocol
69 (Anbinder I, 2009). TY172 is highly resistant to TYLCV, as demonstrated by findings of minimal
70 symptoms following infection and low levels of viral DNA (Lapidot M et al., 1997). Furthermore, TY172,
71 probably due to its high resistance to TYLCV, is a poor source for the acquisition of the virus from
72 whiteflies and its transmission to whiteflies (Lapidot M et al., 2001). These characteristics emphasize the
73 high potential for the use of TY172 in the breeding of TYLCV-resistant tomato cultivars. Classical
74 genetic studies have suggested that the resistance of TY172 is controlled by three genes exerting partially
75 dominant effects (Friedmann M et al., 1998). Specifically, Moshe Lapidot et al. identified the Pelo gene
76 as the gene controlling resistance at the Ty-5 locus. Thus, Pelo, which has been implicated in the
77 ribosome recycling phase of protein synthesis, offers an alternative route to the promotion of resistance to
78 TYLCV and other viruses (Moshe Lapidot et al., 2015). However, the resistance mechanisms of these genes
79 in TYLCV-resistant tomato remains unclear.
80 Viral diseases want to establish a successful infection in planted crops, and thus, viruses must hijack the
81 cellular machinery and prevent or counteract the plant defenses. In response to these viral attacks, plants have

82 developed a variety of resistance mechanisms, which make them either ready to meet incoming pathogens or
83 are induced by infecting viruses. With the advent of molecular biotechnology, particularly high-throughput
84 technologies, changes in gene expression upon viral infection can be monitored at the genome level, and the
85 functions of these genes during infection can be evaluated (Marathe, R et al., 2004; Satoh, K et al., 2010).
86 Moshe Lapidot et al. found that resistance lines inoculated by TYLCV via whiteflies exhibited a less
87 pronounced decline in the abundance of mitogen-activated protein kinases (MAPKs), cellular heat shock
88 proteins and chloroplast protease FtsH and a less pronounced increase in the activities of the pathogenesis-
89 related proteins β-1,3-glucanase and peroxidase compared with a susceptible line (Gorovits R. et al. 2007).
90 Some studies have shown that genes encoding WRKY transcriptional factors, R proteins, protein kinases and
91 receptor (-like) kinases, which are down-regulated DEGs in a susceptible line, are up-regulated or not
92 differentially expressed in a resistant line. Analysis of the up-regulated DEGs in a resistant tomato line after
93 TYLCV infection revealed that the response was characterized by the induction and regulation of a series of
94 genes involved in cell wall reorganization, transcriptional regulation, defense response, ubiquitination, and
95 metabolite synthesis (Tianzi Chen et al. 2013). The screening of cDNA libraries from resistant and susceptible
96 lines before and after TYLCV inoculation identified 69 genes that were preferentially expressed in the resistant
97 line. Twenty-five preferentially expressed genes were tested, and tobacco rattle virus-induced silencing of five
98 of these genes, which encode permease I-like protein, lipocalin-like protein (SlVRSLip), chlorophyll a-b-
99 binding protein 7, thioredoxin peroxidase and hexose transporter (LeHT1), were resulted in resistance collapse
100 (Eybishtz A et al., 2009; Czosnek H et al., 2013; Eybishtz A et al., 2010; Sade D et al., 2012). In recent years,
101 next-generation sequencing (NGS) technologies have emerged as an important genetic tool analysis and
102 have been widely used, providing an unprecedented wealth of high-resolution genotype information
103 (Austin RS et al., 2001). Specifically, these new methods have utilized in studies of sunflower (Livaja M
104 et al., 2013), cucumber (Guangjun Guo et al., 2016), wheat (Yang, Z. et al., 2015), rice (Bai, B et al.,
105 2015), tomato (Jiayin Li et al., 2016; Huanhuan Yang et al., 2017), and bell pepper (Shirani M. K.
106 Widana Gamage et al., 2016). In this study, we used the BGISEQ-500 platform for the comparative
107 transcriptome profiling of resistant and susceptible tomato cultivars to identify differentially expressed
108 genes (DEGs), and the identified DEGs were further verified by qRT-PCR. The results of this study

109 might aid the identification of the key genes and pathways associated with the Ty-5-mediated resistance
110 response and lead to better understanding of the molecular mechanism underlying the contribution of the
111 Ty-5 gene to resistance to TYLCV.

112 Materials and Methods

113 Plant Materials and TYLCV Inoculation

114 Two tomato inbred lines were used: (1) CLN3212A-23, which is also called AVTO1314 and was kindly
115 provided by the Asian Vegetable Research and Development Center (AVRDC), is described as
116 homozygous for the Ty-5 allele and shows high resistance to TYLCV, and (2) Moneymaker, which was
117 kindly provided by the Chinese Academy of Agricultural Sciences, is susceptible to TYLCV.

118 TYLCV was provided by Dr. Xueping Zhou of Zhejiang University, who has thoroughly studied this
119 virus (H Zhang, H Gong & X Zhou, 2009). The reaction of populations to TYLCV inoculation was
120 evaluated in a greenhouse in Zhejiang, China, in the autumn of 2016. The parental lines included 200
121 individuals. The virus was preserved in Agrobacterium and was propagated in YEP medium at 28°C with
122 an inoculation concentration of OD600 = 1. At the four- to five-leaf stage, the stem phloem of the tomato
123 plants was injected with approximately 1 ml of the viral culture solution using a syringe. After the
124 injection, the plants were maintained in an insect-proof greenhouse at a temperature of 26-30°C and a
125 relative humidity of 70-80% for 28 days. As this incubation period, the symptoms for Grade 1 disease,
126 characterized by very slight yellowing of the leaflet margins on apical leaves, could be clearly observed.
127 The symptoms were evaluated according to the disease severity index (DSI) published previously
128 (Lapidot M et al., 1997; Friedmann M et al., 1998).

129 RNA Extraction, RNA-Seq Library Preparation, and Sequencing

130 The total young leaf RNA from plants from each of the four experimental groups, namely, untreated
131 CLN3212A-23 (R group), treated CLN3212A-23 (RT group), untreated Moneymaker (S group) and
132 treated Moneymaker (ST group), was extracted and analyzed according to the manufacturer's
133 recommended protocol, and three biological replicates for each group, which consisted of three plants,
134 were isolated as previously described (Fang, S.M. et al., 2015). The concentration of total RNA and the
135 RNA integrity value (RIN) were determined using the RNA 6000 Pico LabChip for the Agilent 2100

136 Bioanalyzer (Agilent, USA). The obtained total RNA was treated with DNase I, and oligo (dT) was used
137 for mRNA isolation. After purification of the poly-A-containing mRNA molecules using poly-T-oligo-
138 attached magnetic beads, the mRNA was fragmented into small pieces using divalent cations under
139 elevated temperature. The cleaved RNA fragments were copied into first-strand cDNA using reverse
140 transcriptase and random primers, and this procedure was followed by second-strand cDNA synthesis
141 using DNA polymerase I and RNase H. The resulting cDNA fragments then underwent the addition of a
142 single 'A' base and subsequent adapter ligation. The products were then purified and enriched through
143 PCR amplification. The PCR yield was quantified by Qubit, and the samples were pooled together to
144 obtain a single-strand DNA circle (ssDNA circle), which represented the final library. DNA nanoballs
145 (DNBs) were generated from the ssDNA circle by rolling circle replication (RCR) to increase the
146 fluorescent signal during the sequencing process. The DNBs were loaded into patterned nanoarrays, and
147 paired-end reads of 100 bp were obtained using the BGISEQ-500 platform for subsequently data analyses.
148 During this step, the BGISEQ-500 platform combines DNA-nanoball-based nanoarrays with stepwise
149 sequencing using the combinational probe-anchor synthesis sequencing method.

