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Mining of a major QTL and novel genes conferring resistance to SC3 and SC7 strains in soybean

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Abstract

Soybean mosaic virus (SMV) is a serious disease in soybean production, and the cultivation of resistant variety is the irreplaceable strategy to decrease the virus damage. However, the progress of SMV molecular breeding is limited partly due to the diverse types of SMV strains, solely resistance to virus strain of variety and minor genetic effects of QTLs. In the present study, one major pleiotropic additive QTL, *qSMV-D1b*, with phenotypic variation explained that 27.04%–54.23% was identified on chromosome 2, and the favourable allele was from the resistant parent "Qihuang30". Meanwhile, the allele analysis of the flanking markers showed that the favourable genotypes presented high resistance to SC3 and SC7 strains with lower disease index, while the unfavourable genotypes presented high sensitivity with higher disease index. Furthermore, six candidate genes, including four novel resistance genes, *Glyma.02G121000*, *Glyma.02G123000*, *Glyma.02G123200* and *Glyma.02G124700* with different expression levels between resistant and sensitive parents were discovered through transcriptome sequencing and confirmed by quantitative real-time PCR. The resistance genes and the tightly linked molecular markers can be used for SMV breeding in soybean.

KEYWORDS

candidate genes, linkage mapping, pleiotropic QTL, SC3 and SC7 strains, soybean mosaic virus

1 | INTRODUCTION

Soybean is an irreplaceable protein and oil crop in the world, while its growth and development are seriously affected by the soybean mosaic virus (SMV) disease (Adams et al., 2005). Soybean infected with SMV not only shows the typical symptoms of leaf shrinkage, necrosis, plant dwarfing and seed mottling, *etc*., but it also results in serious reduction of seed yield and significant deterioration of seed quality (Wrather et al., 2001). Thus, many countries, including China, always carry out the "SMV-sensitive variety veto policy" in the soybean breeding and variety registration programmes (Li et al., 2013). The cultivation and application of varieties with SMV resistance have been demonstrated as the most economical, effective and environmental friendly strategies to decrease the SMV adverse effects.

To discover the genetic loci used in soybean molecular breeding, the linkage mapping and genome-wide association study (GWAS) strategies have been applied in many studies (Che et al., 2020; Karthikeyan et al., 2017, 2018). Wang et al. (2018) summarized the QTLs associated with SMV resistances in soybean, and found

Jiahao Chu and Wenlong Li contributed equally to this study.

[Correction added on 21 July 2021, after first online publication: The article category has been changed in this version.]

TABLE 1 Disease index to SC3 and SC7 strains of the ZQ-RIL population

	Parents		RILs						
SMV strain	Zheng92116	Qihuang30	Range	Mean ± *SD*	CV (%)	Skew.	Kurt.	Sig.	h_B^2 (%)
SC3	35.0	6.0	3.0–82.0	43.0 ± 24.0	55.8	−0.12	−1.45	**	98.7
SC7	42.0	22.0	4.0–86.3	41.6 ± 22.4	53.8	−0.14	−1.48	**	98.1

Abbreviations: CV, coefficient of variation; h_B^2, broad sense of heritability; Sig., significant of RIL lines in the analysis of variance.
**Significant difference at .01 level.

that the genetic loci of resistances to different SMV strains were mainly located on five chromosomes (Gm02, Gm06, Gm10, Gm13 and Gm14); further analysis showed that there existed ten loci on chromosome 13 (*Rsv1*, *Rsv5*, R_{SC3Q}, R_{SC11} and R_{SC12}, *etc.*), eight loci on chromosome 2 (*Rsv4*, R_{SC5}, R_{SC6}, R_{SC7} and R_{SC8}, *etc.*), two loci on chromosome 6 (R_{SC15} and R_{SC18}) and two on chromosome 14 (*Rsv3* and R_{SC4}). Recently, Lin et al. (2020) identified two QTLs related to resistance to SMV, and the *qTsmv-3* was regarded as a novel genetic locus on chromosome 3.

Based on the genetic loci identified with SMV resistances, some researchers have attempted to find the candidate genes in the QTL regions. Through the comparison of soybean reference genome Williams82, it was found that the QTLs on chromosomes 6, 13 and 14 contained many predicted genes with nucleotide binding site-leucine rich repeat (NBS-LRR) domain, which was the typical domain in resistance genes to plant diseases (Widyasari et al., 2020). Wang et al. (2018) summarized the putative candidate genes on chromosomes 6, 13 and 14, and deduced that there were five potential genes on chromosome 6 (*Glyma.06G182600* as the possible causal gene), 29 potential genes on chromosome 13 (*Glyma.13G184800*, *Glyma.13G184900*, *Glyma.13G187900*, *Glyma.13G190000*, *Glyma.13G190300*, *Glyma.13G190400* and *Glyma.13G190800* as the possible genes), and 17 potential genes on chromosome 14 (*Glyma.14G204500*, *Glyma.14G204600*, *Glyma.14G204700*, *Glyma.14G205000*, *Glyma.14G205200*, and *Glyma.14G205300* as the possible genes).

