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Fine mapping and analyses of the *RSC15ZH* resistance candidate gene for the soybean mosaic virus

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Abstract The *soybean mosaic virus* (SMV) is a viral pathogen caused by *Potyvirus* reported in the major soybean growing areas of the world. SMV strain SC-15 is the most virulent and predominant strain that infects all soybean differentials and is widely distributed throughout southern China. In this study, three elite SMV-resistant soybean varieties that possess better comprehensive traits were identified through a screening test. A mapping population containing 163 F_8 recombinant inbred lines derived from a Zhonghuang24 (resistant) × Huaxia3 (susceptible) cross was used to locate the resistance gene for SMV strain SC-15. A high-density genetic linkage map was developed containing 2639 recombination bins using the restriction site-associated DNA sequencing (RAD-seq) method. Results revealed a novel resistance gene, $R_{SC15}ZH$ identified in a 63 kb region of chromosome 13. This locus was further characterized by quantitative real-time PCR (qRT-PCR). Four candidate resistance genes were identified that were possibly involved in regulating SMV resistance, especially *Glyma13g24461*. These results provide a basic

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foundation for soybean breeding programs to improve resistance to SMV strain SC-15 and for positional cloning of SMV resistant candidate genes.

Keywords Soybean · Soybean mosaic virus · Fine mapping · Resistance gene

Introduction

Soybean mosaic virus (SMV) is a major viral pathogen caused by *Potyvirus* reported in soybean production sites worldwide (Zhang et al. 2012). SMV can infect plants during all growth stages, resulting in yield loss and a reduction in seed quality (Sun et al. 2007). Numerous SMV strains have been classified into different groups. In the United States, 98 isolates of SMV were grouped into seven strains (G1 to G7), with several new strains identified since then (Cho and Goodman 1979, 1982). In Japan, Takahashi et al. classified SMV isolates from four soybean varieties into five strain groups (A–E) (Kato et al. 2016). In China, 22 strains (SC-1 to SC-22) were grouped using reaction to infection to a set of soybean differentials (Guo et al. 2005; Zhan et al. 2006; Li et al. 2010a, b; Wang et al. 2013, 2018). Among them, SC-15 was the most virulent and predominant strain infecting all soybean differentials and is currently widely distributed throughout soybean producing areas of China, especially southern China (Li et al. 2010a, b).

Utilization of soybean accessions resistant to SMV is an economical and environmentally safe way to control infection. Breeding for resistance to SMV using molecular marker-assisted selection is a more efficient approach than traditional methods for selecting resistant varieties. Recently, genetic maps with molecular markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs), have been used to identify the genetic basis for SMV resistance in soybeans. Thus far, three independent resistance genes, *Rsv1*, *Rsv3*, and *Rsv4* were identified and located on chromosomes 13, 14, and 2, respectively (Yu et al. 1994; Hayes et al. 2000; Jeong et al. 2002; Fu et al. 2006). *Rsv1* in PI96983 is resistant to six virulent strains (G1–G6). It was first mapped on soybean chromosome 13 and was considered to be a complex locus within a multigene cluster (Shi et al. 2008). In China, some studies used molecular markers to map SMV-resistant genes, which were mainly concentrated on chromosomes 2 and 13 (Wang et al. 2004; Li et al. 2006a, b; Ma et al. 2010, 2011; Yang et al. 2011; Wang et al. 2011a, b; Zheng et al. 2014; Yan et al. 2015; Li et al. 2015). Many SMV-resistant genes identified in China were located in close proximity to the *Rsv* genes identified in the United States. For instance, Yang et al. (2011) identified two resistant genes. One gene flanked *Rsv1* and elicits resistance for SMV strains SC-3, SC-6, and SC-17, while the other confers resistance for strain SC-7 only. It seems that the SMV resistance region contains a gene that is specific to one SMV strain. Therefore, the relationship between *Rsc* and *Rsv* genes should be further investigated. Additionally, $R_{SC\text{-}15}$ is located on chromosome 6 with the genomic markers, SSR_06_17 and BARCSOYSSR_06_0835, flanking this resistance gene (Yang and Gai 2011; Rui et al. 2017). $R_{SC\text{-}4}$ is located on chromosome 14 between SSR markers, BARCSOYSSR_14_1413 and BARCSOYSSR_14_1416 identified using an $F_{2:3}$ population derived from 'Dabaima' × 'Nannong1138-2' (Wang et al. 2011a).

