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Transcriptome-based discovery of genes and networks related to R_{SC3Q}-mediated resistance to *Soybean mosaic virus* in soybean

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Abstract. *Soybean mosaic virus* (SMV) is a worldwide disease of soybean (*Glycine max* (L.) Merr.) that can cause serious reduction in yield and seed quality. Soybean cv. Qihuang-1 is an important source of resistance to SMV in China, carrying a resistance gene (R_{SC3Q}) against SMV strain SC3. In order to discover genes and networks regulated by R_{SC3Q}-mediated resistance in Qihuang-1, we analysed transcriptome data of a pair of near-isogenic lines, R (R_{SC3Q}) and S (r_{SC3Q}), from the cross Qihuang-1 × Nannong 1138-2 (r_{SC3Q}), after SC3 inoculation. Many differentially expressed genes (DEGs) were identified in the R and S lines at 6, 20 and 48 h post-inoculation. Based on pathway-enrichment analysis of DEGs, three genes encoding calmodulin-like protein (*Glyma03g28650*, *Glyma19g31395* and *Glyma11g33790*) with downregulated expression in the S line were identified in the plant–pathogen interaction pathway at 6 h post-inoculation. Analyses by quantitative real-time PCR were performed to verify that these three genes were not beneficial for SMV infection. Our results also revealed a complex plant-hormone signal network in R_{SC3Q}-mediated resistance during the early stage of SMV infection. Expression of jasmonic acid repressor genes (*TIFY/JAZ*) and abscisic acid-induced genes (*PP2C3a*) was upregulated in the R line but not the S line. More DEGs related to indole-3-acetic acid were found in the R line than the S line, and no salicylic acid-related DEGs were identified. These results suggest that suppression of jasmonic acid or promotion of abscisic acid is important for R_{SC3Q}-mediated resistance against SC3, and that salicylic acid may not act as a main regulator of R_{SC3Q}-mediated resistance during early stages of SC3 infection. Growth and development were greatly affected through R_{SC3Q}-mediated resistance responses after SC3 infection. Our understanding would be enhanced by identification of factors associated with R_{SC3Q} that help to trigger the resistance response.

Keywords: CML, *Glycine max*, NILs, plant-hormone signal-transduction pathway, *Soybean mosaic virus*.

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Introduction

Soybean mosaic virus (SMV) is a member of the largest genus of known plant viruses, *Potyvirus* (Adams *et al*. 2005). SMV is a worldwide disease of soybean (*Glycine max* (L.) Merr.) and can cause serious reduction in yield and seed quality. Based on their differential responses on resistant and susceptible soybean lines, SMV isolates have been grouped into seven strains (G1–G7) in the United States (Cho and Goodman 1979; Cho and Goodman1982), five strains (A–E) in Japan (Takahashi *et al*. 1980; Nakano 1982), and 22 strains in China (Li *et al*. 2010; Wang *et al*. 2018). Soybean has a two-layered innate immune system for combatting SMV: pathogen-associated molecular pattern-triggered immunity (PTI), and effector-triggered immunity (ETI) (Jones and Dangl 2006). ETI is induced when a strain-specific avirulent (Avr) protein from the pathogen associates directly or indirectly with a cognate plant resistance (R) protein (Jones and Dangl 2006). Currently, soybean resistance genes to SMV, *Rsv1*, *Rsv3*, *Rsv4* and *Rsv5*, are identified as single dominant SMV resistance gene (*R*) loci in the USA (Yu *et al*. 1994; Hayes *et al*. 2000; Jeong *et al*. 2002; Klepadlo *et al*. 2017). Resistance to the strains from China is derived from the single dominant SMV *Rsc* loci, and these have been mapped to chromosomes 2, 13, 14, and 6 in the respective cultivars Kefeng-1, Qihuang-1, Dabaima and RN-9 (Ma *et al*. 2011; Wang *et al*. 2011; Zheng *et al*. 2014; Rui *et al*.

2017). To date, only the *Rsv4* gene has been cloned (Ishibashi *et al*. 2019). Some downstream signalling components and pathways of *Rsv*-mediated resistance have been reported; however, there are no reports about *Rsc*-mediated resistance (Fu *et al*. 2009; Zhang *et al*. 2012; Wang *et al*. 2014).