In contrast with the QTLs and candidate genes on chromosomes 6, 13 and 14, the QTLs of resistances to SMV on chromosome 2 did not contain any candidate genes with NBS-LRR typical domain, and were predicted as a novel type of resistant mechanism to SMV in soybean (Saghai et al., 2010). Wang et al. (2018) predicted 21 putative genes on chromosome 2, and *Glyma.02G121400*, *Glyma.02G121500*, *Glyma.02G121600*, *Glyma.02G121800*, *Glyma.02G121900*, *Glyma.02G122000*, *Glyma.02G122100* and *Glyma.02G122200* were considered the most possible genes associated with resistances to SMV strains.

Although some QTLs have been identified to be associated with resistance to different SMV strains in soybean (Klepadlo et al., 2017; Yan et al., 2015; Yang et al., 2013a), the genetic improvements of soybean resistant varieties are still relatively slow and lagging. The reasons for this were partly due to the diverse strains of SMV and complex-infections of various SMV strains in soybean production. Therefore, further studies of genetic loci and candidate genes with pleiotropic effects to control different SMV strains become an important strategy in soybean resistance molecular genetic improvements. In view of this, the objectives of this study were to identify the pleiotropic QTLs for SMV resistance from a recombinant inbred line (RIL) population and identify high confidence candidate genes associated with SMV resistance.

2 | MATERIALS AND METHODS

2.1 | Plant materials and virus strains

A soybean RIL population (ZQ-RIL) was derived from a cross between "Zheng92116" × "Qihuang30", and 279 lines were used in this study to find the QTLs linked with resistance to SMV SC3 and SC7 strains. The two SMV strains were kept in the sensitive germplasm “Nannong 1138-2”. "Zheng92116" was sensitive to SC7 and middle-resistance to SC3, and "Qihuang30" was resistant to both strains (Table 1). The SMV strains SC3 and SC7 were provided by the National Center for Soybean Improvement, Nanjing Agricultural University, China, and the germplasm “Nannong1138-2” was used as sensitive control.

2.2 | Methods

2.2.1 | Assessment of resistance to SC3 and SC7 strains in the RIL population

The RIL lines were grown in an aphid-free greenhouse at 26 ± 2°C temperature under 14/10 hr photoperiod condition in Hebei Agricultural University. The SC3 and SC7 inoculums were prepared by grinding the leaves of "Nannong1138-2", and the unifoliate leaves were inoculated by these two strains at the V1 stage and were inoculated again on the first trifoliate leaves at the V2 stage, respectively. The experiment was conducted by using the randomized complete block experiment design, and the RIL lines were planted with one biological replicate and repeated three times from February to May in 2019. Thirty soybean plants were used for each biological replicate to assess the resistances of RIL lines.

The infection responses of the soybean plants were assessed 30 days after inoculation, and the response of soybean plants was evaluated by a rank of 0–4, whereas 0 means no disease symptom, 1 for slight mosaic or small necrotic spot, 2 for slight shrinkage or

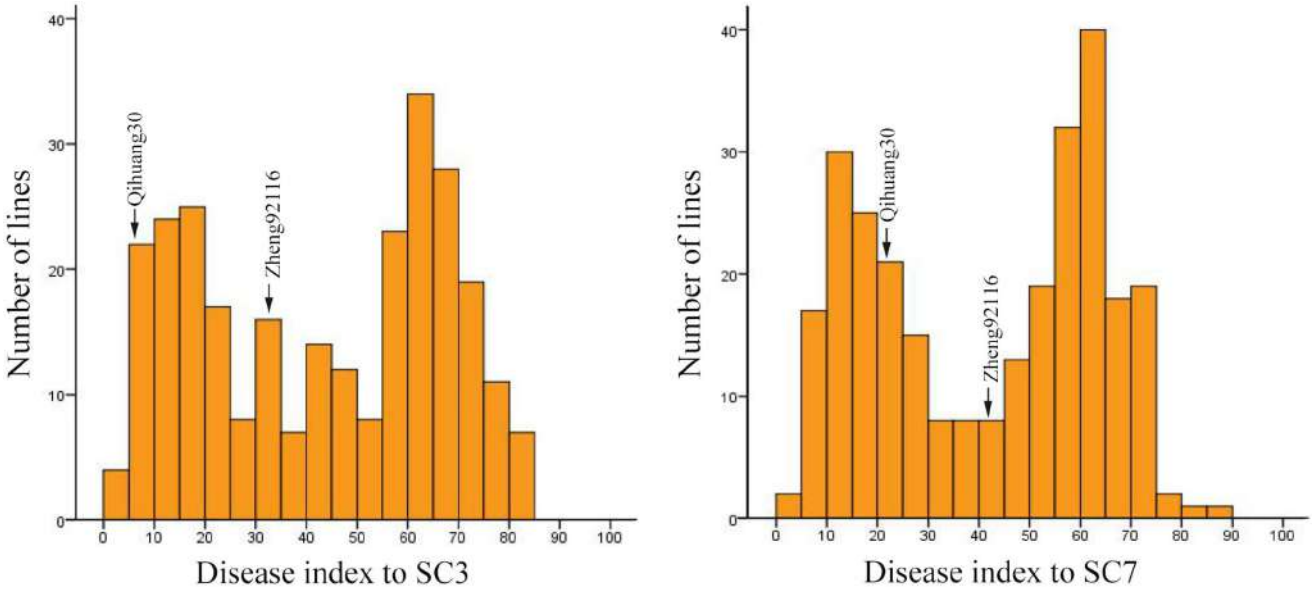

FIGURE 1 Disease index to SC3 and SC7 strains of soybean RIL lines [Colour figure can be viewed at wileyonlinelibrary.com]

the diameter of necrotic spot less than 5 mm, 3 for heavy mosaic or continuous necrotic spots and 4 for leaves shrunk seriously or plant dwarf and dying. The response of plants to SMV was indicated by disease index (DI) as described in reference (Zhi et al., 2005), and DI was calculated via the equation:

