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ORIGINAL PAPER

Fine mapping and analyses of R_{SC8} resistance candidate genes to soybean mosaic virus in soybean

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Abstract Soybean mosaic virus (SMV) in soybean [*Glycine max* (L.) Merr.] is a destructive foliar disease in soybean-producing countries worldwide. In this study, F_2, $F_{2:3}$, and $F_{7:11}$ recombinant inbred lines populations derived from Kefeng No.1 × Nannong 1138-2 were used to study inheritance and linkage mapping of the SMV strain SC8 resistance gene in Kefeng No.1. Results indicated that a single dominant gene (designated R_{SC8}) controls resistance, which is located on chromosome 2 (MLG D1b). A mixed segregating population was developed by selfing two heterozygous plants (RHL153-1 and RHL153-2) at four markers adjacent to the locus and used in fine mapping R_{SC8}. In addition, two genomic-simple sequence repeats (SSR) markers BARCSOYSSR_02_0610 and BARCSOYSSR_02_0616 were identified that flank the two sides of R_{SC8}. Sequence analysis of the soybean genome indicated that the interval between the two genomic-SSR markers is 200 kb. QRT-PCR analysis of the candidate genes determined that five genes (Glyma02g-13310, 13320, 13400, 13460, and 13470) are likely involved in soybean SMV resistance. These results will have utility in cloning, transferring, and pyramiding of the R_{SC8} through marker-assisted selection in soybean breeding programs.

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Introduction

Soybean mosaic virus (SMV) is one of the most devastating viral pathogens in soybeans [*Glycine max* (L.) Merr.]. It causes severe yield loss and a reduction in seed quality. In China, SMV affects all soybean-producing regions in the country. Planting resistant cultivars is the most effective, economical, and environmentally safe approach for controlling the disease.

(Cho and Goodman 1979, 1982) collected 98 isolates of SMV and classified them into seven strains, designated G1–G7 on a set of differential cultivars. In China, SMV has been re-classified into 21 strains based on SMV isolate reactions to a set of soybean differentials (Guo et al. 2005; Li et al. 2010; Wang et al. 2003a). Among the 21 strains, SC8 strain is a moderately virulent strain widespread in the Yangtze River valley and Northern China (Guo et al. 2005; Li et al. 2010).

Wang et al. (2003b) reported that the resistance to SC8 in Kefeng No.1 was controlled by a dominant gene which was designated as R_{SC8}. In order to conduct marker assisted selection (MAS) and clone R_{SC8}, it is necessary to identify the molecular markers tightly linked to the gene.

Different molecular marker systems have been developed and used in resistant gene mapping of soybean. These markers are mainly classified into three types: (1) the type 1 based on the difference of fragment length such as restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs), (2) the type 2 based on the difference of repeat sequence such as simple sequence repeats (SSRs), (3) the type 3 based on the difference of single base such as single nucleotide polymorphisms (SNPs). SMV resistance genes *Rsv1*, *Rsv3*, and *Rsv4* were mapped on respective chromosomes 13, 14, and 2 (formerly MLG-F, B2, and D1b) (Gore et al. 2002; Hayes et al. 2000a;

Hwang et al. 2006; Jeong et al. 2002; Jeong and Saghai Maroof 2004; Yu et al. 1994) using molecular markers, including RFLPs, AFLPs, SSRs, and SNPs. Recently, *Rsv4* was targeted to a 1.3 cM region with a physical interval of less than 100 kb on chromosome 2 using the whole-genome shotgun sequence (Saghai Maroof et al. 2010). In China, several resistance genes were located on chromosomes 2 and 13 using recombinant inbred lines (RILs) and F_2 populations (Bai et al. 2009; Fu et al. 2006; Li et al. 2006; Luan et al. 2006; Wang et al. 2004; Zhang et al. 1999).

Residual heterozygous lines (RHLs) are of utility in fine mapping (Haley et al. 1994). Kobayashi et al. (2006) precisely localized and characterized key quantitative trait locus (QTL) regions for plant types on chromosomes 4 and 6 in rice using heterogeneous inbred families (HIFs, similar to RHLs). The research demonstrated that RHLs represent a powerful tool for isolation, identification, and fine mapping of target loci (Kobayashi et al. 2006; Su et al. 2010; Watanabe et al. 2009; Yamanaka et al. 2005).

Many plant resistant (R) gene sequences contain nucleotide binding sites (NBS) and leucine rich repeat (LRR) domains. Yu et al. (1996) utilized these conserved structural domains to develop 11 different classes of resistance gene analogs for mapping and cloning resistance genes from soybean. Using short NBS5 DNA sequences (Yu et al. 1996) as a probe, Hayes et al. (2000b) screened a cDNA library and identified a candidate resistance gene, L20a. The gene belonged to the Toll/Interleukin-1 receptor (TIR)-NBS-LRR class of R genes, but did not co-segregate with *Rsv1*.