The objectives of this study were to (1) screen elite resistant soybean varieties for SMV strain SC-15, (2) locate the resistance gene, $R_{SC15}ZH$, in a recombinant inbred line (RIL) population derived from a cross between Zhonghuang24 (resistant parent) × Huaxia3 (susceptible parent), and (3) identify candidate genes in the target region using quantitative real-time PCR (qRT-PCR) analysis.

Materials and methods

SMV strain and plant materials

SMV strain SC-15 and susceptible soybean cultivar, Nannong1138-2, provided by the National Center for Soybean Improvement, Nanjing Agricultural University, China were used in this study. Nineteen soybean cultivars, that included 11 cultivars, three introduced cultivars, three mutant cultivars, one landrace cultivar, and one edamame cultivar, were collected from China and Brazil, and screened for SC-15 resistance. Most cultivars were obtained from southern China because SC-15 is common in this region. An RIL population of 163 F_8 lines from the cross Zhonghuang24 × Huaxia3

(Liu et al. 2017) and developed using a single seed descent method (Yue et al. 2017) was used to map resistance genes to SMV strain SC-15.

Inoculation and resistance/susceptible evaluation

Strain SC-15 was cultivated from the susceptible cultivar, Nannong1138-2. Nineteen soybean cultivars and an RIL population were grown in pots and filled with vermiculite and soil in an aphid-free greenhouse. Ten to 12 plants were grown in each pot. Using mechanical inoculation, plants were inoculated when the pair of unifoliolate leaves had unfolded, then inoculated again when the first trifoliate leaves had unfolded (Yan et al. 2015). The reaction of each genotype to SC-15 was determined 4 weeks post-inoculation (Karthikeyan et al. 2018). Based on their reactions to SMV, the plants of each genotype were divided into symptomless (resistant) and mosaic (susceptible) groups when the disease reaction on leaves appeared to be stable.

SNP genotyping and resistance gene detection

Zhonghuang24 is resistant to SMV strain SC-15, whereas Huaxia3 is susceptible to the strain. To fine-map the resistance gene, an RIL population containing 163 individuals was generated by crossing resistant parent Zhonghuang24 with SMV-susceptible variety Huaxia3. All genotyping work was conducted following the methods previously described by Liu et al. (2017). A soybean reference genome, *G. max* Wm82.a1, was used to compare sequencing reads of each individual plant using SOAP software (Li et al. 2009a, b). The input file for realSFS was prepared using SAMtools (Li et al. 2009a, b). The information for each site was performed with realSFS to obtain the genotype of every individual, using Zhonghuang24 and Huaxia3 soybeans as references. Then, the exchange sites for each individual were identified to generate bin information. Finally, a linkage map was constructed using MapChart software (Voorrips 2002). The composite interval mapping (CIM) method was employed to scan bins with WinQTLCart software (Zeng 1993). The LOD threshold was calculated using 1000 replications with an experiment-wise error rate of $p = 0.05$ to determine whether there were any bins linked to a resistance gene.

qRT-PCR analysis

Three plants were mock-inoculated with sodium phosphate buffer, and leaf samples of Zhonghuang24 and Huaxia3 infected by SC-15 were independently collected at 0, 1, 2, 4, 8, 12, 24, and 48 h after the initial inoculation. All samples were immediately frozen in liquid nitrogen and stored at − 80 °C for future analysis.