$\mathrm{DI} = \left[\sum f_i S_i / \left(n \times S_{\max}\right)\right] \times 100$, where, S_i indicated the disease severity, f_i indicated the number of plants with S_i, $S_{\max}$ indicated the highest S_i, n indicated the total number of the detected plants ($n = 30$ in this study).

2.2.2 | Statistical analysis

The broad-sense heritability for disease index was calculated via the equation: $h^2 = \sigma_g{}^2/(\sigma_g{}^2 + \sigma_e{}^2/r)$, and $\sigma_g{}^2$, $\sigma_e{}^2$ and r indicated the genotypic variance, error variance and the number of replications, respectively (Kim et al., 2014; Knapp et al., 1985). The SPSS 25.0 software was used to analyse each variance components, variation coefficients and descriptive statistics.

2.2.3 | QTL mapping of disease index to SC3 and SC7 strains

Using the linkage map of ZQ-RIL population constructed in our previous study (Li et al., 2018a) and disease index to SC3 and SC7 strains, the QTL mapping was processed via the inclusive composite interval mapping (ICIM) method in the ICIMapping v4.2 software (Meng et al., 2015). The threshold of LOD score for evaluating the significant QTL was determined using 1,000 permutations with a LOD score of 3.0 used as a minimum to declare the detection of QTL in a particular region of genome. The walking speed along the chromosome was set to 0.1 cM.

2.2.4 | Transcriptome and qRT-PCR analysis of RIL parents after SMV inoculation

The RIL parents ("Zheng92116" and "Qihuang30") were performed for transcriptome sequencing after the inoculation of SC7 strain. The inoculated and control (mock-inoculated with phosphate buffer) soybean leaves were sampled at 0, 3, 10, 24, 120 and 240 hr post-inoculation (hpi).

Quantitative real-time PCR (qRT-PCR) was used to further verify the expression of candidate genes as described in our previous study (Kong et al., 2018). The inoculated leaves and control were sampled at the same time-point as transcriptome analysis, and the experiments were repeated three times. The primers used for qRT-PCR are listed in the Table S1. The expressions of candidate genes were quantified by using the relative quantification ($2^{-\Delta\Delta Ct}$) method.

3 | RESULTS

3.1 | Genetic variation of disease index to SC3 and SC7 strains

Relatively wide genetic variations of disease index to SC3 and SC7 strains were found in the RIL population with variation coefficients 55.8% and 53.8%, respectively (Table 1). The disease index ranged from 3.0 to 82.0 and 4.0 to 86.3, which indicated the disease gradations of RIL lines ranged from resistance to highly-sensitive. Meanwhile, the broad sense of heritability was 98.7% and 98.1% to these two strains, which indicated that the resistance of RIL population was mainly contributed by the genetic factors (Table 1). In addition, the bi-modal distributions of disease index to SC3 and SC7 strains indicated that there might exist major controlling genes with resistances in the RIL population (Figure 1).

3.2 | Pleiotropic major QTL associated with resistance to SC3 and SC7 strains

The linkage mapping of QTLs to SC3 and SC7 strains were conducted based on the disease index and genetic linkage map of the RIL population, and the linkage map was slightly modified based on our previous study (Li et al., 2018a). One pleiotropic major additive QTL, *qSMV-D1b*, with phenotypic variations explanation of 27.04%–54.23% for resistance to SC3 and SC7 was identified on chromosome 2, and the favourable allele was attributed from the resistant male parent "Qihuang30" (Table 2).

To further analyse the application potentiality of QTL for SC3 and SC7 resistance in soybean breeding, the RIL population was divided into two groups based on the genotypes of QTL flanking markers (ss715580960, ss715581063 and ss715581097). And the results indicated that the favourable genotypes of three flanking markers (TT-type of three linkage markers) presented resistance to SC3 and SC7 strains with significant lower disease index (24.7–26.9), while the unfavourable genotypes (CC-type, CC-type and GG-type of ss715580960, ss715581063 and ss715581097, respectively) were sensitive to SC3 and SC7 with higher disease index (56.2–59.1). The results indicated that the favourable allele of QTL could significantly improve both resistances to SC3 and SC7 strains of RIL lines, and can be used to identify the resistant germplasms in soybean breeding programme (Figure 2, Table S2).