Plant-hormone signalling pathways also play a crucial role in the process of the plant defence response, involving hormones such as salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA). SA, a phenolic compound, is usually required for triggering innate immune responses (i.e. PTI and ETI). Localised resistance responses of plants, activation of programmed cell death, systemic acquired resistance (SAR) and small interfering RNA (siRNA) antiviral machinery are associated with SA and/or SA derivatives (Alamillo *et al*. 2006; Hunter *et al*. 2013; Baebler *et al*. 2014; Shigenaga and Cristiana 2016). JA is a lipid-derived signalling molecule. In the JA signal-transduction process, JASMONATE ZIM-DOMAIN (JAZ) proteins, as a subfamily of TIFY, are key regulators in JA hormonal response (Vanholme *et al*. 2007). Upon stress perception, plants accumulate the bioactive JA-Ile molecule ((+)-7-iso-JA-Ile), which induces the interaction between F-box CORONATINE INSENSITIVE 1 (COI1) and JAZ (Fonseca *et al*. 2009; Sheard *et al*. 2010). The JA-Ile-mediated COI1–JAZ interaction leads to the ubiquitination and subsequent degradation of the JAZ proteins. Several transcription factors including the JA master regulator MYC2 are liberated, which in turn induce JA-specific cellular reprogramming, such as defence responses (Chini *et al*. 2007; Kazan and Manners 2013). JA reportedly has positive and negative effects on defence against viruses (Alazem *et al*. 2018); at the early stage of virus infection, JA seems to support plant defence, but it decreases plant resistance at a later stage when it is induced or applied (Pacheco *et al*. 2012; Garcia-Marcos *et al*. 2013). ABA is a sesquiterpene compound produced by the cleavage of γ-carotene. When ABA receptors PYR/PYL/RCAR bind accumulated ABA, dephosphorylation of SnRK2s (SNF1-related protein kinase 2 family) mediated by protein phosphatase 2C (PP2C) is prevented. The active SnRK2 kinases phosphorylate and activate downstream transcription factors, and then induce the transcription of ABA-responsive genes for developmental process and adaptive stress responses (Hauser *et al*. 2011; Alazem *et al*. 2018). During plant defence, the role of ABA depends on the stage of virus infection. ABA can resist viruses by mediating stomata closure or increasing callose deposition on plasmodesmata to restrict movement of viruses at early stages of infection. However, ABA can suppress hypersensitive response to decrease the production of reactive oxygen species (ROS) and SA, and weak distal SAR and siRNA systems (Alazem *et al*. 2018).

Qihuang-1 is an important resistance source for SMV in China. It carries a resistance gene (R_{SC3Q}) to SMV strain SC3, a prevalent SMV strain in Huang, Huai and Chang Jiang Valleys in China (Wang *et al*. 2003). However, the mechanism underlying SMV resistance in Qihuang-1 is not clear. Next-generation sequencing has become the first choice for researching SMV resistance mechanisms, with a reduction in expense and time needed in sequencing. In this study, we applied transcriptome analysis of a pair of near-isogenic lines (NILs) from the cross Qihuang-1 (resistant to SC3) × Nannong 1138-2 (susceptible to SC3) in order to explore the transcript-accumulation patterns after SMV infection. This enabled us to discover genes and networks in Qihuang-1 that are regulated by R_{SC3Q}-mediated resistance.

Materials and methods

Plant materials and inoculation

Near-isogenic lines (R and S lines) that have similar genetic background were derived from the cross Qihuang-1 × Nannong 1138-2 in a previous study (Zheng *et al*. 2014). The R line carries a resistance gene to SC3 (R_{SC3Q}), like Qihuang-1, whereas S line carries a susceptible gene (r_{SC3Q}), like Nannong 1138–2.

Qihuang-1, Nannong 1138-2, the R line and the S line were planted in a mixture of soil, perlite and vermiculite (volume ratio 3 : 1 : 1). Plants were grown in a greenhouse with temperatures of 25°C (day) and 20°C (night). Plants were inoculated with the SMV strain SC3 or phosphatic buffer solution (control) when the unifoliolate leaves were fully unfolded (V1 stage). The inoculation method was according to the previous study of Li *et al*. (2017).