Jeong et al. (2001) and Gore et al. (2002) identified several sequences, which flanked or co-segregated with *Rsv1* using primers specific to R gene families. Gore et al. (2002) also concluded that one or more resistance genes were tightly linked at intervals adjacent to *Rsv1*. In addition, a candidate *Rsv1* gene (3gG2) in the NBS-LRR gene cluster in PI96983 was identified (Hayes et al. 2004). Based on the analysis of sequences in the *Rsv4* region, Saghai Maroof et al. (2010) thought that *Rsv4* likely belongs to a new class of resistance genes.

The objectives of the present study were to conduct fine mapping of R_{SC8} using RHLs and tightly linked genomic-SSR markers to identify several candidate genes by quantitative real-time polymerase chain reaction (QRT-PCR). The outcome of this study will offer convenience in map cloning and MAS of the resistance R_{SC8} gene.

Materials and methods

Materials and mapping populations

The SC8, a moderately virulent SMV strain, was identified by Guo et al. (2005). Kefeng No.1 and Nannong 1138-2 were, respectively, bred using the pure-line selection method from land variety in Hebei and Shanghai China. They are, respectively, resistant and susceptible to the SMV strain SC8. The F_1, F_2 population with 156 individuals (Fig. 1, population I), $F_{2:3}$ with 100 lines were obtained from Kefeng No.1 × Nannong 1138-2. The $F_{7:11}$ RIL population consisting of 184 lines created by Wang (2001) (Fig. 1, population II).

Two individuals (RHL153-1 and RHL153-2) from line No.153 in RIL population were identified as heterozygous near the R_{SC8} locus based on the genotypes of five DNA markers (Table 1). A mixed segregating population of 181 individuals (secondary population F_2, SP-F_2) was derived by self-pollination of RHL153-1 and RHL153-2 (Fig. 1, population III).

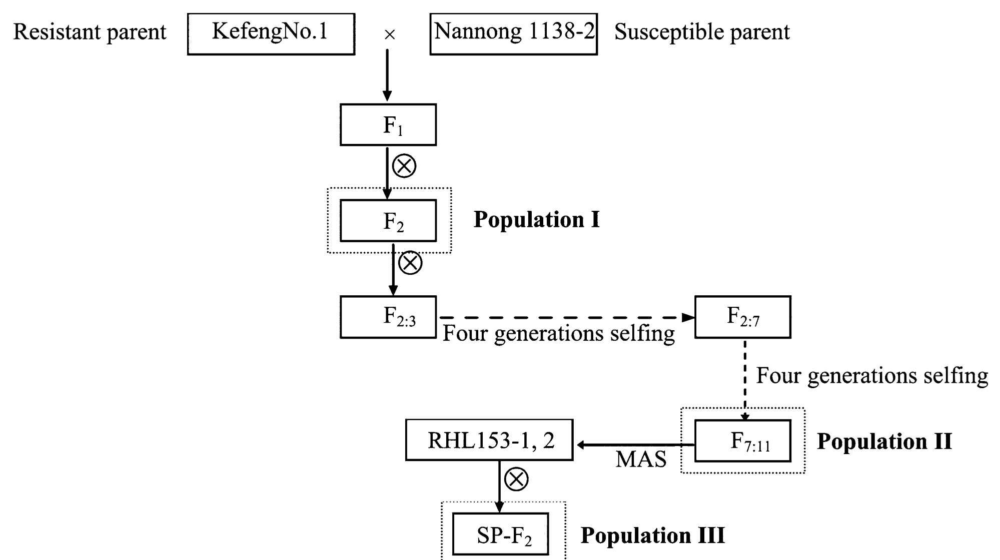

Fig. 1 Construction of three populations used to locate R_{SC8}. The F_2 population (I) was developed from the cross between Kefeng No.1 (resistant parent) and Nannong 1138-2 (susceptible patent). $F_{7:11}$ population (II) was derived by self-pollination of the F_2 population. A mixed segregating population (III) of 181 individuals (SP-F_2) was derived by self-pollination of two F_{11} plants (RHL153-1 and RHL153-2), which were identified as heterozygous adjacent to the R_{SC8} locus by scoring the DNA marker genotypes

Table 1 RHL153-1 and RHL153-2 genotypes from RILs

Individuals	Markers				
	BARCSOYSSR_02_0478	BARCSOYSSR_02_0555	BARCSOYSSR_02_0578	BARCSOYSSR_02_0596	BARCSOYSSR_02_0727
RHL153-1	3/3	3/3	3/3	3/3	1/1
RHL153-2	3/3	3/3	3/3	3/3	1/1

BARCSOYSSR_02_0478, BARCSOYSSR_02_0555, BARCSOYSSR_02_0578, BARCSOYSSR_02_0596 and BARCSOYSSR_02_0727 are linked with resistance gene R_{SC8}

'1/1': The homozygous Kefeng No.1 chromosome segment

'3/3': The heterozygous Kefeng No.1/Nannong 1138-2 chromosome segment

Resistance evaluation

Twenty Kefeng No.1, 23 Nannong 1138-2, 9 F_1, 156 F_2 plants, 100 $F_{2:3}$ (each line containing 20–25 plants), 184 $F_{7:11}$ RIL (each RIL containing 20–25 plants) and 181 SP-F_2 individuals were planted in pots in an aphid-free greenhouse/net-house. SC8 strain was spreaded on the susceptible parent Nannong 1138-2 by inoculating. Inoculum was prepared by grinding infected Nannong 1138-2 fresh leaves in 0.01 mol/L sodium phosphate buffer (approx. 3–5 ml per gram leaf tissue, pH 7.2) using a mortar and pestle. A small amount of 600-mesh carborundum powder was added to the inoculum as an abrasive.