RNA was extracted from samples using the TransZolTm Up (Transgen Biotech, Beijing, P.R.C.) method. cDNA synthesis was performed by using TransScrip II One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen Biotech, Beijing, P.R.C.) following the manufacturer's instructions. qRT-PCR was performed using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using a KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Cape Town, South Africa). All reactions were conducted in 10-µl volumes consisting of 1.0 µl of cDNA as a template. qRT-PCR conditions were as follows: 94 °C for 3 min, followed by 40 cycles at 94 °C for 5 s, then 55 °C for 15 s. Primers of each candidate gene for qRT-PCR analysis were designed by the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). The actin gene of soybean was used as an internal control to normalize each sample. Each PCR assay was replicated three times. The relative expression level of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Results

Screening for resistance lines

Nineteen soybean cultivars were evaluated and results indicated that three cultivars were highly resistant to strain SC-15. These included the two cultivars, Huachun5 and Zhonghuang24 and cultivar BRS135 introduced from Brazil (Table 1). Zhonghuang24 is a high-yielding, high-oil cultivar that has adapted to the Huang-Huai-Hai Valleys in China. Huachun5 was derived from a cross between Guizao1 × BRS135 and is a high-yielding, high-protein cultivar, which has shown high resistant to multiple diseases, such as *Phytophthora sojae* and SMV races. BRS135 is also a high-yielding cultivar that was introduced to China

Table 1 Reactions of 19 soybean accessions to SMV virulent strain SC-15

Variety name	Variety type	Source	IT	RC
BRSMG68	Introduction	Brazil	M	S
BRS135	Introduction	Brazil	–	R
MG/BR 46	Introduction	Brazil	M	S
E2-18	Mutant	Guangdong, China	M	S
EMS-57	Mutant	Guangdong, China	M	S
M3-283	Mutant	Guangdong, China	M	S
V43	Edamame	Guangdong, China	M	S
Wayaohuangdou	Landrace	Guangxi, China	M	S
Guixiadou2	Cultivar	Guangdong, China	M	S
Guizao1	Cultivar	Guangxi, China	M	S
Huachun2	Cultivar	Guangdong, China	M	S
Huachun3	Cultivar	Guangdong, China	M	S
Huachun5	Cultivar	Guangdong, China	–	R
Huachun6	Cultivar	Guangdong, China	M	S
Huaxia1	Cultivar	Guangdong, China	M	S
Huaxia2	Cultivar	Guangdong, China	M	S
Huaxia3	Cultivar	Guangdong, China	M	S
Huaxia5	Cultivar	Guangdong, China	M	S
Zhonghuang24	Cultivar	Beijing, China	–	R

IT, infection type (reaction on inoculated primary leaves); M, mosaic; "–", no symptom; RC, resistance classification; R, resistant; S, susceptible

from Brazil. These SMV-resistant cultivars can be used for parental material for development of high-yielding, multi-disease resistant cultivars with possibly better overall seed quality.

Fine mapping of the SC15-resistance gene

The linkage map used in this study was constructed as previously described (Liu et al. 2017). In total, 47,472 SNPs were integrated into a recombination bin unit, or a high-density linkage map that spanned 2638.24 cM and contained 2639 recombination bins used to locate the target resistance gene (s) for SMV strain SC-15. Based on the genotypic and phenotypic data of the two parental lines and the 163 RIL population, SMV strain SC-15 (designated as $R_{SC15}ZH$) was amplified at the site of bin75 on chromosome 13 using the composite interval mapping method (CIM). This site was located in a physical interval of more than 60 kb with 27,801,314–27,864,011 bp through 12 recombinants: line40, line92, line201, line206, line211, line55, line12, line107, line99, line147, line252, and line264 (Fig. 1).

Possible resistance candidate genes and qRT-PCR analysis

The *Glyma.Wm82.a1.v1.1* gene model from NCBI was used to retrieve gene calls and annotations. There were eight putative genes within the 63 kb target region. Among these genes, the function of *Glyma13g24400* has not been characterized and the annotations of three genes, *Glyma13g24410*, *-24420*, and *-24450*, seem unassociated with plant disease resistance. The remaining four genes, *Glyma13g24440*, *-24461*, *-24470*, and *-24490*, were identified as candidate genes whose expression levels were evaluated by qRT-PCR (Fig. 2). *Glyma13g24440* was significantly up-regulated after 1 h in Zhonghuang24, whereas the expression levels were only slightly up-regulated in Huaxia3 at the same point in time. Down-regulation of this gene was detected at 4 h in both parental lines. *Glyma13g24470* exhibited similar trends across all time points in both parental lines. Expression levels were significantly up-regulated after 2 h, and the magnitude of expression in Zhonghuang24 was higher compared to Huaxia3. After this point in time, expression levels in both parental lines were significantly down-regulated at 4 h then significantly up-regulated at 12 h. *Glyma13g24490* was significantly up-regulated after