Soybean RNA-Seq data

The RNA-Seq data from the NILs (R line and S line) were obtained previously (Li *et al*. 2017; Yuan *et al*. 2020). In brief, leaves were collected as samples from the two lines independently at 0, 6, 20 and 48 h post-inoculation (hpi) with SC3. After extracting total RNA from the samples, libraries were generated from eight samples with Ultra (New England Biolabs, Ipswich, MA, USA), following the manufacturer's instructions, and samples were sequenced on a HiSeq 2000 (Illumina, San Diego, CA, USA). After removing impure data from the raw sequence (using FASTX-Toolkit version 0.0.13; Gordon and Hannon 2010), clean reads were obtained. The clean reads were aligned to the Williams 82 soybean mRNA reference and genome Glyma.Wm82.a1.v1 (Schmutz *et al*. 2010) by using SOAP2 version 2.21 with default settings (Li *et al*. 2009). Differentially expressed genes (DEGs) ($|\log_2(\text{fold-change})| \geq 2$ and $P \leq 0.01$) were obtained by comparison of SMV-infected samples (at 6, 20 and 48 hpi) with 0 hpi in the R line and the S line, using Cufflinks v1.1.0 software (Trapnell *et al*. 2010). All of the original RNA-sequencing data have been submitted to the Sequence Read Archive database (SRA accession no. PRJNA668549, www.ncbi.nlm.nih.gov/sra; BioSample accession no. SAMN16414956, www.ncbi.nlm.nih.gov/biosample).

Soybean pathway enrichment analysis of the DEGs was performed by using EXPath 2.0 (Zheng *et al*. 2017). A heatmap graph was drawn by Origin 2019b (www.originlab.com/2019b) based on $\log_2$(fold-change) values.

RNA extraction and gene expression analysis by qPCR

Extraction of RNA from leaf tissues of soybean plants and quantitative real-time polymerase chain reaction (qPCR) were performed according to the previous study (Zheng *et al*. 2014; Luan *et al*. 2016). Each gene was tested with at least three

biological replicates and the experiment was repeated at least twice. Gene IDs and primer sequences are provided in Supplementary Material Table S1 (available at the journal's website).

Results

Virus content in R and S lines after SMV inoculation

In terms of SMV strain SC3 content in R and S lines after SC3 inoculation (Fig. 1*a*), there was a continuous decrease in the R line after 48 hpi, and virus could hardly be detected at 5 days post-infection (dpi). The SMV level in S line showed a trend of fluctuation. A small amount of virus was detected in inoculated leaves in the S line, but an abundance of virus in uninoculated distal leaves (the first trifoliate leaves at 7 dpi) (Fig. 1*a*). This suggested that the S line without R_{SC3Q} also had some resistance to SC3; however, this resistance did not stop the movement of the virus. Some virus moved along the veins and stems to the distal leaves and replicated, which was detected at 7 dpi in the S line (Fig. 1*a*).

RNA sequencing assembly and assessment of sequencing quality

The eight samples from the R and S lines at four time-points subjected to Illumina sequencing generated 43–67 million clean reads (Table S2), 79.72–92.57% of which were mapped to the soybean reference genome. Around 40 000 genes were found to be expressed in each sample (Table S2).

The reliability of transcriptome analysis was tested via qPCR analysis on eight randomly selected genes. The results showed that seven of the genes had similar expression trends in the transcriptome and qPCR analysis, which indicated that transcriptome data were reliable (Fig. S1).

Identification of DEGs in response to SMV infection

In total, 779, 189 and 84 DEGs were obtained at three time-points (6, 20, and 48 hpi, respectively) in the R line, and 390, 162 and 65 DEGs were discovered at the same three time-points in the S line (Fig. 1*b–d*). The number of DEGs was greater in the R line than the S line at each time-point. Comparable numbers of DEGs with upregulated and downregulated expression were apparent; in the R line, more were upregulated than downregulated at three time-points, but in the S line, more were downregulated (Fig. 1*b–d*). Most of the DEGs were mobilised in response to SC3 infection before 20 hpi in both lines, which was likely an important aspect of soybean defence.