3.3 | Candidate genes responsible for resistance to SC3 and SC7 strains

To further discover the candidate genes responsible for resistance to SC3 and SC7 virus strains in the pleiotropic major QTL interval on chromosome 2, the transcriptome data of the RIL parents ("Zheng92116", "Qihuang30") at six different time-points after inoculation (0, 3, 10, 24, 120 and 240 hpi) was analysed, and four novel genes, *Glyma.02G121000*, *Glyma.02G123000*, *Glyma.02G123200* and *Glyma.02G124700* were firstly discovered to associate with SMV resistances based on their annotations and different expression levels at one or more time-points between the resistant and sensitive RIL parents (Table 3, Figure 3).

Among these four novel genes, *Glyma.02G124700*, encoding a protein of SRG1, showed relatively higher expressions at 3, 10 and 120 hpi in the resistance variety "Qihuang30", while it only showed relatively higher expression level at 10 hpi in the sensitive variety "Zheng92116" (Figure 3). *Glyma.02G123200*, encoding a protein of O-fucosyltransferase family, also showed relatively higher expression levels at 10 and 240 hpi in "Qihuang30", while it had lower expressions in the "Zheng92116" based on the transcriptome sequencing analysis (Figure 3).

In contrast with these two genes above, *Glyma.02G123000*, encoding a protein of serine carboxypeptidase family, showed relatively higher expression levels at 3, 10, 24 and 120 hpi in the sensitive variety "Zheng92116" and lower expressions in the resistance variety "Qihuang30" (Figure 3). Meanwhile, *Glyma.02G121000*, encoding a protein of SAM dependent carboxyl methyltransferase, showed higher expression levels at 3 and 240 hpi in the "Zheng92116" and lower expressions in the resistance variety "Qihuang30" (Figure 3). In addition, the two previously reported candidate genes, *Glyma.02G122100* and *Glyma.02G121800*, were also found to show different expression levels between the RIL parents through the transcriptome sequencing (Figure 3).

Moreover, the expression levels of four novel resistance genes and the reported gene *Glyma.02G122100* were further demonstrated via qRT-PCR technology, and the results showed that the expressions of four novel genes and *Glyma.02G122100* exhibited significant differences between "Zheng92116" and "Qihuang30" (Figures 4 and 5; Figure S1). These results not only demonstrated the transcriptome results above, but also indicated that these four novel candidate genes might have important functions to defend the SMV infection in soybean.

4 | DISCUSSION

Soybean yield and quality have been seriously influenced by the SMV in many countries (Hill & Whitham, 2014), and the issues have been put forward while no effective strategies have been deployed by now. Although some genetic loci associated with the resistances to SMV have been identified through linkage mapping or GWAS studies (Che et al., 2020; Karthikeyan et al., 2017, 2018; Klepadlo

TABLE 2 The pleiotropic QTLs of disease index to SC3 and SC7 strains in the RIL population

QTL[a]	Position (cM)	Marker interval	Region (cM)	Physical interval (bp)	LOD	PVE(%)	ADD.
qSC3-D1b	99.49	ss715580960-ss715581063	97.13–104.92	10,935,557–12,334,435	54.13	54.23	−19.35
qSC7-D1b	99.29	ss715580960-ss715581063	97.13–104.92	10,935,557–12,334,435	51.98	29.39	−17.64
	105.63	ss715581063-ss715581097	104.92–106.16	12,334,435–12,506,411	51.53	27.04	−16.91

Abbreviations: ADD., Additive effect of QTL;LOD, Likelihood odds ratio; PVE, Proportion of phenotypic variation explained by QTL.
[a]The nomenclature of QTL included QTL, SMV strain, and linkage group.

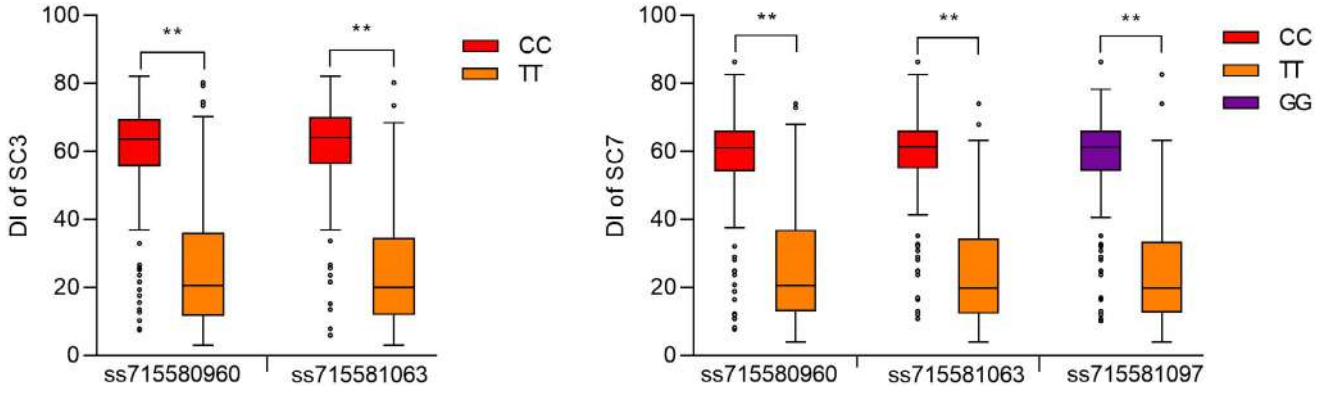

FIGURE 2 Analysis of elite allele at flanking markers associated with disease index to SC3 and SC7 on chromosome 2 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Candidate genes conferring resistance to SC3 and SC7 strains in the QTL region