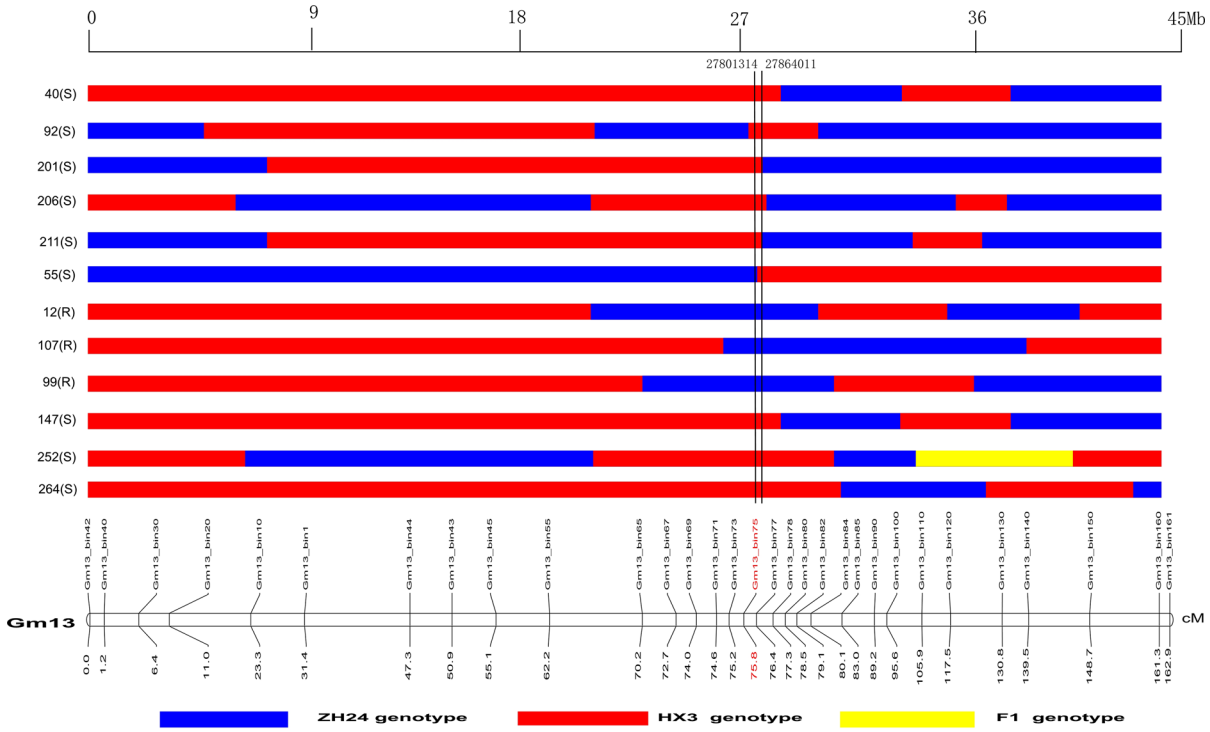

Fig. 1 The position of the resistance gene RSC15ZH on chromosome 13. Blue blocks designate Zhonghuang24 genotype on chromosome 13, red blocks designate Huaxia3 genotype on chromosome 13 and yellow blocks designates F1 genotype. (Color figure online)

1 h and 24 h in Zhonghuang24, while there was no significant change observed in Huaxia3 after the first 4 h; after this point in time, expression levels increased gradually. *Glyma13g24461* exhibited its highest up-regulated expression levels after 1 h, but was significantly down-regulated at 4 h in Zhonghuang24. The relative expression levels in Huaxia3 appeared to be suppressed at all points in time. Thus, *Glyma13g24461* is the most likely to be involved in response to SC-15 infection.