150 **Quantification and Analysis of Transcripts**

151 Raw reads were filtered using SOAPnuke software (https://github.com/BGI-flexlab/SOAPnuke) to
152 acquire clean reads. These were obtained after removing reads containing adapter sequences and low-
153 quality reads, as defined based on the percentage of bases in a read with a quality less than 15% or a
154 quality greater than 20% but a sequencing quality less than 5. The reference genome and gene model
155 annotation files were downloaded from the Ensembl Genomes Database
156 (ftp://ftp.ensemblgenomes.org/pub/release-23/plants/fasta/solanum_ lycopersicum/dna/). The clean reads
157 were mapped to the tomato genome assembly SL2.50 using HISAT2 software
158 (http://www.ccb.jhu.edu/software/hisat). An index for the reference genome was built using Bowtie
159 v2.2.3 (Langmead. B & Salzberg. S.L. 2012). Gene expression levels in terms of transcripts were
160 quantified by RNA-Seq by expectation maximization (RSEM) and fragment per kilobase per million
161 mapped (FPKM) (Trapnell, C. et al., 2012; Li, B. & Dewey, C. N. 2011). DEGs were detected using
162 DEseq2 methods with an adjusted p value (p_{adj}) < 0.05. Genes with adjusted p-value (PNOI) ≤ 0.08 and

163 fold change ≥ 2 were defined as significantly enriched (Michael I Love, Wolfgang Huber &
164 Simon Anders 2014). Cufflinks v2.1.1 (Trapnell, C. et al., 2010) was used to construct and identify both
165 known and novel transcripts from the HISAT2 alignment results.

166 **Gene Ontology and KEGG Enrichment Analysis of DEGs**

167 The DEGs were classified by function based on Gene Ontology (GO) annotation results, and an
168 enrichment analysis was performed using R software according to ITAG2.4. GO terms with $p_{adj} \leq 0.05$
169 were considered significantly enriched in the DEGs (Chen, Z.Z., et al., 2005). The DEGs were classified
170 by biological pathway based on KEGG annotation results, and an enrichment analysis was performed
171 with R software. A p-value ≤ 0.05 indicated significant enrichment (Kanehisa, M. et al., 2017).

172 **Quantitative Real-Time PCR Validation**

173 Twelve randomly selected DEGs were analyzed by RT-qPCR to verify the expression profiles obtained
174 by RNA-Seq. The primer pairs used to amplify the selected genes were designed using Primer Premier
175 6.0 (Premier Biosoft, Canada; File S1). The RNase H Reverse Transcription kit (TaKaRa) was used
176 according to the manufacturer's instructions. The tomato GAPDH gene was used as a reference control.
177 The Analytik Jena qTOWER 2.2 qRT-PCR instrument (Germany) was used in this experiment. Each
178 sample was repeated three times, and relative expression levels were evaluated using the $2^{-ÄÄCt}$ method
179 (Livak, K.J. & Schmittgen, T.D, 2001). The amplification conditions were denaturation for 8 min at 95ºC
180 followed by 39 cycles of 95ºC for 10 s, 58ºC for 30 s, and 72ºC for 20 s. Following amplification, melting
181 curves were performed by increasing the temperature from 60 to 95ºC at an interval of 1ºC every 4 s to
182 confirm the specificity of the PCR amplification.

183

184 **Results**

185 Summary **and Analysis of the RNA-Seq Data**

186 Raw data corresponding to an average of 6.97 Gb were generated for each of the three replicates of each
187 of the four groups using the BGISEQ-500 platform. In addition, an average of 6.56 Gb of clean reads was
188 obtained for each sample, and 90.38% of the clean reads were at the Q30 level. The average percentage of
189 clean reads was 93.82% across all samples (File S2). Moreover, at least 90.1% of these reads were

190 mapped to the tomato reference genome, and 79.4% of the mapped reads were uniquely mapped (Table 1).
191 Furthermore, a total of 21,739 novel transcripts were generated using the Cufflinks tool (Trapnell, C. et al,
192 2012), and the CPC software predictions showed that these novel transcripts included 17,560 novel forms,
193 537 novel genes, 3,642 non-coding transcripts and 18,097 coding transcripts (Kong, L. et al. 2007). A
194 total of 27,863 genes, including 27,329 known and 537 unknown genes, were generated.

195 We used Venn diagrams to analyze the differences in gene expression between samples and between
196 groups (Fig. 1). The biological replicates R1, R2 and R3 expressed 21,921 genes in common, whereas
197 RT1, RT2 and RT3 had 22,137 genes expressed in common, S1, S2 and S3 showed the expression of
198 21,851 genes in common, and ST1, ST2 and ST3 expressed 22,249 genes in common. The number of
199 genes that were expressed in both the R and RT groups and in both the S and ST groups were reduced, i.e.,
200 21,921 and 21,851, which are lower than 22,137 and 22,249, respectively. This finding suggests that the
201 expression of groups of genes is activated or deactivated upon infection with TYLCV, and the number of
202 activated genes is higher than that of deactivated genes.

203 **Analysis of DEG Responses to TYLCV**

204 The DEGs were identified using DEseq2 methods (Files S3, S4, S5, and S6). A total of 8,739 DEGs were
205 detected between the R and RT groups (R-VS-RT comparison), 747 DEGs were detected between the R
206 and S groups (R-VS-S comparison), 2,819 DEGs were detected between the RT and ST groups (RT-VS-
207 ST comparison), and 8,899 DEGs were detected between the S and ST groups (S-VS-ST comparison).
208 These results indicated that the number of DEGs in the non-inoculated CLN3212A-23 or Moneymaker
209 line was higher than that in the corresponding treated group, which suggested that genes were activated
210 by TYLCV infection. To observe the overall changes in gene expression levels, the DEGs were subjected
211 to hierarchical clustering using FPKM analysis. Analysis of the R-VS-RT and S-VS-ST comparisons
212 showed that the same genes could be up- or down-regulated depending on the line, and we hypothesized
213 that the genes that showed increased up-regulation in the R-VS-RT comparison compared with the S-VS-
214 ST comparison (i.e., the S-VS-ST comparison showed no change or little up-regulation) are likely
215 correlated with resistance (Fig. 2). Similarly, the RT-VS-ST and R-VS-S comparisons showed
216 differences in down-regulation and up-regulation for the same genes, and we hypothesized that the genes

217 that showed increased down-regulation in the RT-VS-ST comparison compared with the R-VS-S
218 comparison (i.e., the R-VS-S comparison showed up-regulation, down-regulation or no change) are
219 potential resistance genes (Fig. 2). Because CLN3212A-23 shows a resistant phenotype after inoculation,
220 the Ty-5 gene, which is carried by this line, likely activated the expression of multiple genes to contribute
221 to the process of resistance, and the identified DEGs therefore represent a series of molecular mechanisms
222 involved in resistance to TYLCV.