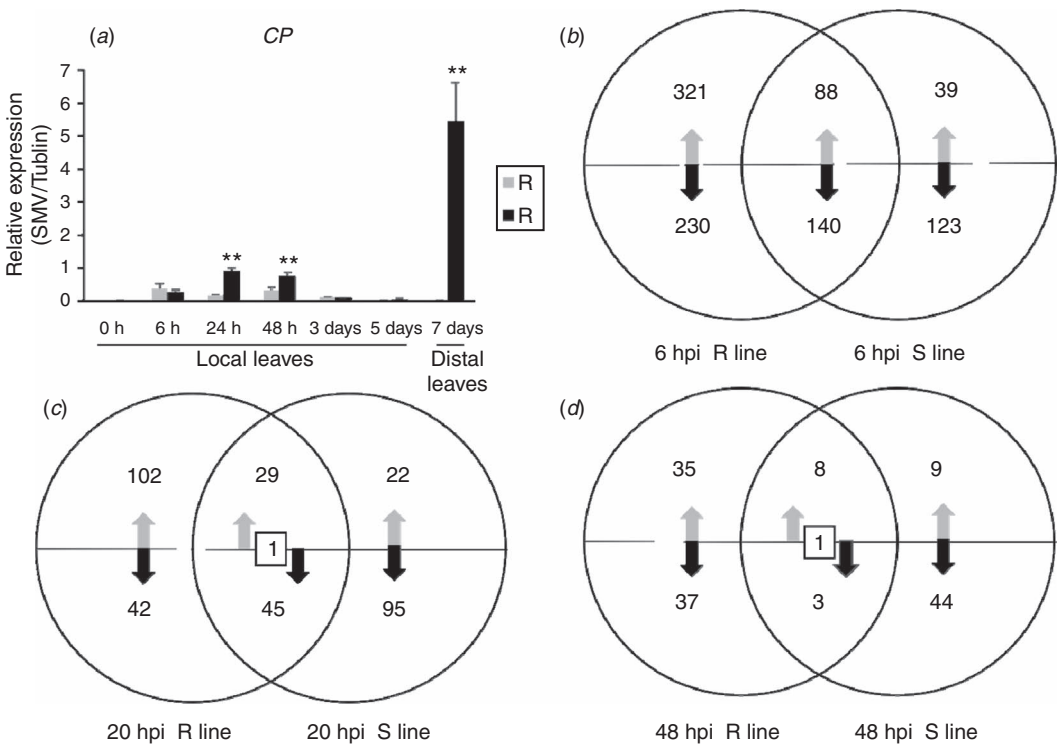

Fig. 1. (*a*) Content of virus in soybean after inoculation with *Soybean mosaic virus* (SMV) strain SC3 of seedlings of R and S lines; and (*b–d*) Venn diagrams illustrating the clustering of differentially expressed genes (DEGs). Samples were collected at 0, 6, 24, 48 h post inoculation (hpi) and at 3 and 5 days post-inoculation (dpi) from inoculated leaves (local leaves), and at 7 dpi from the first trifoliate leaves (distal leaves). SMV content was determined by quantitative real-time PCR analysis using SMV-specific primers, with soybean tubulin transcript level as an internal control; $^{**}P < 0.01$ (*t*-test). DEGs (fold-change ≥ 2 or ≤ 0.5, and $P \leq 0.01$) were obtained by comparison of SMV-infected sample at 6, 20 and 48 hpi with 0 hpi in R and S lines. Diagrams show number of DEGs with upregulated (↑) and downregulated (↓) expression from both lines. Boxed 1 indicates that one DEG had the opposite expression pattern in R and S lines.

Pathway enrichment analyses

Pathway enrichment analyses by EXPath 2.0 showed that, whether in the R or S line, DEGs involved in metabolic-related pathways represented a large proportion at the three time-points (6, 20 and 48 hpi; Fig. S2). Among the different pathways, plant–pathogen interaction and plant-hormone signal-transduction pathways play important roles in plant defence.

Genes encoding calmodulin-like (CML) protein involved in the response to SMV in soybean

Three *CML* genes (*Glyma03g28650*, *Glyma19g31395* and *Glyma11g33790*) with downregulated expression were found in the plant–pathogen interaction pathway in the S line only. CMLs are the members of the Ca^{2+} sensors, which interact with Ca^{2+} and regulate the function of diverse target proteins by direct binding or through phosphorylation (Aldon *et al*. 2018). CMLs are reported to be important regulators of plant defence against pathogens (Xu *et al*. 2017; Zhu *et al*. 2017; Lu *et al*. 2018). Therefore, we speculated that the three identified *CML* genes in the S line at 6 hpi probably positively regulated the early resistance response of soybean to SC3. Further, qPCR analysis was performed on the parent plants of R and S lines (Qihuang-1 and Nannong 1138-2). As shown in Fig. 2, expression of the three *CML* genes increased in Qihuang-1 but decreased significantly in Nannong 1138-2 at 6 hpi, indicating that inhibition of expression of the three *CML* genes was very likely to promote SMV infection.