Discussion

SMV strain SC-15 is the most virulent *Potyvirus* strain that causes severe infection in soybeans throughout southern China. From the screening test, among the 19 identified soybean cultivars, 16 were highly susceptible, while three cultivars were asymptomatic. Three soybean accessions, Huaxia3, Huaxia5, and Guixiadou2, were developed from crossing Guizao1 × BRSMG68. These cultivars and their parents were highly susceptible to infection. Three elite cultivars, Huachun3, Huachun6, and Huaxia1, and their common parents, Guizao1 and MG/BR 46, inoculated with SC-15 were all susceptible. BRS135 and Huachun5 were resistant to SC-15. Use of SMV-resistant parents and selecting for SMV resistance should be an effective and economical method for controlling infection of this disease. The cultivars that exhibited dominant resistance to SC-15 can be used as resistance sources and could greatly assist research for exploiting elite cultivars with better agronomic traits, as well as SMV resistance.

The goal of this study was to locate the resistance gene for SMV strain SC-15. Most previous studies have reported mapping resistant SMV genes using RFLP, AFLP, and SSR markers, however, these methods did not have high enough efficiency to precisely detect genomic regions of resistance genes (Lee et al. 2015). SNP genotyping is a novel form of molecular technology that has been widely used for mapping resistance genes mapping due to their high-density and relatively even distribution throughout