223 **GO Assignments and Enrichment Analysis of DEGs**

224 To identify the functions of the DEGs involved in the tomato response to TYLCV, GO assignments and
225 enrichment analysis were performed using GOseq. Most of the assigned functions of the identified DEGs
226 belonged to the biological process (BP), cellular component (CC) and molecular function (MF) categories.
227 A total of 23 GO terms in the BP category, 15 GO terms in the CC category and 13 GO terms in the MF
228 category were found (Fig. S1). Thus, both CLN3212A-23 and Moneymaker showed underwent major
229 changes in the expression of genes involved in the BP, CC and MF categories after inoculation. Table 2
230 shows the results of the GO enrichment analysis for the R-VS-RT versus S-VS-ST comparisons, which
231 revealed common enrichment terms related to TYLCV infection, and the difference in the p-values
232 between the groups was partly due to the contribution of the Ty-5 gene. The GO enrichment analysis for
233 the R-VS-RT versus RT-VS-ST comparisons revealed only one common enrichment term, "response to
234 stimulus" in the BP category(Fig. S2, S3, S4), which suggests that this term is related to the main effect of
235 the Ty-5 gene.

236 **KEGG Enrichment Analysis of DEGs**

237 To investigate the major pathways containing DEGs, we aligned all DEGs to KEGG pathways (Qvalue <
238 0.05), and the four scatter plots in Figure 3 show the 20 KEGG pathways showing the greatest enrichment
239 of the DEGs in each experimental group. The comparison of these four diagrams demonstrates that the R-
240 VS-RT and RT-VS-ST comparisons presented a similar DEG enrichment pattern: the DEGs were
241 enriched in the "Plant hormone signal transduction", "Carbon metabolism", "Carbon fixation in
242 photosynthetic organisms", and "Glutathione metabolism" pathways. The DEGs identified from the S-
243 VS-ST comparison were also enriched in the "Plant hormone signal transduction", "Carbon metabolism",

244 and "Carbon fixation in photosynthetic organisms" pathways, but the DEGs identified from the R-VS-S
245 comparison were not enriched in the "Plant hormone signal transduction", "Carbon metabolism", or
246 "Carbon fixation in photosynthetic organisms" but were enriched in "Glutathione metabolism". Thus, the
247 "Plant hormone signal transduction", "Carbon metabolism", "Carbon fixation in photosynthetic
248 organisms", and "Glutathione metabolism" might be the major metabolic pathways involved in the
249 response of Ty-5-carrying tomato to TYLCV infection. Therefore, DEGs related to disease-resistance
250 pathways were significantly down-regulated in the RT-VS-ST comparison but up-regulated in the R-VS-
251 RT comparison and showed either up-regulation or no change in the S-VS-ST comparison. Based on the
252 these analyses, the DEGs related to the TYLCV-resistance gene Ty-5 were screened from the "Plant
253 hormone signal transduction" (Fig. S5, Files S3, S4, and S5), "Carbon metabolism" (Fig. S6, Files S3, S4,
254 and S5), "Carbon fixation in photosynthetic organisms" (Fig. S7, Files S3, S4, and S5) and "Glutathione
255 metabolism" (Fig. S8, Files S3, S4, and S5) pathways, and the results identified 16, nine, one, and two
256 DEGs in the significantly enriched KEGG pathways "Plant hormone signal transduction" (Table 3),
257 "Carbon metabolism" (Table 4), "Carbon fixation in photosynthetic organisms" (Table 5), and
258 "Glutathione metabolism" (Table 6), respectively.

259 **RT-qPCR Validation of RNA-Seq Data**

260 The gene expression levels of 12 randomly selected genes obtained by RNA-Seq were validated by real-
261 time
262 qPCR using the same total RNA preparations used for the RNA-Seq library preparation. The expression
263 values of the 12 genes in the four groups (S, R, RT and ST) obtained by RNA-Seq and RT-qPCR analysis
264 were compared (File S7). Due to differences in the statistical methods used for determining the expression
265 levels, the numerical expression values obtained using these two techniques cannot be directly compared,
266 but the trends in the expression patterns of the selected genes indicated the reliability of the sequencing
267 data. As shown in Figure 4 with superimposed bars, the trends for increasing and decreasing expression
268 for the 12 genes in the four groups (S, R, RT and ST) were nearly identical, indicating the reliability of
269 the RNA-Seq data.

270 **DISCUSSION**

271 In this study, we used RNA-Seq to investigate the transcriptome profiles of resistant cv. CLN3212A-23
272 and susceptible cv. Moneymaker in response to TYLCV. A total of 21,739 transcripts and a number of
273 significant DEGs were identified in our four pairwise comparisons (RT-VS-ST, R-VS-RT, S-VS-ST, and
274 R-VS-S). A comparative analysis of GO enrichment terms identified common terms in the R-VS-RT and
275 S-VS-ST comparisons, suggesting that the responses of both resistant cv. CLN3212A-23 and susceptible
276 cv. Moneymaker to TYLCV infection included changes in the expression levels of genes involved in
277 three major functional domains (BP, MF and CC). However, the p-values for the GO enrichment terms
278 showed notable differences in these two comparisons because CLN3212A-23 and Moneymaker have
279 different genetic backgrounds, which results in different response levels. Furthermore, the presence of the
280 Ty-5 gene was likely responsible for the lower p-values obtained in the R-VS-RT comparison compared
281 with the S-VS-ST comparison. Specifically, the "protein kinase activity", "kinase activity" and
282 "phosphotransferase activity, alcohol group as acceptor" terms in the MF category were found to be related
283 to disease resistance. The "chlorophyll binding" and "pigment binding" terms have been found to play a
284 critical role in plant hormone signal transduction (Goyer A et al., 2015). In the BP category, the "response
285 to stimulus" and "signaling" terms were also identified as related to disease resistance. The "response to
286 radiation" and "photosynthesis, light harvesting" terms are important for plants because these
287 photosynthetic organisms must achieve a delicate balance between the light energy absorbed and their
288 capacity to channel that energy into productive photochemical reactions to improve photosynthetic
289 efficiency. In addition, a previous study of the infection of potato with potato Y virus showed that
290 transcripts classified with the GO term "photosynthesis, light harvesting" were enriched during the early
291 phase of the infection (Shirani M. K. Widana Gamage et al., 2016). Analysis of the CC category
292 identified the "integral component of membrane", "intrinsic component of membrane", "membrane",
293 "membrane part" and "chloroplast thylakoid membrane" terms, which are related to the functions of
294 biological membranes; cell membranes can protect cells and carry communication signals between cells,
295 and inner membranes create orderly conditions for biochemical reactions. In a complex environment,
296 where multiple biotic and abiotic stresses might seriously restrict the growth and development of plants,
297 changes in membrane construction and components occur, indicating the participation of membranes in

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298 stress resistance. For example, plastoglobules are attached to the thylakoid membrane by the shared outer
299 lipid leaflet, and previous studies have shown that a large proportion of the total plastid tocopherol is
300 accumulated in the plastoglobule core, which enlarges during oxidative stress (Vidi, P.A. et al., 2006;
301 Brehelin, C., Kessler, F. & VanWijk, K.J, 2007) and that plastoglobules play a role in metabolite repair
302 through the tocopherol redox cycle (Lucia EugeniPiller et al., 2014).