Young plants were inoculated by gently rubbing the two unifoliolate leaves of a plant at V1 stage with the inoculum, then by re-inoculation on the first tripoli late at V2 stage. The inoculated leaves were rinsed with tap water following inoculation. The observations were conducted 7–10 days after the first inoculation, when the susceptible checks were heavily infected. Symptom reactions were noted at 1-week intervals for 2 months after inoculation. Reactions of the plants to SMV were divided into symptomless (resistant) and mosaic (susceptible). The resistant and susceptible plants were calculated. Chi-square (χ^2) tests were performed to determine the goodness of fit between observed segregation ratios and expected genetic ratios.

DNA extraction and SSR marker analysis

DNA was extracted from fresh leaves using the CTAB method (Saghai Maroof et al. 1984) with minor modifications. PCR was conducted in a total reaction mixture of 10 μl including approximately 50 ng of genomic DNA, 10× PCR buffer (500 mmol/L KCl, 100 mmol/L Tris–HCl, pH 8.0, 0.1% gelatin), 0.25 μmol/L of each primer, 0.2 mmol/L dNTPs and 1 U Taq polymerase in double distilled water. PCR reaction conditions were as follows: denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, extension at 72°C for 50 s; and a final extension at 72°C for 10 min before cooling to 4°C. PCR products were visualized after electrophoresis on an 8% polyacrylamide gel followed by silver staining; or on a 1% agarose gel followed by ethidium bromide staining. The polymorphic markers observed between resistant and susceptible parents and F_2 population resistant (R) and susceptible (S) bulks were used to screen populations I, II, and III (Michelmore et al. 1991).

Development and utilization of molecular markers for fine mapping

Five CSSR and CSTS (SSR and STS markers developed by Chiba University), six EST-SSR (Hwang et al. 2009; Xia

Table 2 Primer sequences used for fine mapping or QRT-PCR

Name of markers/genes	Types	Tm (°C)	PCR size (bp)	Forward primers (5′→3′)	Reverse primers (5′→3′)	References
BE822457AT	SNP	58	123	GCGTTGTGAGCGACATCTT	GCCGTCAGAGACCAGCAAT	This study
BE822457AG	SNP	58	203	TGCATCTTAGACCTTGGAAAA	GACGGAGTATTGGCTGTCA	This study
BM954695AG	SNP	58	224	GACATGCAAATTGCTGAGGA	TCTCACTTGACCATGCCTATTA	This study
BARC-025183-06457	Indel	58	419/403	TTCATTGGACCAACCTAACATGC	TTGTGCTTGATCCTTGGATTGTT	Choi et al. (2007)
BARCSOYSSR_02_0606	SSR	60	282	CAACATGCTGTTTGGAGCAG	CGTTGCCAATCCTTTGATTT	Song et al. (2010)
BARCSOYSSR_02_0610	SSR	60	167	GATGGGGGAGTGGTCATTTA	AATACCCGTGGGTCCTTACC	Song et al. (2010)
BARCSOYSSR_02_0616	SSR	60	245	ACGTGTTTGATACAGGCTGC	CCAAGGCTCCATAACTGCAT	Song et al. (2010)
BARCSOYSSR_02_0618	SSR	56	247	TGCGCATTACGATGAATGTT	AGCAGGGTATGTGATCCAGC	Song et al. (2010)
Glyma02g13310	–	60	120	GGGTGGAGAAGAAAGACTTGGA	CAAAGTAGTGAAGGAAAAGGCTGAT	This study
Glyma02g13320	–	60	224	GCGCTCACCGGTAGTATTCC	GAGAGGCGGTTCCCAGAGA	This study
Glyma02g13400	–	60	74	CGGTATCACAGATGCAGTTATGG	TGGTAGCGCCTCTGTGTCTCT	This study
Glyma02g13430	–	60	110	GATGCCTGTCCTACATGTTTTGAA	CGTCTCTCCTGCCACTCAAGT	This study
Glyma02g13450	–	60	100	TTATGAACTTGCTTCCTCCTTCAA	TTTTGGACCCTTTTCCTTTCC	This study
Glyma02g13460	–	60	101	CCGCTCGAGGACTACATTATCTC	GGCCACCCAATTCTGATCTAATA	This study
Glyma02g13470	–	60	192	GGGAGGCCAGGAGTTGCTAT	GGGCCCTGCAAGATTCCTAT	This study
Tubulin[a]	–	60	189	GGAGTTCACAGAGGCAGAG	CACTTACGCATCACATAGCA	This study

[a] Genbank accession No. AY907703

et al. 2007) markers and 15 SNP markers reported in the MLG D1b target region (Choi et al. 2007) were tested based on co-mapped SSR markers in the region (Song et al. 2004) across different maps.