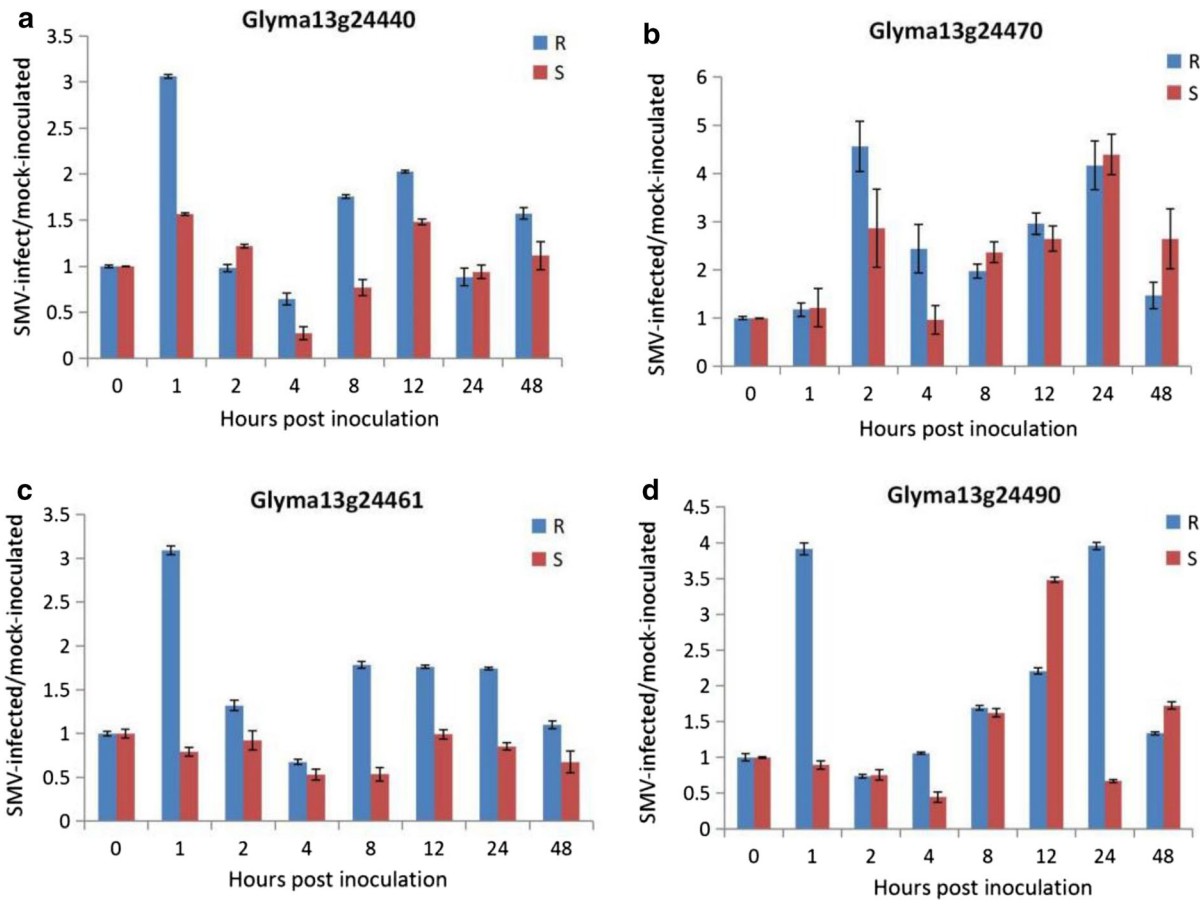

Fig. 2 Expressional changes of four candidate genes based on quantitative real-time PCR analysis. *Y*-axes indicate the ratios of mRNA expression levels between samples infected with SMV and samples inoculated with phosphate-buffer. *X*-axes indicate different time points of collecting samples. *R* indicates resistant parent Zhonghuang24 and S indicated susceptible parent Huaxia3

various genomes. In this study, the high-density genetic map of resistance genes for SMV was based on the RAD-seq method, and more than 47,000 SNPs were integrated into 2639 recombination bin units, suggesting that SNPs are more accurate for detecting resistance genes, and that reliable results can be obtained using this methodology.

Some studies have suggested that resistance genes are not randomly distributed throughout the entire genome, but generally exist as members of gene clusters in a certain linkage group or chromosome (Li et al. 2006a, b). Similarly, in this study, $R_{SC15}ZH$ was mapped to an interval of 27,801,314–27,864,011 bp on chromosome 13, located in a region that includes different SMV resistance genes (Table 2). For example, R_{SV1} was first identified in soybean line PI96983 as a single allele and was mapped on chromosome 13 (Yu et al. 1994). Li et al. (2006a, b) found that SMV strain SC-14 from Qihuang No.1 was also mapped on chromosome 13, and Ma et al. (2010) reported the resistance gene R_{SC-12} from Qihuang22 was also mapped on chromosome 13. Moreover, a series of SMV resistance genes, R_{N1}, R_{N3}, R_{SC-3}, R_{SC-6}, R_{SC-7}, R_{SC-11}, R_{SC-12}, R_{SC-14}, R_{SC-17}, R_{SC-18}, and R_{SC-20}, have been mapped on chromosome 13 (Li et al. 2006a, b; Li et al. 2015; Ma et al. 2010; Yang et al. 2011; Karthikeyan et al. 2018). Several important molecular markers, including Sat_234, Sat_154, Satt334, and Sct_033, have been linked to these resistance genes. Therefore, it can be inferred that this region may not consist of a single resistant gene, but a cluster of SMV resistance genes. Previously, Yang et al. (2011) reported that the SC-15 resistance gene was located between Sat_213 and Sat_286 on chromosome 6 in a

Table 2 Loci of SMV resistance genes on chromosome 13

Gene	Parent	Molecular marker	Locus genomic map position	References
$R_{sv1\text{-}f/r}$	J05 × Essex	Sat_154/Satt510	27,312,436–27,312,485/ 30,590,369–30,590,395	Shi et al. (2008)
R_{SC3}	PI96983 × Nannong1138-2	BARCSOYSSR_13_1128/ BARCSOYSSR_13_1136	28,919,973–28,920,014/ 29,264,742–29,264,795	Yang et al. (2011)
R_{SC3Q}	Qihuang 1 × Nannong1138-2	Satt334/Sct_033	28,415,974–28,416,021/ 30,739,608–30,739,666	Zheng et al. (2014)
R_{SC6}	PI96983 × Nannong1138-2	BARCSOYSSR_13_1128/ BARCSOYSSR_13_1136	28,919,973–28,920,014/ 29,264,742–29,264,795	Yang et al. (2011)
R_{SC7}	PI96983 × Nannong1138-2	BARCSOYSSR_13_1140/ BARCSOYSSR_13_1155	29,301,702–29,301,734/ 29,682,501–29,682,520	Yang et al. (2011)
R_{SC12}	Qihuang22 × Nannong1138-2	Satt334/Sct_033	28,415,974–28,416,021/ 30,739,608–30,739,666	Ma et al. 2010)
R_{SC14}	PI96983 × Nannong1138-2	Sat_154/Sct_033	27,312,436–27,312,485/ 30,739,608–30,739,666	Li et al. (2006a, b)
R_{SC17}	PI96983 × Nannong1138-2	BARCSOYSSR_13_1128/ BARCSOYSSR_13_1136	28,919,973–28,920,014/ 29,264,742–29,264,795	Yang et al. (2011)
R_{SC18}	Qihuang22 × Nannong1138-2	SOYHSP176/ Satt334	–/28,415,974–28,416,021	Li et al. (2015)
R_{SC20}	Qihuang 1 × Nannong1138-2	gm_indel_13-3/gm_SSR_13-14	30,875,000–30,795,177	Karthikeyan et al. (2018)
$R_{SC15}ZH$*	Zhonghuang24 × Huaxia3	Bin75	27,801,314–27,864,010	The present study