303 Plant hormones are known to regulate the expression of gene networks related to defense responses
304 (Bari, R. et al., 2009). In the present study, 16 hormone-responsive DEGs were found to be abundantly
305 expressed in resistant cv. CLN3212A-23 after infection (Table 3), and these DEGs are involved in the
306 auxin, gibberellin (GA), abscisic acid (ABA), ethylene (ET) and jasmonic acid (JA) metabolic pathways.
307 Auxin-regulated plant growth and development are likely initiated by the rapid response of specific genes
308 to local changes in the auxin concentration. These genes are denoted early auxin response genes. SAUR
309 family proteins have been identified in diverse plant species, including tomato (Zurek, D.M. et al., 1994),
310 and Aux/IAA genes are one of the several different types of early auxin response genes in Arabidopsis
311 (Guilfoyle, T.J, 1999; Hagen, G. & Guilfoyle, T. J., 2002). Although substantial progress has been made
312 toward understanding the functions of Aux/IAA proteins in auxin response (Tiwari, S.B. et al., 2007;
313 Calderon Villalobos et al., 2012), functional studies of SAURs have lagged. The functions of Aux/IAA
314 genes in regulating plant growth and development have largely been revealed through the characterization
315 of dominant gain-of-function mutations in Aux/IAA protein domain II (Nagpal, P. et al., 2000; Rinaldi, M.
316 A. et al., 2012). In tomato, phenotypic changes have been observed in knockdown mutants of Aux/IAA
317 genes (Wang, H. et al., 2005a; Bassa, C. et al., 2012; Deng, W. et al., 2012; Su, L. Su, L. et al., 2014),
318 which suggests that the functions of Aux/IAA proteins might be depend on the particular plant species.
319 Type-B phospho-accepting response regulator (ARR) family members serve as DNA-binding
320 transcriptional regulators as part of a phosphorelay, and their activities are most likely regulated by
321 phosphorylation/dephosphorylation (Oshinori Tajima et al., 2004). Exogenous GA can affect plant
322 growth and regulation, and two genes, including the GA receptor GIBBERELLIN INSENSITIVE
323 DWARF1 (GID1), which directly binds GA, regulate GA signaling (Ueguchi-Tanaka, M. et al., 2005).
324 Phytochrome-interacting factor is one component of light-absorbing photoreceptors (Carvalho, R. F.,

325 Campos, M. L. & Azevedo, R. A, 2011) and can thus affect the photosynthetic efficiency of plants.
326 Abscisic acid (ABA) is known to participate in a wide range of adaptive responses to diverse
327 environmental abiotic stresses, such as drought, osmosis, and low temperatures, and has been implicated
328 in plant-pathogen interactions (Grant M & Lamb C, 2006). ABA signaling is initiated by its receptors,
329 PYR/PYL/RCARs, which are soluble proteins with a conserved START domain that can bind ABA and
330 trigger downstream signaling. The involvement of JA and ET in several plant-pathogen interactions has
331 been extensively studied (Bari R & Jones JD, 2009; Pieterse CM et al., 2009; Grant M & Lamb C, 2006),
332 and a number of ethylene-responsive factor (ERF) transcription factors have been shown to play critical
333 roles in regulating immune responses in plants. A previous study showed the functions of Arabidopsis At
334 ERF15 in immune responses against *Pseudomonas syringae* pv. Tomato (Pst) DC3000, a (hemi-)
335 biotrophic bacterial pathogen, and *Botrytis cinerea*, a necrotrophic fungal pathogen (Huijuan Zhang et al.,
336 2015). MYC2 activity is enhanced by the effects of ABA on JA-induced transcriptional activation, and
337 ABA is known to exert synergistic effects on the MYC family (Anderson, J.P. et al., 2004; Kazan, K &
338 Manners, J.M, 2013). In addition, these plant hormone pathways can interact, and the SA–JA backbone of
339 the immune signaling network can be modified by other hormones, such as ET and ABA (VanHulten, M.
340 et al., 2006; Ton, J., Flors, V. & Mauch-Mani, B, 2009; Robert-Seilaniantz, A., Grant, M. & ones, J.D.G,
341 2011). Brassinosteroids (BRs), GA, JA, and ABA have been reported to regulate the expression of some
342 SAUR genes, indicating that SAURs likely contribute to other hormone-regulated aspects of plant growth
343 and development. Similar to auxin, BRs are growth-promoting hormones that regulate many
344 physiological and developmental processes (Fa` bregas, N. & Can˜ o-Delgado, A.I., 2014; Wang, W., Bai,
345 M.Y. & Wang, Z.Y, 2014b).

346 The pairwise group comparison analysis performed in this study found that nine DEGs were
347 significantly enriched in the KEGG pathway “Carbon metabolism”. These genes are likely related to the
348 mechanisms of Ty-5 resistance and were annotated as follows. Glutathione (GSH), an important cellular
349 antioxidant, plays a critical role in plant disease resistance (Parisy, V. et al., 2007), and the absence of S-
350 (hydroxymethyl)glutathione dehydrogenase makes cells more sensitive to oxidants and antibiotics.
351 Alcohol dehydrogenases (ADH, EC 1.1.1.1) belong to the dehydrogenase enzyme superfamily, are widely

352 distributed in all types of organisms (Chase, T, 1999; Alka, K. et al., 2013), and participate in an
353 astonishingly wide range of metabolic processes (Strommer, J, 2011). Certain abiotic and biotic stresses
354 can induce the specific expression of these alcohol dehydrogenases in different tissues of soybean, wheat
355 and barley, implying that their potential participation in differential tissue development under stress
356 (Komatsu, S. et al., 2011; Pathuri, I. P. et al., 2011; Yamauchi, T. et al., 2014). Formate dehydrogenase,
357 which been proven to be a shock protein, shows significantly increased levels under various stresses, such
358 as drought, hypoxia and low temperature, and thus plays an important role in responses to stress in plants.
359 This enzyme can catalyze formic acid to produce carbon dioxide and NADH, and under stress conditions,
360 formic acid might act as a signal compound (Appling DR, 1991; Hourton-Cabassa C et al., 1998; Suzuki
361 K et al., 1998). L-cysteine plays crucial roles in the structure, stability and catalytic function of many
362 proteins and is also an important amino acid used in the pharmaceutical, food and cosmetics industries.
363 Serine o-acetyltransferase plays a catalytic and regulatory role in the synthesis of L-cysteine (Kredich NM,
364 1983). Phosphoserine aminotransferase (PSA) is involved in the phosphorylated pathway of L-serine
365 biosynthesis. Isocitrate lyase (ICL), one of the key enzymes in the unique metabolic process of the
366 acetaldehyde acid cycle, has been found to be important in human, animal, and plant pathogenesis. In
367 particular, for several agricultural crops, including cereals, cucumbers, and melons, increased expression
368 of the gene encoding ICL has been found to be important for fungal virulence (Dunn, MF, Ramirez-
369 Trujill JA & Hernandez-Lucas I, 2009). In plants, malate dehydrogenase (oxaloacetate-decarboxylating)
370 (NADP+) isoforms show specific subcellular localizations and have specific coenzymes. In fact, plant
371 MDHs are classified according to their subcellular localizations as chloroplast, mitochondrial,
372 peroxisomal, plastidial, and cytosolic MDHs (Gietl, C, 1992), and the different MDH isoforms play
373 various roles depending on their subcellular localizations and the metabolic activities that occur in their
374 respective cellular compartments. For example, chloroplast MDHs are key enzymes in Chinese cabbage
375 that show marked effects on plant growth, and their gene is also involved in aluminum resistance (Jing
376 Zhao et al., 2016). Additionally, the pH levels control the specificity of substrate binding by malate
377 dehydrogenase due to proton transfer during the catalytic mechanism. 2,3-Biphosphoglycerate-
378 independent phosphoglycerate mutase performs a key enzymatic activity in glycolysis and catalyzes the