The soybean Williams82 genome sequence (http://www.phytozome.net/soybean) was used to develop genomic-SSR markers. Song et al. (2010) provided the target region primer sequences for the genomic-SSR markers (Table 2).

DNA sequence analysis for SNP identification

PCR products were separated by electrophoresis on a 1% agarose gel. The expected size (400–1,200 bp) band was excised and purified with the Quick PCR gel purification kit (Bio Dev-Tech., Beijing, China). Purified PCR products were cloned into the pGEM-T-easy plasmid vector (Promega, Madison, WI) and transformed into DH5α *E. coli* competent cells.

Plasmids were purified using the Wizard Miniprep kit (Promega) and both strands sequenced with sequence specific primers. All sequences were verified manually and edited based on the chromatograms. SNaPshot technology was applied for SNP detection (Möhring et al. 2005; Missaoui et al. 2007) according to the protocol provided with the ABI PRISM SNaPshot Multiplex kit (Applied Biosystems).

Linkage analysis

The genetic distances between markers (the resistance gene served as a morphological marker) were calculated by JoinMap3.0 software (Van Ooijen and Voorrips 2001). Marker distances were calculated with the Kosambi function (Kosambi 1944) and the linkage map was drawn with Map Chart v. 2.1 (Voorrips 2002).

Quantitative real-time PCR (QRT-PCR) analysis

Two or three mock-inoculated (inoculated with phosphate buffer) and SMV-infected leaves from Kefeng No.1 and Nannong 1138-2 were independently collected at 0, 1, 2, 4, 8, 12, 24, and 48 h post inoculation (hpi) and stored at −80°C until QRT-PCR analysis. Total RNA was extracted from treated plants with the RNA simple total RNA Kit (Tiangen, Beijing) according to the manufacturer's protocols. First-strand cDNA was synthesized with Oligo (dT) primer using PrimeScript^{TM} 1st strand cDNA Synthesis Kit D6110A (TaKaRa, Dalian) following the manufacturer's instructions.

QRT-PCR was conducted in a total reaction mixture of 20 μl including 0.8 μl (approximately 50 ng) of first-strand cDNAs, 0.5 μl of 10 μmol/L gene-specific primers (forward and reverse primers), 10 μl of 2× SYBR Green I Master Mix, and 8.2 μl of ddH_2O. PCR reaction conditions were as follows: 50°C for 2 min; 95°C for 10 min; followed by 40 cycles at 95°C for 20 s and 60°C for 60 s (96-well 7500 Real Time PCR System).

The gene-specific primers (Table 2) were designed from soybean genome sequences (http://www.phytozome.net/soybean) for every candidate gene. Tubulin (NCBI accession No. AY907703) was employed as an internal control to normalize the total amount of cDNA in each reaction. Gene expression was quantified using the relative

quantification ($\Delta\Delta C_T$) method and data was compared with internal controls. Each sample was replicated three times, and the resulting data were analyzed using the mock inoculation conditions at each time point after inoculation as calibrators, with the aid of the Sequence Detection System (SDS) software version 1.4 (graph error ± SD = 0.1, normalized expression $\Delta\Delta C_T = (C_{T,Target} - C_{T,tubulin})\ Time_x - (C_{T,Target} - C_{T,tubulin})\ Time_0$) from 7500 Real Time PCR System (Applied Biosystems).

Results

SMV strain SC8 resistance inheritance in soybean

Kefeng No.1 and all F_1 plants of Kefeng No.1 × Nannong 1138-2 were resistant (symptomless) to SC8 and Nannong 1138-2 was susceptible (mosaic). Segregation of the F_2 and SP-F_2 population fit a 3R:1S ratio. The $F_{2:3}$ and $F_{7:11}$ RIL population segregated in a 1R:2seg.:S and 1R:1S ratio, respectively (Table 3). These results firmed that a dominant gene, designated as R_{SC8} controls Kefeng No.1 resistance to SC8.

R_{SC8} linkage mapping

Five SSR markers, BARCSOYSSR_02_0478, BARCSOYSSR_02_0555, BARCSOYSSR_02_0578, BARCSOYSSR_02_0596, and BARCSOYSSR_02_0727 (formerly Satt698, Satt558, Sat_254, Satt634, and Satt266) exhibited polymorphisms and R and S bulks between Kefeng No.1 and Nannong 1138-2. Therefore, these data were used to assess linkage with the resistance gene R_{SC8} based on 156 individuals from population I. The results linked R_{SC8} with the five SSR markers and mapped the gene to chromosome 2. SSR markers BARCSOYSSR_02_0478, BARCSOYSSR_02_0555, BARCSOYSSR_02_0578, and BARCSOYSSR_02_0596 were located on one side of R_{SC8} and BARCSOYSSR_02_0727 was positioned on the other side of the gene (Fig. 2a). BARCSOYSSR_02_0596 was the closest marker to R_{SC8}.