RIL population derived from RN-9 (R) × 7605 (S). Subsequently, Rui et al. (2017) used the same RIL population to narrow down this region, and *Rsc15* was mapped to a 95 kb region on chromosome 6. Therefore, it is presumed that the same SMV strain in different resistant parents was inherited independently and exist on different chromosomes. A similar conclusion was confirmed by a previous study (Wang et al. 2018). Thus, in this study, the region of $R_{SC15}ZH$ can be considered a newly discovered resistance gene locus for the SMV strain SC-15.

Within the 63 kb region identified in this study, four candidate genes were selected for further analysis (Tables 3, 4). The *Glyma13g24470* gene model encodes a highly conserved 76 amino acid protein ubiquitin that is covalently attached to substrate proteins that are targeted most for degradation. The ubiquitin/26S proteasome system (UPS) was one of the main protein degradation pathways found to play a critical role in plant–pathogen interactions. UPS is involved in almost every step of the defense mechanism process in plants, regardless of the type of pathogen (Dielen et al. 2010). In this study, the relative expression level of UPS was significantly up-regulated at 2 h in both parental lines; however, the magnitude of the expression level in the resistant parent was significantly higher compared to the susceptible parent. UPS was up-regulated again at 24 h at a high expression level in both parents. Gene ontology (GO) analysis revealed that this gene participated in the biological processes of salicylic acid, which is an important endogenous signal molecule in the activation of plant defense responses (Zhang et al. 2001; Li et al. 2006a, b; Chaturvedi and Shah 2007). Despite these findings, the process of this gene's involvement in response to SMV infection is complicated. Molecular mechanisms for plant disease resistance are complex and controlled by diverse and interrelated networks, and are not simple linear relationships.

Small heat shock proteins (sHSP) are widespread and found in all organisms. These stress proteins help protect plants from damage, or repair damage caused by a wide range of stressors, including heat, drought, and pathogens (Lu et al. 2003; Murakami et al. 2004).

Table 3 The information of eight candidates' annotations

Gene name	Position	Arabidopsis homologues	Annotation description
Glyma13g24400	27,818,918–27,822,569	AT3G17950.1	Uncharacterized protein
Glyma13g24410	27,826,688–27,828,514	AT2G33845.1	Nucleic acid-binding/OB-fold-like protein
Glyma13g24420	27,830,405–27,834,313	AT3G05700.1	Drought-responsive family protein, response to water deprivation
Glyma13g24440	27,839,025–27,840,078	AT1G53540.1	small heat-shock protein, response to oxidative stress
Glyma13g24450	27,841,590–27,846,322	AT5G64680.1	Protein maturation, photomorphogenesis
Glyma13g24461	27,847,776–27,854,806	AT1G53540.1	small heat-shock protein Hsp26/Hsp42,response to endoplasmic reticulum stress, response to hydrogen peroxide
Glyma13g24470	27,850,355–27,853,113	AT4G05320.2	Ubiquitin family, response to salicylic acid stimulus
Glyma13g24490	27,862,779–27,863,758	AT1G07400.1	small heat-shock protein Hsp26/Hsp42,response to oxidative stress