379 reversible interconversion of 3-phosphoglycerate to 2-phosphoglycerate (Zhao, Z. & Assmann, S.M,
380 2011), and this enzyme might play important roles in energy metabolism under stress.
381 Enolase, also known as phosphopyruvate hydratase, is a metalloenzyme responsible for one of the
382 catalytic reactions of glycolysis. Acetyl-CoA synthetase is an enzyme (EC 6.2.1.1) involved in the
383 metabolism of acetate. It catalyzes the formation of a new chemical bond between two large molecules,
384 and its activity is usually associated with metabolic pathways. Overall, the nine DEGs significantly
385 enriched in the KEGG pathway "Carbon metabolism" were annotated as enzymes involved in carbon
386 metabolism, the synthesis or degradation of new materials, and energy generation, and such materials and
387 energy can improve crop resistance to biotic or abiotic stresses and promote vigorous crop growth.
388 Notably, a common significantly enriched gene (Solyc05g050120.3.1) with the same functional
389 annotations in the KEGG pathways "Carbon metabolism" and "Carbon fixation in photosynthetic
390 organisms" was found. MDH exists as a homodimeric molecule in most organisms, which suggests that the
391 gene is active and sensitive to TYLCV and that the Ty-5 gene affects its regulation.
392 Two significantly enriched DEGs (Solyc02g093830.3.1 and Solyc09g011590.3.1) were found in the
393 KEGG pathway "Glutathione metabolism", and these genes were annotated as glucose-6-phosphate
394 dehydrogenase (G6PDH) and GSH S-transferase (GST), respectively. G6PDH is widely distributed in
395 many species from bacteria to humans and is involved in regulating the metabolic rates of many
396 physiological processes (Kruger, N.J. & vonSchaewen, A, 2003). Some studies have reported that
397 G6PDH genes respond to various environmental stresses, such as salt (Zhang, L. et al., 2013; Cardi, M. et
398 al., 2015) and drought (Liu, J.et al., 2013). In recent years, the study of G6PDHs has mainly focused on
399 its transcription and activity under various stresses. GSTs are a class of detoxification enzymes found
400 throughout the eukaryotic kingdom that catalyze the conjugation of reduced GSH to both natural and
401 synthetic xenobiotics (Li, X., Schuler, M.A. & Berenbaum, M.R, 2007). GSTs are involved in flavonoid
402 metabolism (Kitamura, S., Shikazono, N., & Tanaka, A, 2004), signaling (Chen, I.-C. et al., 2007), and
403 responses to biotic and abiotic stress and plant hormones (Moons, A., 2005). We thus speculate that
404 TYLCV infection and the presence of the TY-5 gene resulted in the observed enrichment of these two
405 DEGS.

406 Similar to results in other plants, a complicated gene network might be involved in the Ty-5-mediated
407 resistant response of tomato to TYLCV, which may being with a basal response and the production of
408 general pathogen-associated molecular pattern molecules. This experimental study showed that 16 of the
409 identified genes were significantly enriched in the “Plant hormone signal transduction” pathways, and
410 these genes were annotated to some transcription factors, such as transcription factors of the abscisic acid
411 receptor PYR/PYL family, SAUR auxin-responsive protein IAA R family, and two-component response
412 regulator ARR-B family, as well as ethylene-responsive transcription factor 1, which are related to signal
413 transduction, regulation, growth, and plant-pathogen interactions, among other functions. We then
414 speculated that the transduction and regulation of plant hormone signals activated 12 DEGs in the
415 “Carbon metabolism”, “Carbon fixation in photosynthetic organisms” and “Glutathione metabolism”
416 pathways, and these genes were annotated to enzymes, such as glutathione dehydrogenase, enolase,
417 malate dehydrogenase, acetyl-CoA synthetase, glutathione S-transferase, that have strongly been
418 associated with plant disease resistance in previous studies. Thus, an in-depth study of the 28 genes is
419 necessary because these might be associated with the Ty-5-mediated resistance to TYLCV. By studying
420 the molecular mechanism of The ty-5 gene, we can improve the anti-TYLCV management of tomato
421 varieties.

422 **CONCLUSION**

423 In summary, a transcriptome sequencing analysis of the responses of the resistant tomato cultivar
424 CLN3212A-23 and the sensitive cultivar Moneymaker to TYLCV infection revealed many DEGs.
425 Furthermore, based on GO term and KEGG pathway analyses, some of the significantly enriched DEGs
426 were associated with the Ty-5 resistance gene: because these DEGs showed a decreased up-regulation or
427 no change in expression in the Moneymaker cultivar after inoculation, we concluded that the presence of
428 Ty-5 in CLN3212A-23 after inoculation affected the expression of these DEGs. More specifically, the
429 annotation of these significantly enriched DEGs indicated association with plant hormone level changes,
430 carbon and glutathione metabolism, sugar metabolism, and some photosynthetic factors, all of which
431 strengthen plants and provide energy to support pathogen resistance. Based on these analyses and
432 conclusions, we hypothesize that these significantly enriched DEGs are related to resistance to TYLCV

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433 disease, either directly or indirectly.