Four SSR markers (BARCSOYSSR_02_0478, BARCSOYSSR_02_0555, BARCSOYSSR_02_0578, and BARCSOYSSR_02_0596) identified two RHLs (RHL153-1 and RHL153-2) as heterozygous in the region adjacent to R_{SC8} (Table 1). Population III, derived from the two RHLs was used to refine the position of R_{SC8}. These results confirmed that four SSR markers were at one side of R_{SC8} (Fig. 2c). The distance between BARCSOYSSR_02_0596 and R_{SC8} was approximately 0.9 cM in population III. SSR marker BARCSOYSSR_02_0727 was homozygous with a resistant parent.

Polymorphisms were tested for 15 SNPs derived from the soybean transcription map (Choi et al. 2007) in the region of interest on chromosome 2. The results showed that only BARC-025183-06457 was polymorphic in the mapping population. Subsequent sequence analyses revealed that BARC-025183-06457 was an Indel (insertion/deletion, 16 bp) marker between resistant and susceptible parents. Primer pairs of the other 14 SNPs (Choi et al. 2007) were used to sequence Kefeng No.1 and Nannong 1138-2. Four SNPs and one Indel (2 bp) were identified between the two parents based on amino acid sequence alignment. Linkage analysis found that three SNP (Table 2) loci, putatively linked to R_{SC8} were also mapped using three populations (populations I, II, and III) (Fig. 3a–c).

One hundred and thirty-one genomic-SSR primers (Song et al. 2010) in the target region were identified to screen for polymorphisms between the resistant and susceptible parents. The results detected 33 polymorphic genomic-SSR markers, which were used to map R_{SC8} in populations I, II, and III (Fig. 3a–c). In population III, R_{SC8} was located on chromosome 2 with a distance of 0.1 cM from BARCSOYSSR_02_0610 and 0.3 cM from BARCSOYSSR_02_0616 on the other side. The interval was estimated to be 200 kb based on the soybean Williams82 (http://www.phytozome.net/soybean) genome sequence.

Candidate genes for R_{SC8} and QRT-PCR analysis

Williams82 (http://www.phytozome.net/soybean) sequence annotation database identified 17 putative genes in the

Table 3 Segregation of resistance to SMV strain SC8 in Kefeng No.1

Parent or progeny	No. of plant (line)				χ^2	*P*
	Total	R	Seg.	S		
Kefeng No.1 (P1)	20	20				
F_1 (Kefeng No.1 × Nannong 1138-2)	9	9				
Population I	156	114		42	0.31 (3:1)	0.58
$F_{2:3}$ (Kefeng No.1 × Nannong 1138-2)	100	27	52	21	0.88 (1:2:1)	0.64
Population II	184	89		95	0.20 (1:1)	0.66
Population III	181	130		51	0.97 (3:1)	0.32
Nannong 1138-2 (P2)	23			23		

R resistance, *Seg.* segregating, *S* susceptible

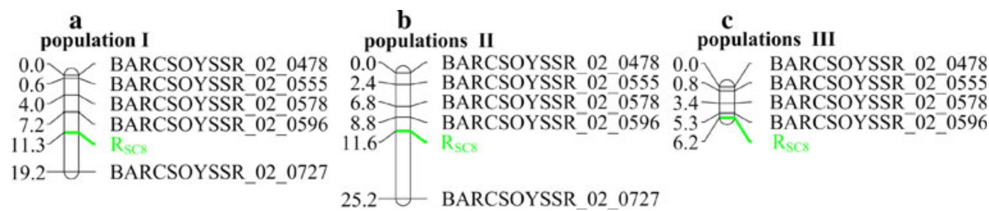

Fig. 2 Genetic linkage map constructed with SSR markers. **a** Map derived from the Kefeng No.1 × Nannong 1138-2 F_2 population. **b** Map derived from the Kefeng No.1 × Nannong 1138-2 RIL population. **c** Map derived from the Kefeng No.1 × Nannong 1138-2 SP-F_2 population. Genetic distance in centimorgans (cM) was calculated using the Kosambi function. The *green font* shows the position of the resistance gene R_{SC8} to SMV strain SC8