Table 4 Primer sequences for four candidate genes and reference gene

Gene name	Forward primers(5′ → 3′)	Reverse primers(5′ → 3′)
Glyma13g24440	GTGGAGCGTAGCAGTGGTAA	GGCCTTGACATCAGGCTTCT
Glyma13g24461	CCAGGGCTGAAGAAAGAGGA	CTTACCACTGCTACGCTCCA
Glyma13g24470	CCTTCGTCTGAGGGGAGGTA	TCCTTCCATCCTCCAGTTGC
Glyma13g24490	AACACTCTTTCTTCAGCTTCCTTT	TTGAATACGTGTGCTTCTGGG
Actin	CAGAGAAAGTGCCCAAATCATGT	TTGCATACAAGGAGAGAACAGCTT

A dominant gene, RTM2, which has also been cloned (Whitham et al. 2000), encodes a sHSP that restricts long-distance virus movement by active mechanisms in *Arabidopsis*. In this study, three gene models (i.e., Glyma13g24440, -24461, and -24490) were found to encode a protein that is likely to be involved in defending against SMV. For instance, *Glyma13g24440* and *Glyma13g24461* were significantly up-regulated at 1 h in the resistant parent, but their relative expression levels in the susceptible parent were almost entirely suppressed at each point in time. Another gene model, *Glyma13g24490*, was up-regulated at 1 h and again 24 h with higher levels expressed in Zhonghuang24 compared to Huaxia3. Per the GO enrichment analysis, these three genes were related to the process of 'response to oxidative stress or hydrogen peroxide'. Oxidative burst is one of the earliest responses identified in resistance to pathogen attack in plants (Li et al. 2006a, b). The accumulation of hydrogen peroxide (H_2O_2) exists in multiple plant disease systems. Previous research has suggested that H_2O_2 could influence plant disease resistance responses in a number of ways, including (1) inhibiting and poisoning the pathogen directly, (2) inducing host membrane lipid peroxidation, leading to a hypersensitive response, and, (3) inducing the synthesis of phytoalexins (Li et al. 2006a, b). Moreover, a previous study demonstrated that H_2O_2 concentration is involved in SMV infection (Rui et al. 2017). The *Glyma13g24461* gene is also presumed to participate in endoplasmic reticulum stress, which is a kind of subcellular pathological state associated with many diseases. Thus, these candidate genes, especially *Glyma13g24461*, may be correlated with activating plant signal pathways that regulate defense responses to SMV infection.

Nucleotide binding site-leucine-rich repeat (NBS-LRR)-type genes have also been demonstrated to be involved in genetic disease resistance (Gore et al. 2002; Hayes et al. 2004). However, in this study, no NBS-LRR resistance genes were observed within the target region. Similar results have been previously

reported for *Rsv4*, *Rsc8*, and *Rsc15* (Hwang et al. 2006; Maroof et al. 2010; Ilut et al. 2016; Zhao et al. 2016; Rui et al. 2017). The newly identified and cloned resistance gene at the *Rhg4* locus of soybean cyst nematode does not belong to the NBS-LRR family either, and exhibited a completely different plant resistance mechanism (Liu et al. 2012). Thus, the SMV strain SC-15 resistance locus likely belongs to a new class of resistance genes, and its role in the underlying molecular mechanism of soybean SMV resistance requires further functional investigation.

Conclusions

Three elite SMV-resistant varieties to strain SC-15 were identified from 19 soybean cultivars in this study. The whole soybean genome was resequenced in order to exploit the bins associated with SC-15 resistance using a population consisting of 163 RILs from the cross Zhonghuang24 (resistant) × Huaxia3 (susceptible). A novel resistance gene, *RSC15ZH*, was located within a 63 kb region that fell within a hotspot of resistance genes on chromosome 13, and this locus for SC-15 was identified using different populations and methods compared to earlier reports. Moreover, four candidate resistance genes were analyzed by qRT-PCR, among which, *Glyma13g24461* was the gene most likely to be involved in defending against SMV. These results will provide a foundation for cloning genes and confirming molecular resistance mechanisms in future studies.

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Author contributions ML and HN designed and directed the project. ML, NL, and QM participated in the analysis of phenotypic data. TL and ZC helped perform experiments. TL and QM was responsible for mapping the QTLs. ML and ZC performed the qRT-PCR analysis. ML and NL wrote the manuscript with input from all authors. NL designed the figures and tables in the manuscript. HN helped supervise the project. All authors discussed the results and contributed to the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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