434 **Supporting Information**

435 File S1. Primers used for RT-qPCR

436 File S2. Quantitative analysis of raw RNA-Seq data

437 File S3. DEGs identified in the S-VS-ST comparison by DEseq2 methods

438 File S4. DEGs identified in the RT-VS-ST comparison by DEseq2 methods

439 File S5. DEGs identified in the R-VS-RT comparison by DEseq2 methods

440 File S6. DEGs identified in the R-VS-S comparison by DEseq2 methods

441 File S7. Expression values of 12 genes in the S, R, RT and ST groups obtained by RNA-Seq and RT-

442 qPCR analysis

443

444 FIGURE S1| Gene Ontology and enrichment of DEGs identified from the RT-VS-ST, R-VS-RT, R-VS-S

445 and S-VS-ST comparisons

446 FIGURE S2| GO enrichment of DEGs in the biological process categories obtained from the RT-VS-ST,

447 R-VS-RT and S-VS-ST comparisons

448 FIGURE S3| GO enrichment of DEGs in the cellular component categories obtained from the RT-VS-ST,

449 R-VS-RT and S-VS-ST comparisons

450 FIGURE S4| GO enrichment of DEGs in the molecular function categories obtained from the RT-VS-ST,

451 R-VS-RT and S-VS-ST comparisons

452 FIGURE S5| "Plant hormone signal transduction" pathway

453 FIGURE S6| "Carbon metabolism" pathway

454 FIGURE S7| "Carbon fixation in photosynthetic organisms" pathway

455 FIGURE S8| "Glutathione metabolism" pathway

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Figure 1

Venn diagrams of gene expression between experimental replicates

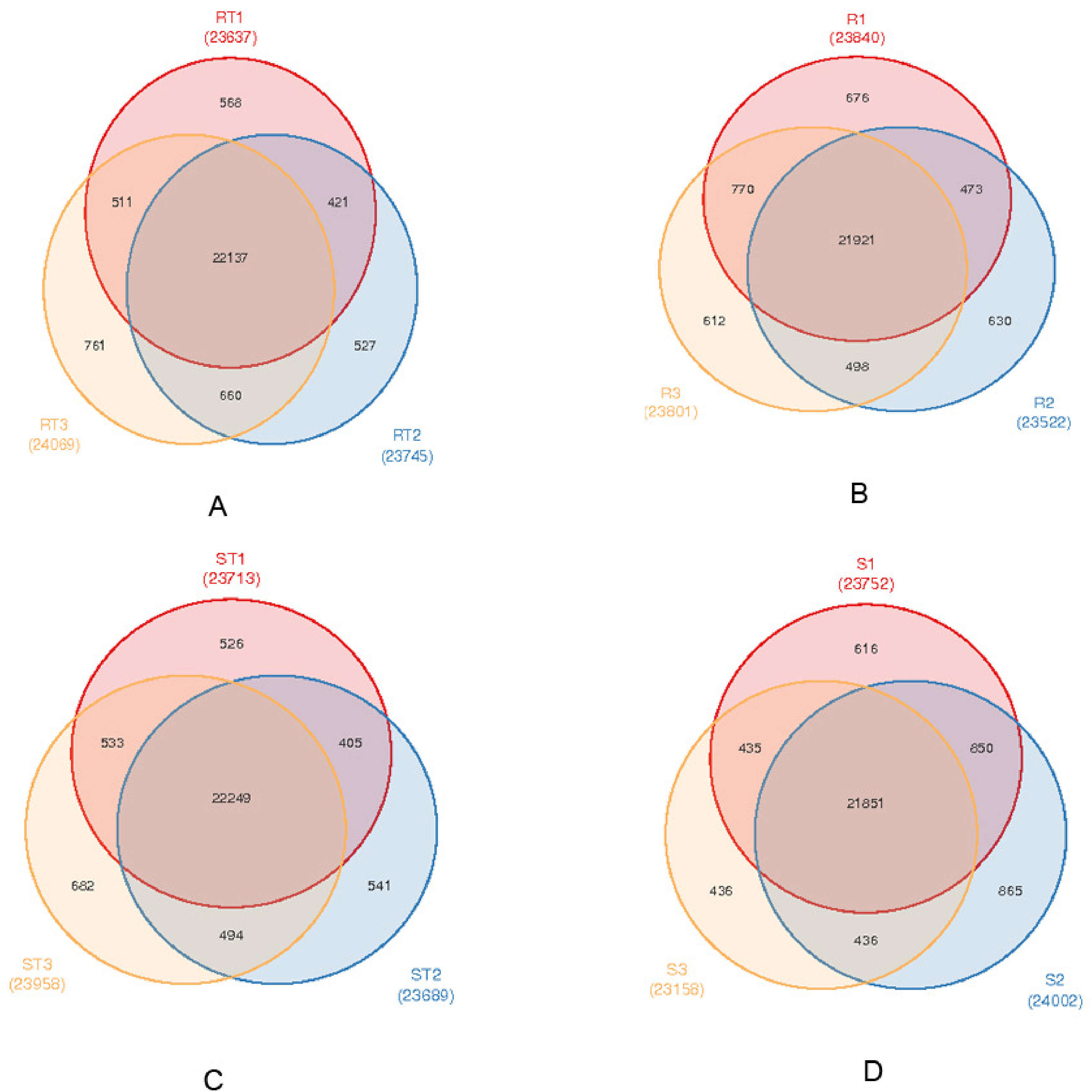

Figure 2

Hierarchical clustering of DEGs

The color represents the expression fold change after log2 transformation. More intense red color indicates more strongly up-regulated DEGs. More intense blue color indicates more strongly down-regulated DEGs. The union represents a comparison of expression changes in the same DEGs in different treatment pairs.

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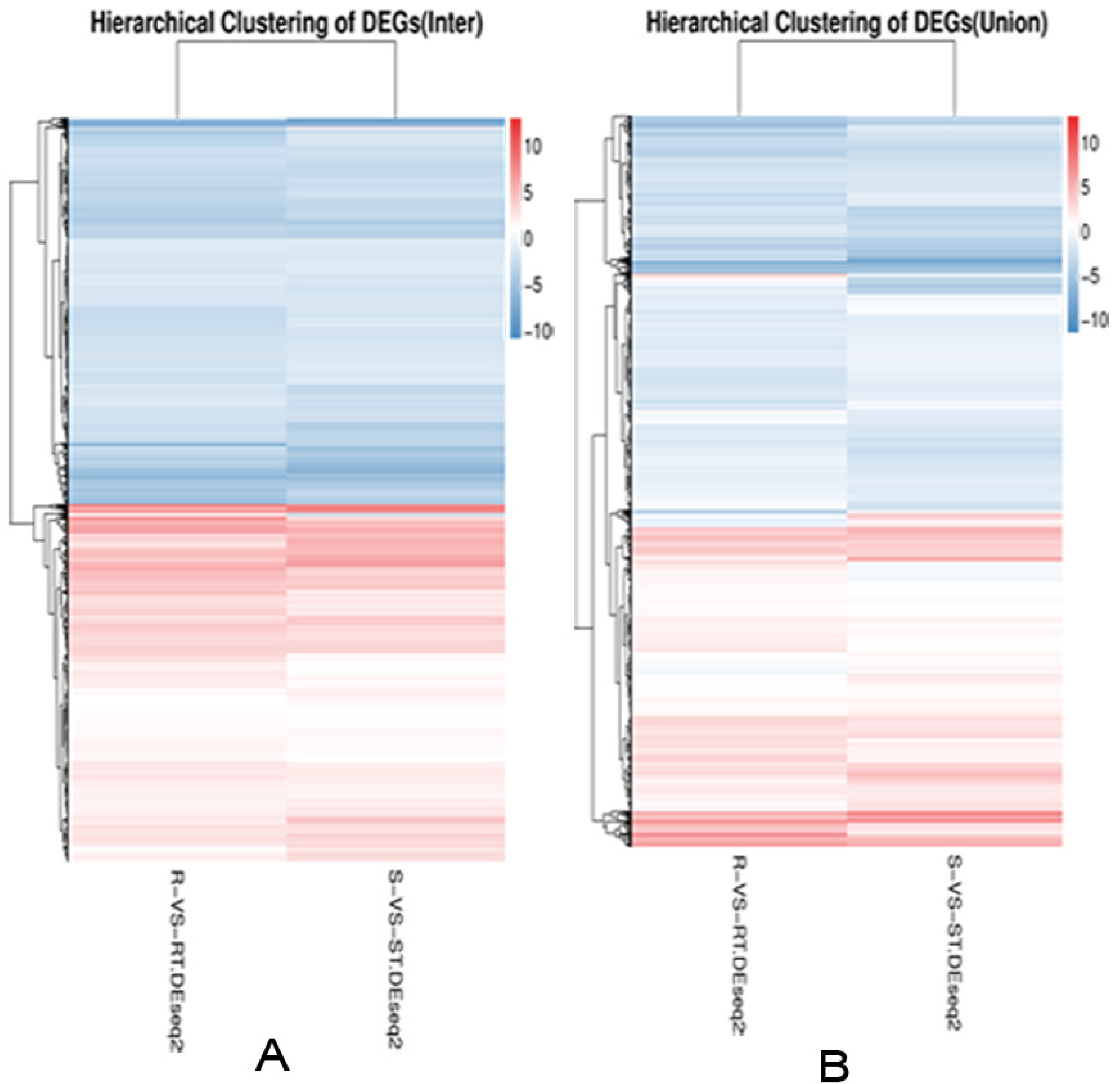