Plant-hormone effects on soybean defence against SMV

According to the pathway enrichment analyses, 14 DEGs were found in the plant-hormone signal-transduction pathway (Fig. 3). *Glyma01g41290* and *Glyma11g04130* encoding TIFY/JAZ proteins had downregulated expression in the S line, and upregulated in R line (Fig. 3), indicating that induction of *Glyma01g41290* and *Glyma11g04130* expression is advantageous for soybean resistance during early stages of SC3 infection. TIFY/JAZ proteins are a key negative regulator in JA hormonal response (Fonseca *et al*. 2009). This means that JA probably plays a role in response to SC3 infection, and repression of JA probably enhances soybean resistance to SC3 during early stages of SC3 infection.

Two DEGs (*Glyma14g06100* and *Glyma14g32430*) were related to the ABA-signalling pathway. *Glyma14g06100* encodes a PYR1-like protein (PYL), which is an ABA receptor and positively responds to ABA regulation (Hauser *et al*. 2011). Its expression was significantly downregulated in the S line. *Glyma14g32430*, as an ABA-induced *PP2C3a*

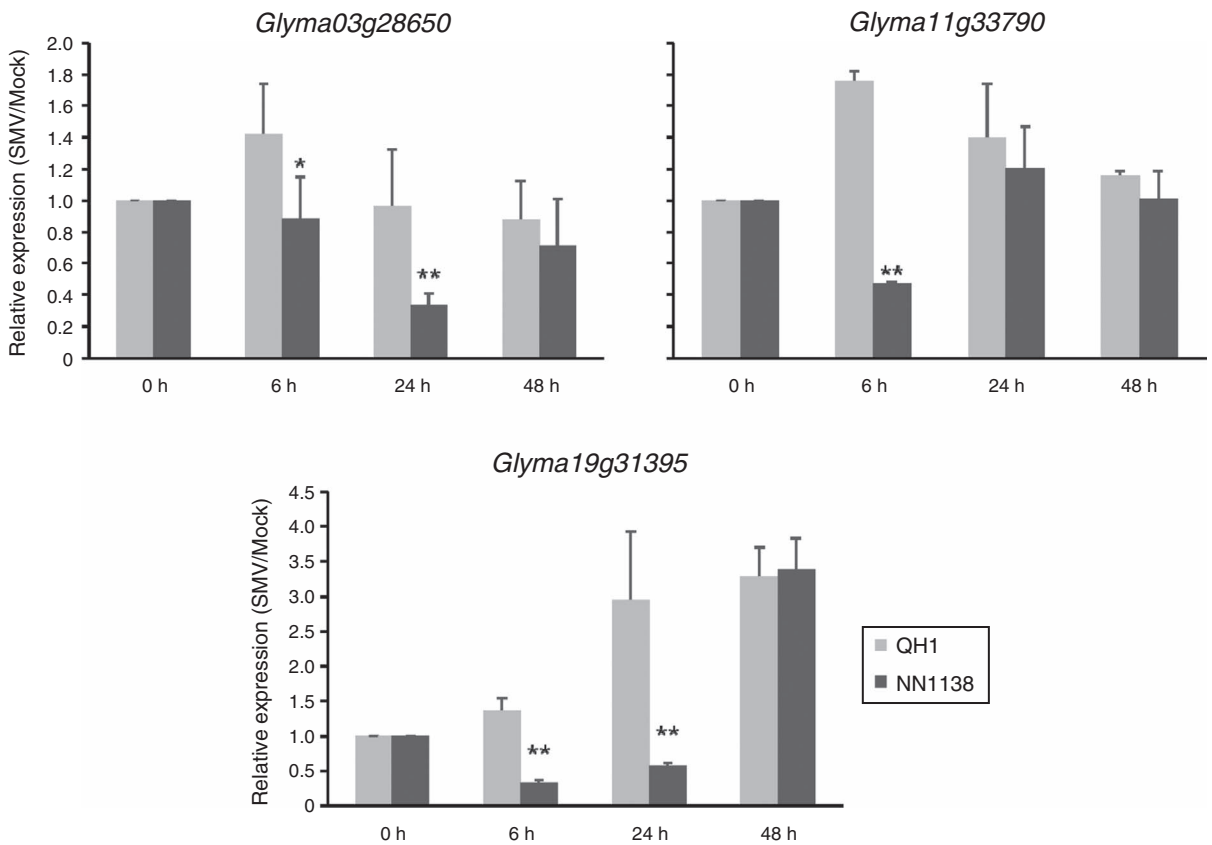

Fig. 2. Detected expression of genes for calmodulin-like protein (CML) by quantitative real-time PCR, at 0, 6, 24, and 48 h after inoculation with *Soybean mosaic virus* strain SC3 of Qihuang-1 (QH1, resistant to SC3) and Nannong 1138-2 (NN1138-2, susceptible to SC3). *$P < 0.05$, **$P < 0.01$ (*t*-test).