Candidate gene	Start position	End position	Length	Annotation
Glyma.02G121000	11,953,827	11,956,583	1,448	SAM dependent carboxyl methyltransferase
Glyma.02G121800	12,106,084	12,107,743	1,178	Adenine nucleotide alpha hydrolases-like protein
Glyma.02G122100	12,134,374	12,137,612	1,315	Copper transport protein atox1-related
Glyma.02G123000	12,271,120	12,276,647	1,782	Serine carboxypeptidase S28 family protein
Glyma.02G123200	12,289,339	12,294,930	3,205	O-fucosyltransferase family protein
Glyma.02G124700	12,466,663	12,469,681	1,631	Protein SRG1

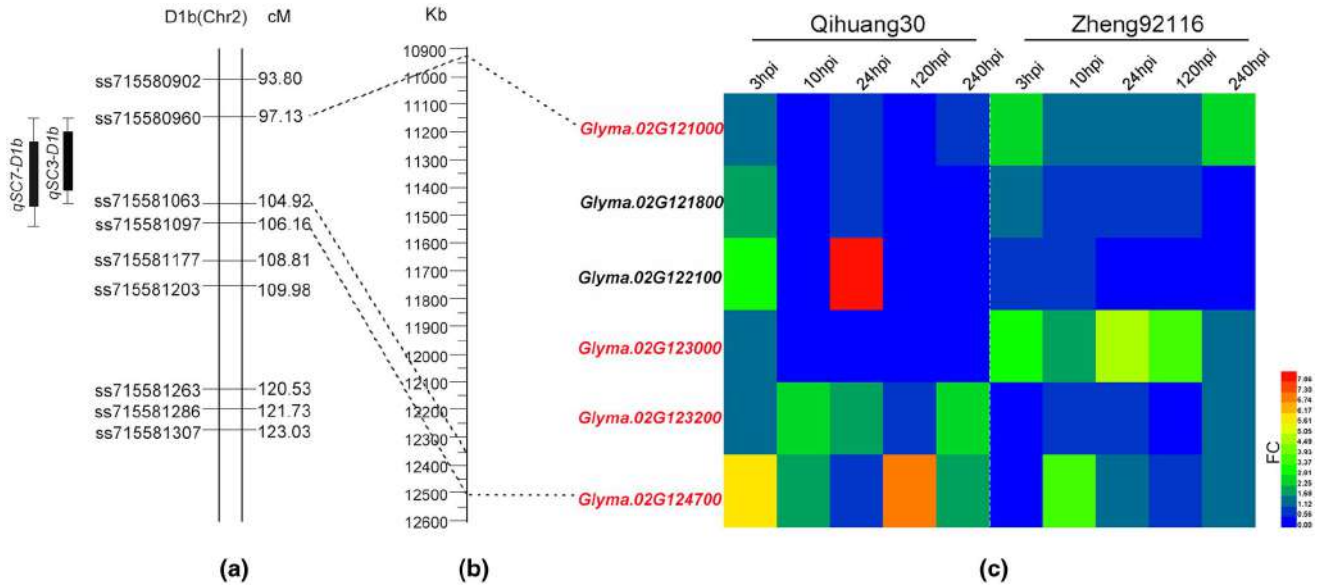

FIGURE 3 Pleiotropic major QTL and candidate genes for resistances to SC3 and SC7 strains on chromosome 2 in soybean. (a) Major QTL mapped in ZQ RIL population; (b) Physical position of major QTL on chromosome 2 (*Gmax2.0*); (c) Differential expressions of candidate genes based on transcriptome sequencing [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2017; Yan et al., 2015; Yang et al., 2013a; Yang et al., 2013b), the implementation of SMV resistance in soybean breeding is still slow in progress. The reasons for this might be owing to: (a) The relatively diverse types of SMV strains (more than 30 at present) in different eco-regions and the potential increasing of variations of SMV strains with the soybean planting in the future; (b) The mixed and complex infection of different virus strains on host plants in soybean production, such as the SC3 and SC7 strains in Huang-huai-hai eco-region in China; (c) The minor genetic effect of QTL and sole function of candidate genes. Thus, the mining of pleiotropic QTLs with major genetic effects and candidate genes with multiple-resistance to different SMV strains become more and more important.