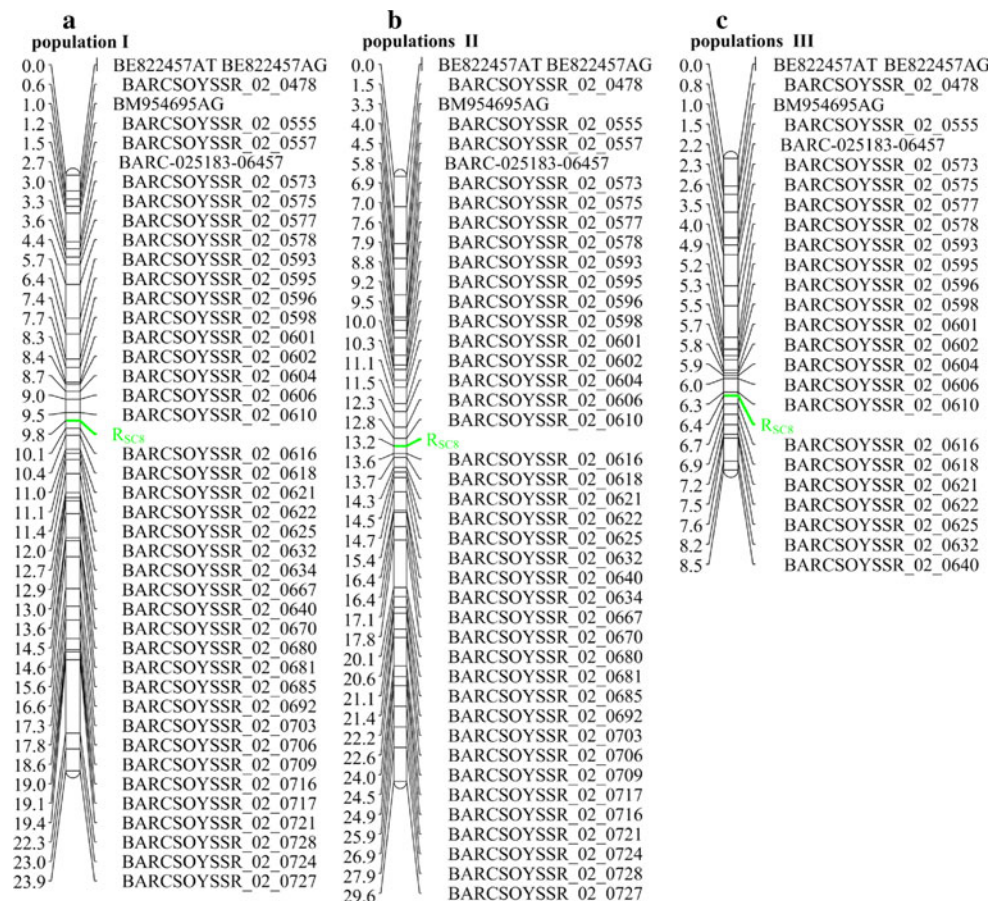

Fig. 3 Chromosome 2 genetic linkage map of the R_{SC8} locus. **a** Map derived from the Kefeng No.1 × Nannong 1138-2 F_2 population with 156 individuals. **b** Map derived from the Kefeng No.1 × Nannong 1138-2 RIL population with 184 lines. **c** Map derived from the Kefeng No.1 × Nannong 1138-2 SP-F_2 population with 181 individuals. Genetic distance in centimorgans (cM) was calculated using the Kosambi function. The *green font* shows the position of the resistance gene R_{SC8} to SMV strain SC8

200 kb target region (Table 4). Among them, the functions of two genes are unknown, three have no transcript, and the others are involved in disease defense, signal transduction, transcription, and metabolism (Table 4).

Based on the predicted functions, three disease defense, two transcription factors and two signal transduction genes were selected as the candidate resistance genes to SC8, and were further analyzed by QRT-PCR. The results showed low Glyma02g13430 expression levels. The other six genes (Glyma02g13310, 13320, 13400, 13450, 13460, and 13470) expressed normally and were further analyzed using QRT-PCR.

The expression level of each genotype and mock-inoculated treatments were compared at different times (0, 1, 2, 4, 8, 12, 24, and 48 hpi). Results detected significant differences in the expression levels between the resistant and susceptible parent for Glyma02g13310, 13320, 13400, 13460, and 13470 (Fig. 4).

Disease defense genes Glyma02g13310 and Glyma02g13320 had similar expression levels. The

Table 4 The 17 candidate genes on the R_{SC8} region of chromosome 2

Gene name[a]	Position[a]	Exon(s)	AA	Predicted function (BlastX)[b]	Functional classification
Glyma02g13310	11,567,483–11,574,109	9	440	Cytochrome P450 monooxygenase (*Pisum sativum*)	Disease/defense
Glyma02g13320	11,590,875–11,593,592	1	906	LRR receptor protein kinase (*Arabidopsis thaliana*)	Disease/defense
Glyma02g13330	11,601,951–11,605,281	6	395	Reversibly glycosylated polypeptide (*Oryza sativa*)	Metabolism
Glyma02g13340	11,617,438–11,619,983	5	364	SAM transferase-like protein (*Glycine max*)	Metabolism
Glyma02g13350	11,646,986–11,648,684	5	160	YABBY protein (*Arabidopsis thaliana*)	Transcription
Glyma02g13360	11,651,991–11,661,761	13	1,387	Unknown protein (*Arabidopsis thaliana*)	Unknown
Glyma02g13370	11,671,023–11,677,144	7	512	SBP DNA-binding protein (*Arabidopsis thaliana*)	Transcription
Glyma02g13380	11,692,937–11,694,242	2	355	Unknown protein (*Arabidopsis thaliana*)	Unknown
Glyma02g13390	11,730,022–11,730,199	–	–	No transcript	No transcript
Glyma02g13400	11,734,620–11,736,851	3	77	MADS box protein (*Glycine max*)	Transcription
Glyma02g13410	11,746,279–11,746,718	–	–	No transcript	No transcript
Glyma02g13420	11,749,041–11,752,996	9	243	MADS box transcription factor (*Pisum sativum*)	Transcription
Glyma02g13430	11,757,828–11,758,642	3	87	Ring finger containing protein (*Arabidopsis thaliana*)	Transcription
Glyma02g13440	11,767,761–11,768,963	–	–	No transcript	No transcript
Glyma02g13450	11,770,447–11,771,944	5	237	USP family protein (*Arabidopsis thaliana*)	Disease/defense
Glyma02g13460	11,776,527–11,778,734	1	736	Serine/threonine protein kinase (*Arabidopsis thaliana*)	Signal transduction
Glyma02g13470	11,779,801–11,782,246	1	814	Serine/threonine protein kinase (*Arabidopsis thaliana*)	Signal transduction