(encoding a subset of the Type 2C protein phosphatase), is reported to be a key positive regulator of *Rsv3*-mediated extreme resistance against SMV strain G5H in soybean (Seo *et al*. 2014). In our study, *PP2C3a* had significantly upregulated expression at 6 hpi in the R line, but it had almost had no expression in the S line (Fig. 3). These results suggest that ABA was involved in plant response after SC3 infection and probably promoted R_{SC3}-mediated resistance.

The auxin (IAA)-related DEGs with different expression patterns were mostly in the plant-hormone signal-transduction pathway (Fig. 3). This shows that the expression of DEGs was greatly affected by SMV infection at early time-points in the R line, but not in the S line. We speculate that growth and development were greatly affected through R_{SC3Q}-mediated resistance responses during the early stages of SC3 infection. There was one DEG in each of the ethylene (ET) and gibberellin (GA) signalling pathways, encoding ET receptor EIN4 (ETHYLENE INSENSITIVE4) and GA receptor GID1B (GIBBERELLIN INSENSTIVE DWARF1), respectively (Fig. 3). This indicated that ET and GA participated in response to SC3 inoculation.

Other defence pathways possibly effecting soybean against SMV

Mitogen-activated protein kinase (MAPK) signalling pathway plays a critical role in plant immunity. Five DEGs were identified in the MAPK signalling pathway at 6 hpi in the R and S lines (Fig. 3). We speculated that these DEGs were probably regulated by R_{SC3Q}-mediated resistance. Three of them (*Glyma05g05540*, *Glyma14g32430* (*PP2C3a*) and *Glyma19g42220*) had significantly upregulated expression at 6 hpi in the R line, but they had almost no expression in the S line (Fig. 3). We speculate that these three DEGs maybe more important in R_{SC3Q}-mediated resistance. Autophagy responses have also been reported in plant defences (Alazem *et al*. 2018). Only one DEG was involved in the autophagy pathway (*Glyma01g32400*), which had downregulated expression in both R and S lines.

Discussion

Qihuang-1 (R_{SC3Q}) is an important resistance source for SMV strain SC3 in China, but the published literature