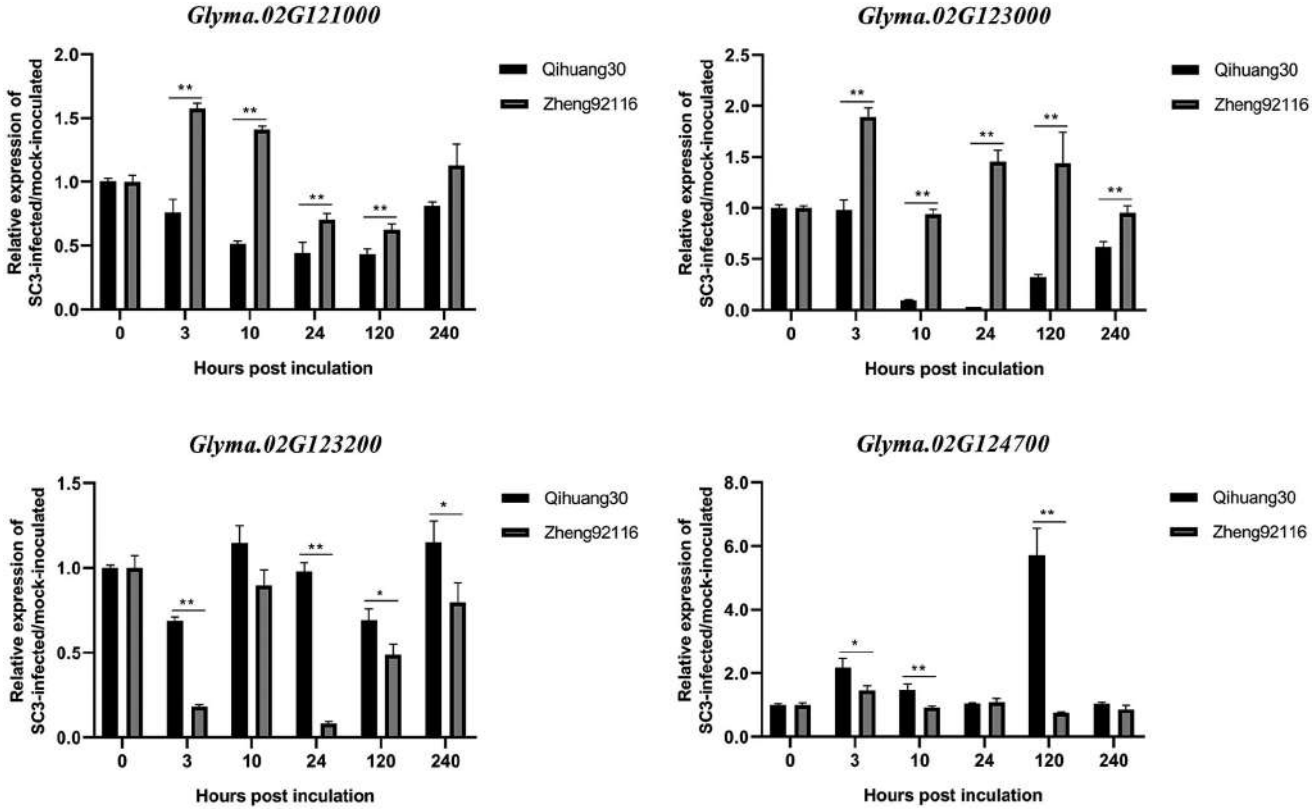

FIGURE 4 Expressions of novel candidate genes for resistance to SC3 strain in soybean based on the qRT-PCR analysis. *t test, $p < .05$. **t test, $p < .01$