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Figure 3

Scatter plot of the KEGG pathway enrichment of DEGs

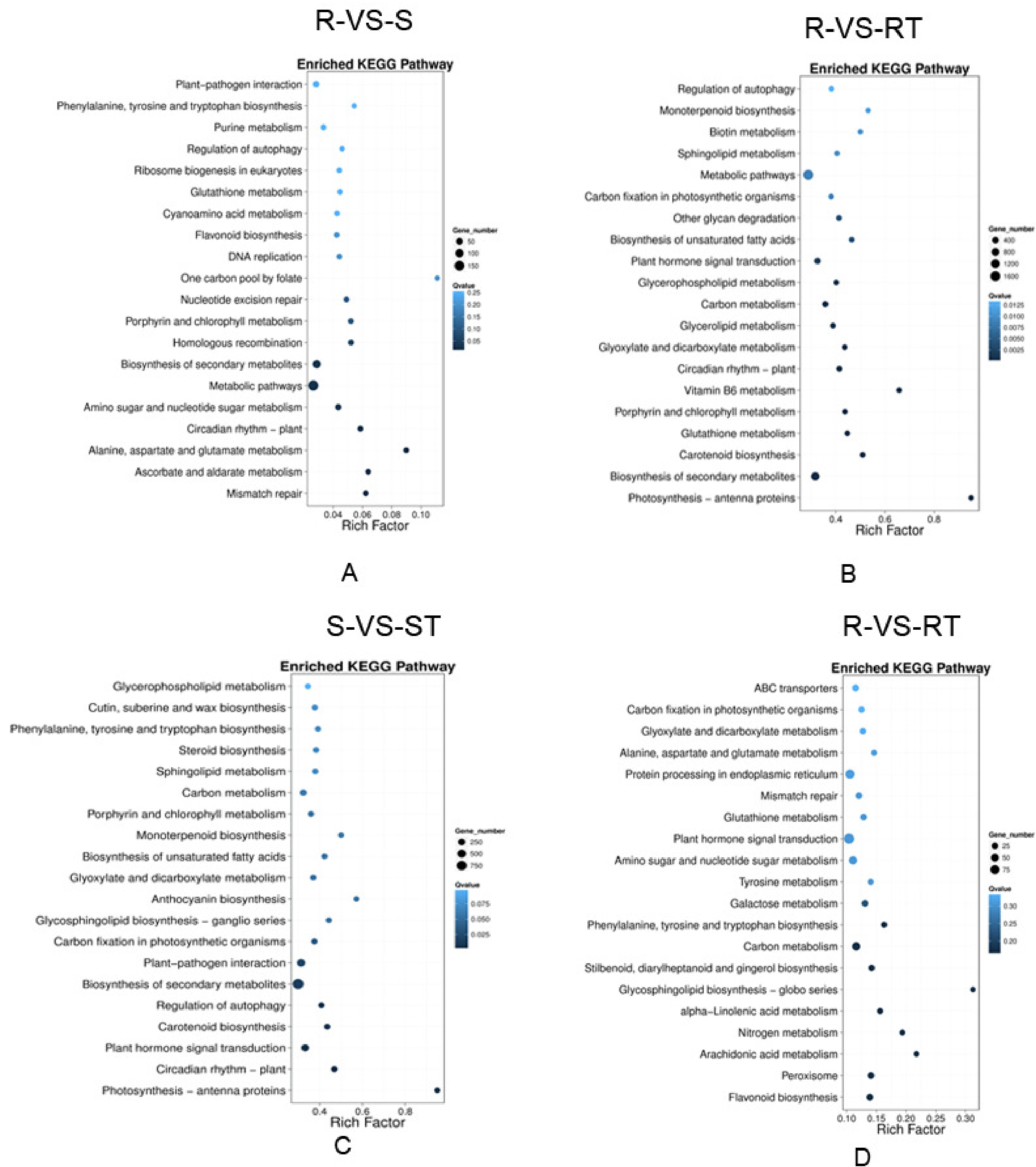

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Figure 4

The expression values of the 12 genes in the four treatments (S, R, RT and ST) by RNA-Seq and RT-qPCR analysis

The X-axis represents the four samples, S, R, RT and ST; the Y-axis represents the FPKM values obtained by RNA-Seq or the 2-ΔΔCt values obtained by RT-qPCR. The asterisk above the bars indicates statistically significant differences between R-VS-RT or S-VS-ST by RNA-seq and RT-PCR analysis by t test ($p < 0.05$) .

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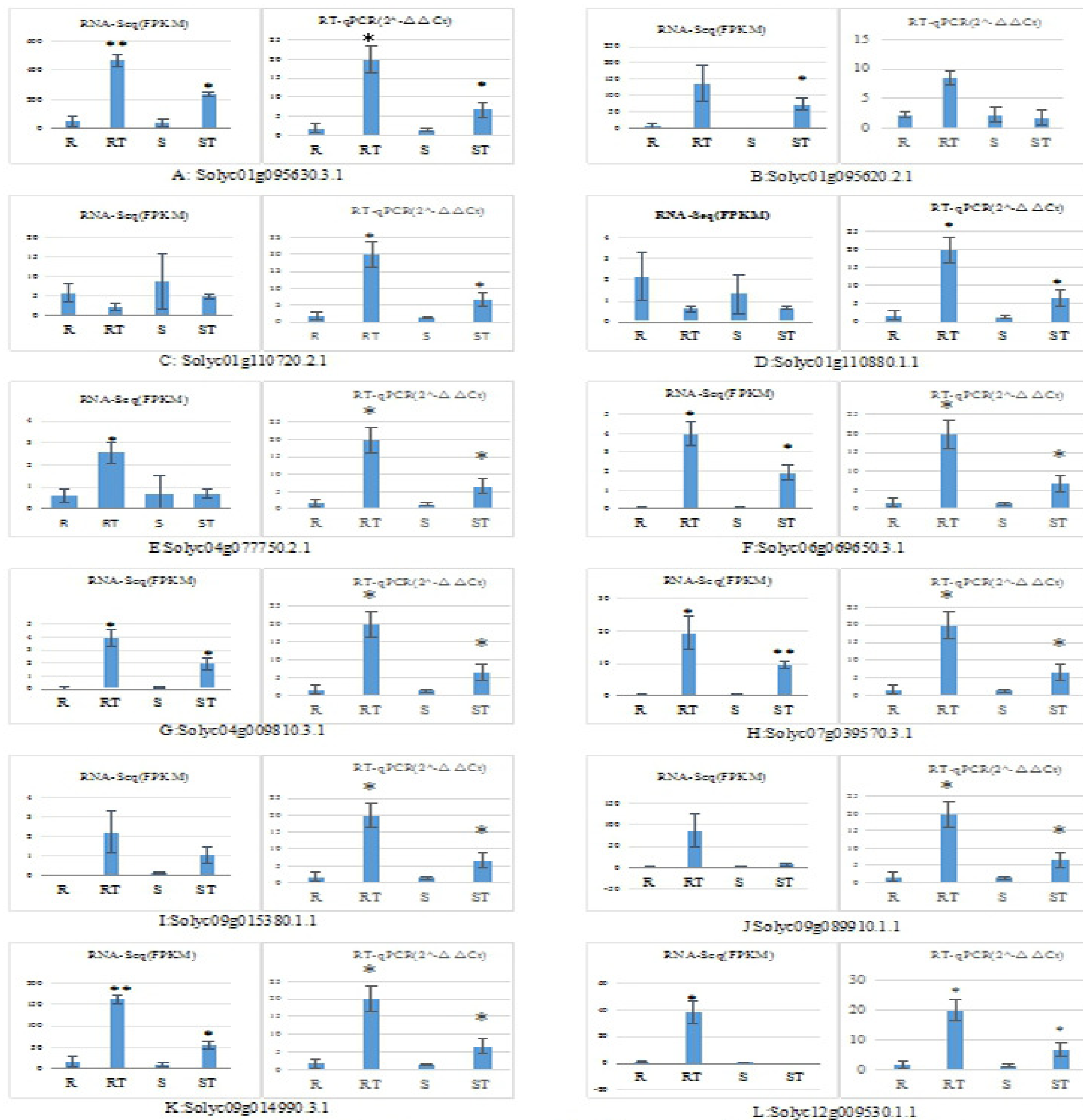

FIGURE 4 | The X-axis represents the four samples, S, R, RT and ST; the Y-axis at left represents the FPKM values obtained by RNA-Seq; the Y-axis atright represents the $2^{-\Delta\Delta Ct}$ values obtained by RT-qPCR.