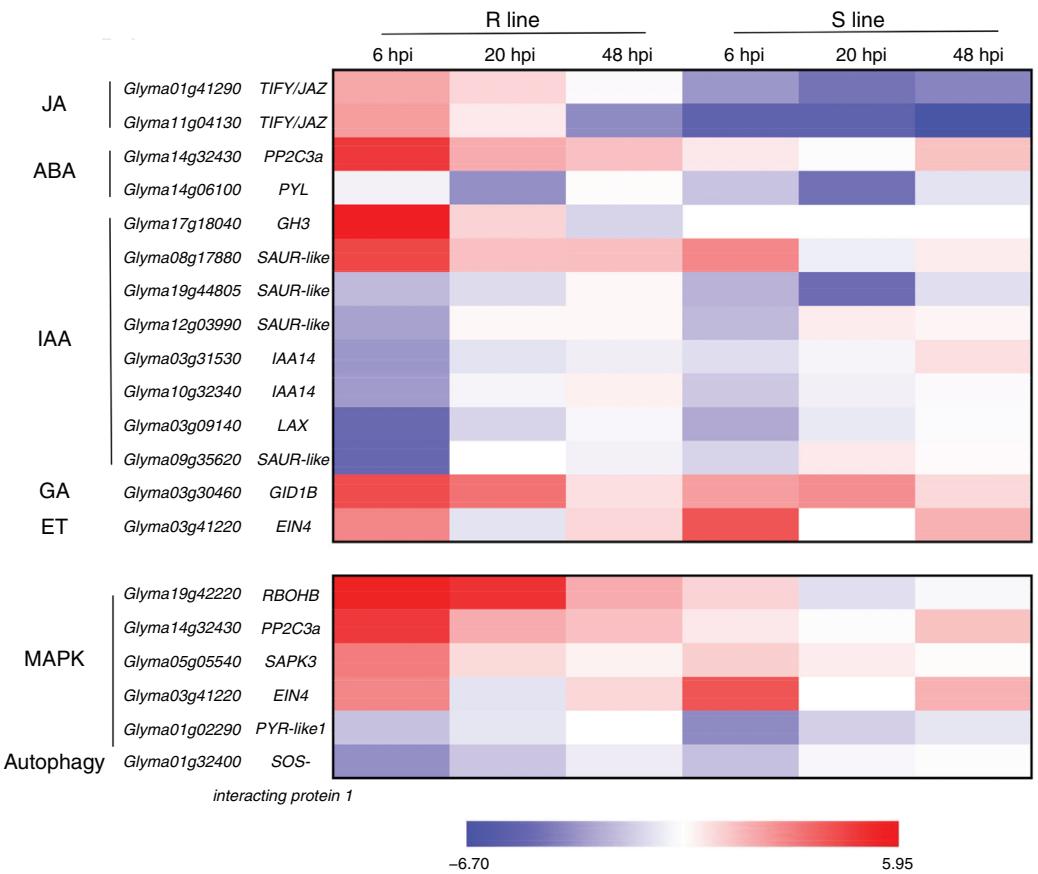

Fig. 3. Heatmap of differentially expressed genes (DEGs) in plant hormone, MAPK and autophagy signal pathways at different times after inoculation. DEGs ($|\log_2(\text{fold-change})| \geq 2$, and $P \leq 0.01$) were obtained by comparison of SMV-infected sample at 6, 20, and 48 h post inoculation (hpi) with 0 hpi in R and S lines. Hierarchical cluster heatmap was drawn by Origin 2019b software, and $\log_2$(fold-change) values for these DEGs are indicated in different colours.

includes little information on R_{SC3Q}-regulated resistance mechanisms or pathways. Our findings provide some R_{SCQ}-mediated, resistance-related genes and a general understanding of transcriptional activation of defence-related genes. Immediately downstream of the initial elicitor–receptor recognition, the activation of ion fluxes (including Ca^{2+}) and the production of H_2O_2 are the initial responses detected in plant cells, which occur before transcriptional activation of defence-related genes (Ramos *et al*. 2008). In response to the stimuli, cytosolic free Ca^{2+} rises and binds to a plethora of sensors, including CML, which in turn activate subsequent reactions of plant immunity (Aldon *et al*. 2018). In our study, three *CML* genes (*Glyma03g28650*, *Glyma19g31395* and *Glyma11g33790*) were enriched in the plant–pathogen interaction pathway in the S line only. Their expression was downregulated in the S line and the susceptible parent Nannong 1138-2 at 6 and 24 hpi, but was upregulated in resistant parent Qihuang-1 (Fig. 2). We consider that these three *CML* genes are beneficial for R_{SC3Q}-mediated resistance. The functions of *CML* genes are diverse. *CML8* and *CML9* can promote *Arabidopsis* resistance against the phytopathogenic bacteria *Pseudomonas syringae* pv. *tomato* (strain DC3000) (Zhu *et al*. 2017). Disease-resistance pathways involving *CML8* and *CML9* are different. *CML8* plays a role in SA-dependent processes, probably by modulating the effect of bacterial effectors. *CML9* contributes to PTI (via a flagellin-dependent pathway) but also to SA-dependent processes (Zhu *et al*. 2017). *CML9* as a negative regulator is involved in drought and salt stress. *Arabidopsis CML42* and *CML37* are also involved in plant defence and drought response (Vadassery *et al*. 2012*a*, 2012*b*; Scholz *et al*. 2014). Therefore, the functions of the three *CML* genes in this study can be investigated jointly from the aspects of plant defence and abiotic stress in subsequent experiments. These three genes might have different functions.

Some DEGs identified had involvement in plant-hormone signal transductions (Fig. 3). Plants defend against pathogen attack by modulating plant-hormone signalling pathways, such as those involving SA, JA, ET and ABA (Robert-Seilaniantz *et al*. 2011; Pieterse *et al*. 2012; Vos *et al*. 2013).