[a] From http://www.phytozome.net/soybean

[b] Predicated functions were obtained using BlastX (http://blast.ncbi.nlm.nih.gov/Blast.cgi) based on the predicted genes sequences

up-regulated expression level was higher in the resistant parent than in the susceptible parent at 4, 8, and 12 hpi. For most hpi, no significant difference was detected in the expression level for the disease defense gene Glyma02g13450 between resistant and susceptible parents.

The expression level of gene Glyma02g13400 was significantly up-regulated at 1 and 2 hpi in the resistant parent. In the susceptible parent, the gene expression was only up-regulated at 2 hpi compared with the control (Fig. 4).

Glyma02g13460 and Glyma02g13470 were down-regulated in the resistant parent at five of the seven hpi times, and no significant difference was found in the susceptible parent at most hpi time periods (Fig. 4). These results suggested that the expression level of five of the six genes, i.e., Glyma02g13310, 13320, 13400, 13460, and 13470 was regulated by SMV infection and could be involved in disease defense mechanisms.

Discussion

Fine mapping using RHLs

In this study, two RHLs (RHL153-1 and RHL153-2) from a RIL population consisting of 184 lines were used to fine map the SMV resistance locus R_{SC8}. The RHLs were heterozygous at the R_{SC8} resistance locus. Most of the other loci were homozygous and integrated into the population. When mapping *Flwdt7* conferring days to flowering with the RHL population, Su et al. (2010) also mixed RH79-1, RH79-3, and RH79-4 into a population, RH79-m.

Comparison between different maps targeting the resistance gene

In this study, we completed three high-resolution maps for the R_{SC8} resistance gene. The three linkage maps covered 8.5–29.6 cM in length and the average distance between markers was 0.3–0.7 cM. There were slight differences in marker order and interval size among the three maps. This can be explained by differences in mapping population size and type.

Our high-resolution genetic map will facilitate MAS for R_{SC8} in cultivar breeding programs. Because of rapid changes in predominant virus isolates in nature (Choi et al. 2005; Gagarinova et al. 2008), single-gene resistance in a cultivar may become ineffective soon after it is released. Stacking two or more genes in one cultivar can enhance durability and the level of SMV resistance (Saghai Maroof et al. 2008; Shi et al. 2009).

The relationship of R_{SC8} and *Rsv4*

In this study, the resistant gene R_{SC8} was found to be linked to the SSR markers BARCSOYSSR_02_0478, BARCSOY