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Table 1 (on next page)

Clean reads and reference genome alignment results by sample

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1 Table 1 Clean reads and reference genome alignment results by sample

Sample	Total Clean Reads	Total Mapping Ratio	Uniquely Mapping Ratio
R1	65,470,972	92.83%	72.87%
R2	65,339,662	86.09%	69.45%
R3	65,020,130	91.98%	72.38%
RT1	66,064,474	90.48%	72.81%
RT2	65,924,602	91.28%	72.95%
RT3	65,013,388	91.38%	73.19%
S1	65,115,760	88.12%	70.24%
S2	65,255,430	91.01%	72.85%
S3	65,057,484	89.16%	70.94%
ST1	66,004,210	89.95%	72.98%
ST2	67,033,562	87.70%	67.29%
ST3	66,008,854	90.14%	72.33%

2

Table 2 (on next page)

Common GO enrichment terms in the R-VS-RT and S-VS-ST groups

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1 Table 2 Common GO enrichment terms in the R-VS-RT and S-VS-ST groups

		GO terms: p-value ≤ 0.05	
Functional classification	GO terms	R-VS-RT	S-VS-ST
molecular function	protein kinase activity	0.02987	5.32e-05
	kinase activity	0.00943	0.00081
	phosphotransferase activity, alcohol group as acceptor	0.04500	0.00134
	chlorophyll binding	0.00248	0.02056
	pigment binding	0.00238	0.03575
biological process	response to radiation	0.00162	0.00042
	photosynthesis, light harvesting	0.00292	0.02228
	response to stimulus	2.85e-05	0.04397
	signaling	1.58e-05	0.05510
cellular component	integral component of membrane	2.97e-16	8.36e-16
	intrinsic component of membrane	9.73e-10	2.22e-12
	photosystem I	2.70e-8	2.42e-07
	membrane	5.52e-6	1.71e-07
	membrane part	8.20e-6	1.38e-08
	chloroplast thylakoid membrane	0.00047	0.00137
	plastoglobule	0.00282	0.02305

2

Table 3 (on next page)

Differentially expressed genes in the significantly enriched KEGG pathway “Plant hormone signal transduction” by group analysis 1

NOTE: “-----” indicates that S-VS-ST did not show differential expression of this gene

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1 Table 3 Differentially expressed genes in the significantly enriched KEGG pathway
2 "Plant hormone signal transduction" by group analysis

Gene ID	Annotations	Fold change (log_2 ratio)		
		RT-VS-ST	R-VS-RT	S-VS-ST
Solyc10g084020.1.1	SAUR family protein	-2.1	2.97	-----
Solyc03g120390.3.1	auxin-responsive protein IAA	-1.1	3.94	1.94
Solyc05g054390.3.1	two-component response regulator ARR-B family	-1.4	2.59	-----
Solyc05g014320.3.1	gibberellin receptor GID1	-1.3	1.62	-----
Solyc09g098110.3.1	phytochrome-interacting factor	-1.4	1.98	-----
Solyc09g090970.3.1	abscisic acid receptor PYR/PYL family	-2.3	5.65	3.75
Solyc05g052420.2.1	abscisic acid receptor PYR/PYL family	-2.1	5.24	-----
Solyc03g007320.3.1	abscisic acid receptor PYR/PYL family	-1.2	6.61	3.2
Solyc05g054380.2.1	abscisic acid receptor PYR/PYL family	-1.2	6.08	5.5
Solyc10g085310.1.1	abscisic acid receptor PYR/PYL family	- 1.2	1.55	1.23
Solyc03g096670.3.1	protein phosphatase 2C	-1.1	3.6	-----
Solyc03g005520.1.1	ethylene-responsive transcription factor 1	-2.2	6.3	3.2
Solyc05g051200.1.1	ethylene-responsive transcription factor 1	-1.8	1.64	-----
Solyc01g008140.3.1	protein brassinosteroid insensitive 1	-1.1	1.79	-----
Solyc08g076930.1.1	transcription factor MYC2	-1.3	1.06	-----
Solyc07g039570.3.1	transcription factor MYC2	-1.0	5.89	5.80

3 NOTE: "-----" indicates that S-VS-ST did not show differential expression of this gene
4

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Table 4 (on next page)

Differentially expressed genes in the significantly enriched KEGG pathway "Carbon metabolism" by group analysis

NOTE: "-----" indicates that S-VS-ST did not show differential expression of this gene

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1 Table 4 Differentially expressed genes in the significantly enriched KEGG pathway
2 "Carbon metabolism" by group analysis

Gene ID	Annotations	Fold change (log_2 ratio)		
		RT-VS-ST	R-VS-RT	S-VS-ST
Solyc04g082170.3.1	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	-2.5	2.77	-----
Solyc02g086880.3.1	formate dehydrogenase	-1.1	1.93	-----
Solyc07g065340.1.1	serine O-acetyltransferase	-1.2	1.71	-----
Solyc02g082830.2.1	phosphoserine aminotransferase	-1.9	1.73	-----
Solyc07g052480.3.1	isocitrate lyase	-1.3	1.19	-----
Solyc05g050120.3.1	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+)	-1.3	4.59	3.33
Solyc04g072800.3.1	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	-2.0	2.7	-----
Solyc03g114500.3.1	enolase	-1.5	3.04	1.43
Solyc07g017860.3.1	acetyl-CoA synthetase	-1.1	1.87	1.43

3 NOTE: "-----" indicates that S-VS-ST did not show differential expression of this gene
4
5

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Table 5 (on next page)

Differentially expressed gene in the significantly enriched KEGG pathway "Carbon fixation in photosynthetic organisms" by group analysis

NOTE: "-----" indicates that S-VS-ST did not show differential expression of this gene

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1 Table 5 Differentially expressed gene in the significantly enriched KEGG pathway
2 "Carbon fixation in photosynthetic organisms" by group analysis

Gene ID	Annotations	Fold change (log_2 ratio)		
		RT-VS-ST	R-VS-RT	S-VS-ST
Solyc05g050120.3.1	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+)	-1.3	4.59	3.33

3 NOTE: "-----" indicates that S-VS-ST did not show differential expression of this gene
4

Table 6(on next page)

Differentially expressed genes in the significantly enriched KEGG pathway “Glutathione metabolism” by group analysis ?

NOTE: “-----” indicates that S-VS-ST did not show differential expression of this gene

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1 Table 6 Differentially expressed genes in the significantly enriched KEGG pathway
2 "Glutathione metabolism" by group analysis

Gene ID	Annotations	Fold change (log2 ratio)		
		RT-VS-ST	R-VS-RT	S-VS-ST
Solyc02g093830.3.1	glucose-6-phosphate dehydrogenase	-1.0	3.7	2.2
Solyc09g011590.3.1	glutathione S-transferase	-1.2	4.31	3.59

3 NOTE: "-----" indicates that S-VS-ST did not show differential expression of this gene
4