The role of JA in plant defence against viruses is controversial (Alazem and Lin 2015). JA has been known for its positive roles in a few compatible interactions; for example, the silencing of JA receptor gene *COI1* can enhance virulence of *Potato virus X* and *Potato virus Y* in *Nicotiana benthamiana* (Garcia-Marcos *et al*. 2013). However, for incompatible interactions between *Tobacco mosaic virus* (TMV) and *N. benthamiana*, JA had negative roles. Oka *et al*. (2013) found that N-mediated resistance to TMV was enhanced in NtCOI1-RNAi line. There was an incompatible interaction between the R line and SC3, and a compatible interaction between the S line and SC3. Two DEGs (*Glyma01g41290* and *Glyma11g04130*) encoding TIFY/JAZ, a negative regulator in JA pathway, had significantly downregulated expression in the R line and upregulated in S line following SC3 infection (Fig. 3). Therefore, we speculate that JA probably had a positive role in R_{SC3Q}-mediated resistance during the early stages of infection, which was similar to previously reported findings as mentioned above (Oka *et al*. 2013; Alazem *et al*. 2018).

Xun *et al*. (2019) found that ABA might be related to *R*-mediated resistance to SMV in soybean. Overexpression of a candidate *R* gene against SMV in soybean increased ABA accumulation. In the present study, we found that ABA-related genes *PYL* (*Glyma14g06100*) and *PP2C3a* (*Glyma14g32430*) were differentially expressed. As shown in Fig. 3, *PP2C3a* was rapidly induced in the R line at 6 hpi, but not in the S line. *PP2C3a*, encoding a Type-2C protein phosphatase, was predicted to participate not only in the ABA signalling pathway, but also in the MAPK signalling pathway (Fig. 3). When plants are invaded by pathogens, callose deposition increases on plasmodesmata and restricts cell-to-cell movement of viruses (Nakashima *et al*. 2003). In this process, the MAPK signalling pathway plays a positive regulatory role. Inhibition of the MAPK signalling pathway will decrease callose deposition and reduce resistance of plants (Xu *et al*. 2019). It has been verified that *PP2C3a* is specifically involved in *Rsv3*-mediated extreme resistance in an ABA-dependent manner against SMV (strain G5H) in soybean. It controls the rapid accumulation of callose at the points of G5H infection to stop virus spread (Seo *et al*. 2014; Alazem *et al*. 2019). All of the above evidence indicates that ABA plays an important role in *R*-mediated resistance against SMV and that *PP2C3a* might be a key gene connecting the MAPK and ABA signalling pathways. The role of *PP2C3a* in two pathways needs further verification.

In plant defence against viruses, SA signalling constitutes the major defensive pathway, and is tightly connected to the majority of *R* genes (Alazem and Lin 2015). Although SMV multiplication was reported to be inhibited by SA treatment (Zhao *et al*. 2018), no SA-related DEGs were detected in our study. Perhaps SA did not act as a main regulator of R_{SC3Q}-mediated resistance during the early stages of SC3 infection.

In addition to *PP2C3a*, *Glyma19g42220* (encoding respiratory burst oxidase homologue B, RBOHB) was significantly upregulated at 6 and 20 hpi in the MAPK signalling pathway in the R line. RBOH is also called NADPH oxidase (NOX), a key enzyme of ROS generation, and playing vital roles in various biological processes including plant immunity. The important role of NOX/RBOH in plant immunity has been well reported, especially in *Arabidopsis*, tobacco and rice (Hu *et al*. 2020). However, there is little research into the NOX/RBOH–MAPK pathway in plant immunity. In *N. benthamiana*, *NbRBOHB* is an important player in both the PTI ROS burst and the ETI ROS burst. It is noteworthy that MAPK is responsible for the ETI ROS burst by transactivation of *NbRbohB*, but not for the PTI ROS burst (Yoshioka *et al*.2016). We assume that *Glyma19 g42220* (*RBOHB*) has a similar function to *NbRBOHB*, which participates in the ETI ROS burst (the *R*-mediated resistance) through the MAPK cascade-signalling pathway.

In summary, CML, JA, ABA and MAPK, but not SA, are involved in the plant defence response during the early stages of SC3 infection. Suppression of JA or induction of ABA probably increased R_{SC3Q}-mediated SMV resistance. Soybean resistance against SMV was mediated by complex gene families at different loci, and the resistance was specified

via both SMV strain and soybean variety. Our understanding of R_{SC3Q}-mediated resistance against SC3 would be enhanced by identification of factors associated with R_{SC3Q} that help to trigger the resistance response.

Conflicts of interest

We declare no conflict of interest.

Author contributions

HZ, Y Yuan and Y Yang conceived and designed the experiments; Y Yuan wrote the article and performed major parts of the experiments; JL modified the language and provided technical support for RNA-Seq; LW performed parts of RNA-Seq experiment; YS and BL performed parts of plants cultivation and qRT experiment; HZ re-edited the article.

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