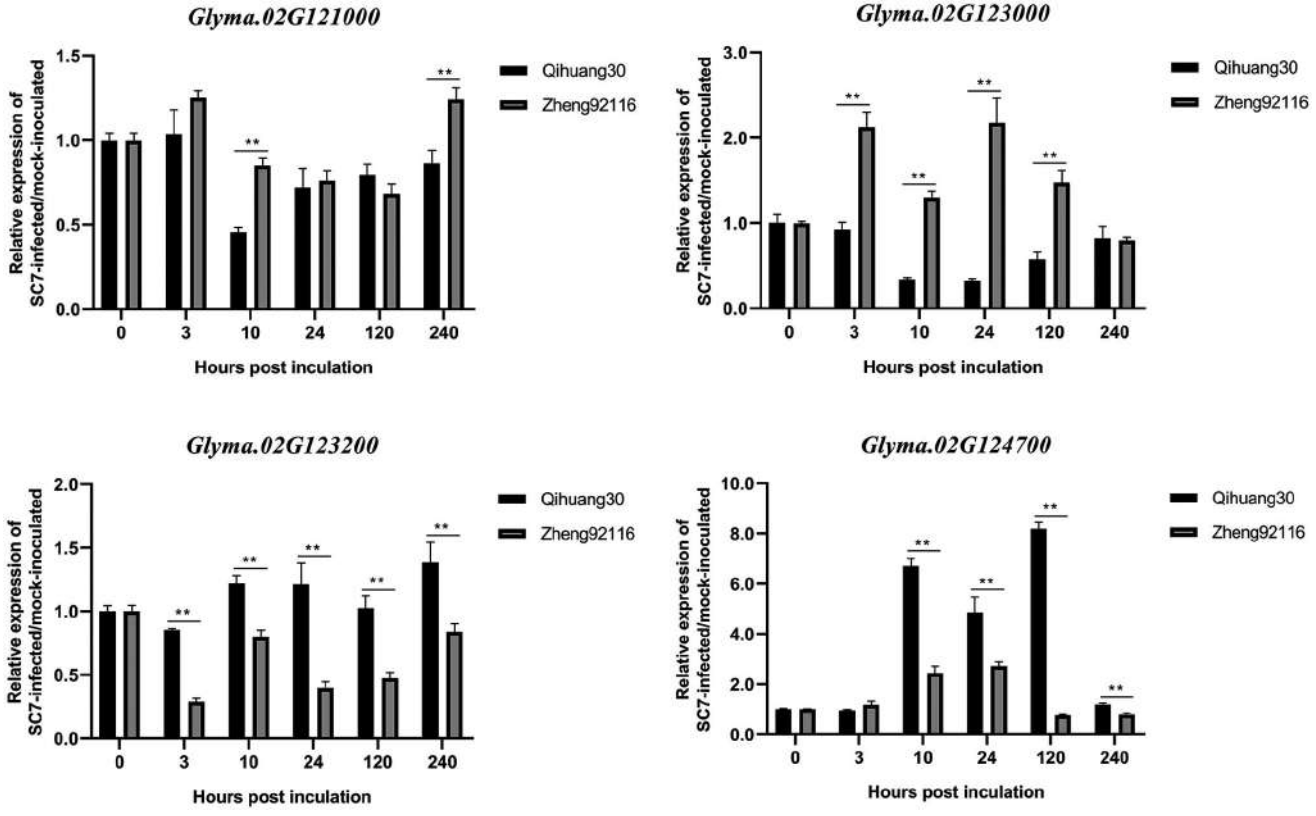

FIGURE 5 Expressions of novel candidate genes for resistances to SC7 strain in soybean based on the qRT-PCR analysis. **t test, $p < .01$

In view of this, one pleiotropic major QTL with PVE 27.04%–54.23% (more above 10%) controlling the resistance to SC3 and SC7 strains, simultaneously, was identified on chromosome 2 (physical position 10,935,557–12,506,411) in the present study. Furthermore, the favourite alleles of the linkage markers could not only significantly decrease the disease index of RIL lines to SC3 and SC7 strains, but they could also change the reaction properties of RIL lines to SC3 and SC7 strains. In another word, the favourite alleles of flanking markers could convert the sensitive characteristics into resistant characteristics of RIL lines, and suggested that the QTL detected in the present study had potential application in SMV resistance molecular breeding in soybean.

As to the SMV resistance QTLs on chromosome 2, there were some others studies that reported QTLs controlling different SMV strains nearing to the QTL in the present study. Luan et al. (2020) reported the SMV locus *RSC7* (SC7 strain) in the marker BARCSOYSSR_020667 and BARCSOYSSR_020670 interval with physical position 13.02–13.11 Mb, and also mapped the *Rsc13* locus (SC13 strain) in the marker BARCSOYSSR_020610 and BARCSOYSSR_020621 interval with physical position 11.96–12.16 Mb, which justly located in the QTL region detected in our study. Meanwhile, some other QTLs were also identified co-locating with our mapping results (Ilut et al., 2016; Karthikeyan et al., 2017; Li et al., 2015; Yan et al., 2015; Zhao et al., 2016), such as the Rsc7 locus (SC7 strain) in the region of BARCSOYSSR_020621~BARCSOYSSR_020632 (physical position 12.15–12.32 Mb), Rsc5 locus (SC5 strain) in the region of Bin352 and Bin353 (physical position 11.39–12.15 Mb), Rsv4 locus (G1-G7 strain) in the region of ZL-42 and ZL-52 (physical position 12.06–12.10 Mb), Rsc8 locus (SC8 strain) in the region of Sms3 and S6ac (physical position 12.07–12.17 Mb). Furthermore, it was found that there existed one pleiotropic QTL controlling SC3 and SC7 strains in our present study.

Based on these consistent mapping results, we deduced that there existed causal genes for SMV resistance in this region, and the candidate genes were further searched via the transcriptome data of two RIL parents, "Zheng92116" and "Qihuang30", and six associated genes including four novel genes were found according to their different expressions after inoculation. Among these genes, *Glyma.02G122100*, encoding a ATOX1-related protein, was associated with resistance to SMV SC5 and G1~G7 strains in previous studies (Karthikeyan et al., 2017; Ilut et al., 2016), because the ATOX1 protein had close interactions with SOD1 (Consortium, 2011; Wan et al., 2015), and SOD1 could significantly improve the adapt-abilities of plants under different biological and abiotic stresses (Møller et al., 2010; Song et al., 2009; Wang et al., 2016). Meanwhile, the ATOX1 protein had been found to significantly inhibit the phosphorylation of mitogen-activated protein kinases (MAPKs) in plants, and MAPKs played an important role in the plant adaption process to various stresses (Meng & Zhang, 2013). There were many studies demonstrating that the MAPKs attributed to the early signals in response to different plant pathogen infections, and the MAPKs could be activated by the pathogen infection, and then resulted in the switch on or switch off of the plant defense pathway via the phosphorylation process of target proteins (Meng & Zhang, 2013). In addition, the candidate gene, *Glyma.02G121800*, was predicted to encode adenine nucleotide alpha hydrolases-like superfamily protein, and was reported to have functions in the resistance to SMV SC6 and G1~G7 strains in previous studies (Ilut et al., 2016; Yang et al., 2013b).

Apart from these two genes reported above, there were four novel candidate genes that were first identified in the present study. The candidate gene *Glyma.02G124700*, encoding a protein of SRG1, showed relatively higher expression levels at 3, 10 and 120 hr after SC7 inoculation in the resistance variety, while it showed relatively higher expression levels only at 10 hr after inoculation in the sensitive variety (Figure 3). Therefore, the resistant variety had a relatively earlier and longer time expression compared to the sensitive variety with relative later and shorter time expressions. The SRG1 protein was reported to participate in the progress of plant cell senescence or apoptosis, and had important functions in the organism immune responses to many adverse stresses (Cui et al., 2018). The molecular mechanism analysis of SRG1 protein found that it was an important target molecule of nitric oxide (NO) and was induced by NO; furthermore, it could regulate the expressions of a series of download genes, which resulted in the plant cell apoptosis, and lead to the positive immune response in plants against the biotic and abiotic stress (Cui et al., 2018). Thus, we deduced that the relatively earlier and longer time expressions of *Glyma.02G124700* in resistant soybean variety might induce the infected soybean cells to a relatively earlier and longer time apoptosis, and effectively limit the extension of SMV strains in a relatively narrow region, while the later and shorter time expression in sensitive variety of this gene induced a relatively wide range extension of SMV, which led to the sensitive effects to SMV infection.

In addition, the candidate gene, *Glyma.02G123200*, encoding a protein of O-fucosyl transferase, showed relatively higher expression levels at 10 and 240 hr in the resistance variety after SC7 inoculation (Figure 3). The O-fucosyltransferase participates in the cell wall formation during plant development against environmental stress, and cell wall had been regarded as the essential physical plant defenses to multiple stresses (Li et al., 2018b). Many studies had demonstrated the important functions of cell wall to defend the pathogenic bacterial invasion, insect herbivory and physical damage during plant growth and development (Zhang & Li, 2018). Meanwhile, the O-fucosyltransferase was also reported to show functions in the cell-to-cell adhesion and cell wall remodelling in plants (Verger et al., 2016). In addition, the O-fucosyltransferase had been demonstrated to play an important role in the resistance to many physiological and pathological diseases in animals (Liang et al., 2021). Thus, we deduced that the higher expression levels of *Glyma.02G123200* in resistant soybean variety might involve in the bio-synthesis of cell wall to avoid the serious harm of SMV.

Meanwhile, the candidate gene, *Glyma.02G123000*, encoding a serine carboxypeptidase family protein, showed relatively higher expression levels at 3, 10, 24 and 120 hr in the sensitive variety “Zheng92116” after SC7 inoculation (Figure 3), and the serine

carboxypeptidase was reported to play an important role in the disease resistance response of plants. Liu et al. (2013) reported that the expression of serine carboxypeptidase gene *ZmSCP* in maize was up-regulated after the JA, ABA, low temperature, and salt treatments, and also presented the relative higher expression levels after the *Rhizoctonia solani* inoculation, which indicated that the serine carboxypeptidase participated in the resistances to these biotic and abiotic stresses. Meanwhile, one serine carboxypeptidase was identified in tomato leaves during the course of detection of the wound-induced proteins, and the enzyme activity was increased in response to the wounding (Moura et al., 2001). Based on this, Moura et al. (2001) isolated the cDNA of serine carboxypeptidase gene from tomato leaves, and found that it was induced by the wounding, systemin and methyl jasmonate; furthermore, the serine carboxypeptidase gene in tomato leaves was regarded as the "late wound-inducible genes" with higher expressions at 4–12 hr after wounding, which was different from some other "early wound-inducible genes" with higher expressions within 30 min after wounding. Thus, we deduced that the higher expression levels of *Glyma.02G123000* in sensitive soybean variety might be due to the relative heavy injury of sensitive variety after SMV strain inoculation and infection.

In addition, the candidate gene, *Glyma.02G121000*, encoding a protein of SAM dependent carboxyl methyltransferase, showed relatively higher expressions in "Zheng92116", and the SAM dependent carboxyl methyltransferase was a class of methyltransferase to catalyse many substrates such as the salicylic acid and jasmonic acid via the transfer of a methyl group from SAM to their substrates. It was reported that the SAM dependent carboxyl methyltransferase could be induced by many biotic and abiotic stresses in plants. In summary, the results in the present study not only validated the candidate genes reported previously, but also added the novel genes for SMV resistance breeding in soybean.

5 | CONCLUSION

One pleiotropic major additive QTL, *qSMV-D1b*, with PVE 27.04%–54.23% was discovered on chromosome 2 controlling resistance to SC3 and SC7 strains, simultaneously. Allele analysis of the QTL flanking markers showed that the favourable genotypes presented resistance to SC3 and SC7 strains, while the unfavourable genotypes presented sensitive to these two strains. Six candidate genes, including four novel resistance genes, *Glyma.02G124700*, *Glyma.02G123200*, *Glyma.02G123000* and *Glyma.02G121000* were identified in the pleiotropic QTL interval with different expressions between the resistant and sensitive varieties. The results identified the candidate genes and effective selection markers for SMV resistance in soybean molecular breeding programme.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

CZ and XL designed the methods and experiments. JC, WL, YK and HD conducted SMV inoculations and resistance evaluation. JC and XL performed genotype analysis. JC, WL and FL analysed the data. JC and WL drafted the manuscript. CZ and XL reviewed the manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

The experiments were performed in compliance with the current laws of China.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article and its supplementary information files.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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