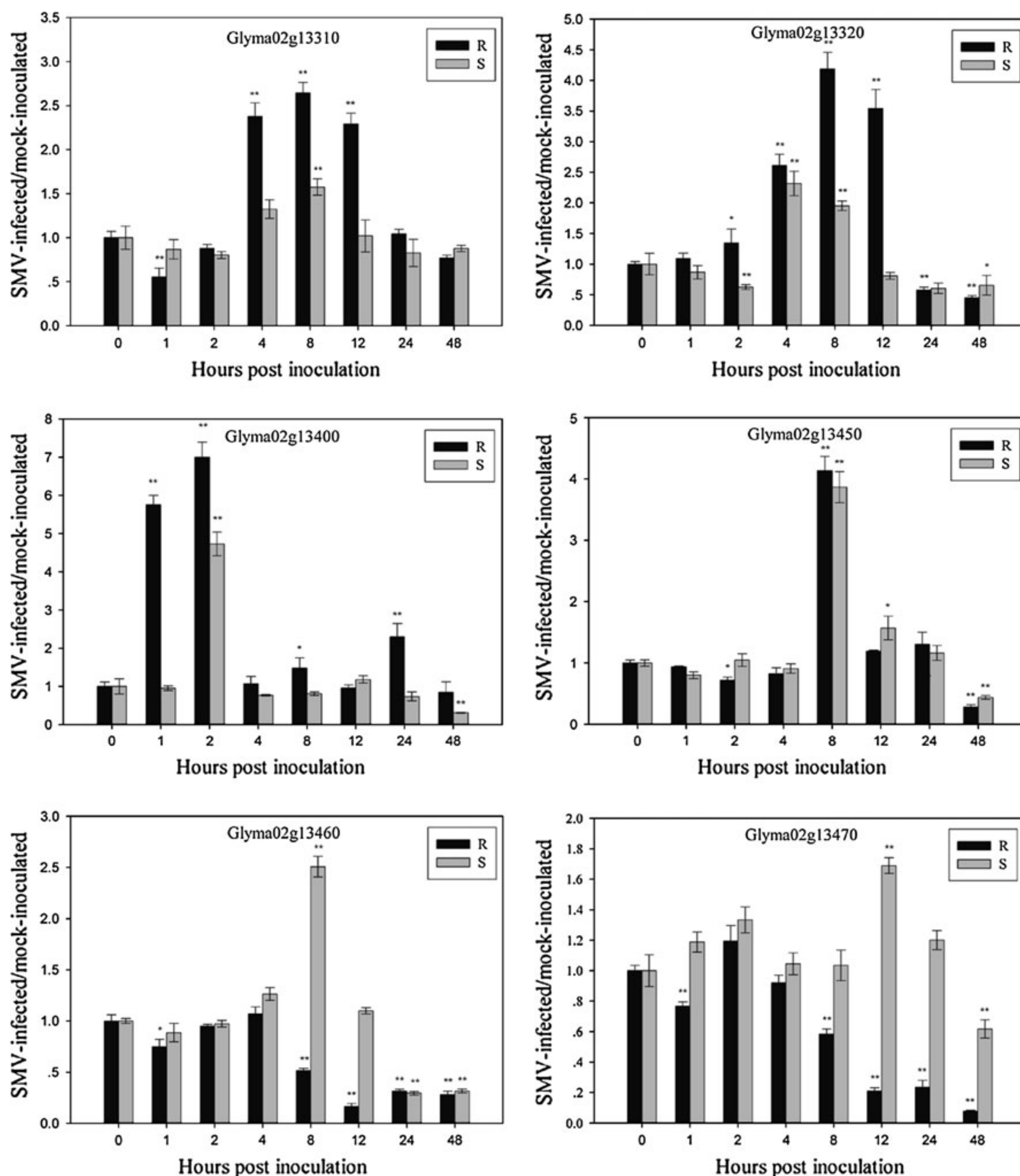

Fig. 4 Expression analysis of six candidate genes for R_{SC8} by QRT-PCR. Y-axes indicate the ratio of mRNA between samples infected with SMV and inoculated with phosphate buffer at different time points (0–48 hpi). *R* the ratios of mRNA of resistant cultivar Kefeng No.1 inoculated with SMV and phosphate buffer. *S* the ratios of mRNA of susceptible cultivar Nannong 1138-2 inoculated with SMV and phosphate buffer. *Asterisks* represented statistical significance (*$P < 0.05$; **$P < 0.01$)

SSR_02_0555, BARCSOYSSR_02_0578, BARCSOYSSR_02_0596, and BARCSOYSSR_02_0727 and was further mapped to an interval (between BARCSOYSSR_02_0606 and BARCSOYSSR_02_0618) of 200 kb from 11,573,385 to 11,791,629 on soybean chromosome 2 (http://www.phytozome.net/soybean) based on SNP and genomic-SSR markers. Hayes et al. (2000a) mapped *Rsv4* to chromosome 2 between SSR markers BARCSOYSSR_02_0555 and BARCSOYSSR_02_0676 (formerly Satt542). Hwang et al. (2006) employed a comparative genomics approach further to map *Rsv4* between the two markers BARCSOYSSR_02_0596 and AW471852R. Saghai Maroof et al. (2010) mapped of *Rsv4* to chromosome 2 in a physical interval of less than 100 kb from 11,651,991 to 11,771,944 using the whole-genome sequence of soybean.

The resistance genes R_{SC8} and *Rsv4* linked with SSR markers BARCSOYSSR_02_0555, BARCSOYSSR_02_0596, and BARCSOYSSR_02_0727. They were mapped to the same interval from 11,573,385 to 11,791,629 on soybean chromosome 2. It is possible that *Rsv4* and R_{SC8} share the same or tightly linked resistance loci.

Candidate genes for R_{SC8} in soybean

The cytochrome P450 monooxygenase gene Glyma02g13310 and the LRR receptor protein kinase gene Glyma02g13320 were up-regulated to a higher level in R than in S genotypes at 4, 8, and 12 hpi. Ohkawa et al. (1998) reported that cytochrome P450 monooxygenase plays important roles in preventing the potentially harmful effects imposed on plants by stress and pathogens. It has been suggested the P450 super family was associated with SMV resistance (Cheng et al. 2010, Yang et al. 2010). Hulbert et al. (2001) reported the LRR domains are involved in the interaction with effector proteins and are the major determinant of resistance specificity. LRR domain exchange experiments between Cf-4 and Cf-9 provide strong evidence that the LRR domain is critical in distinguishing resistance specificity (Van et al. 2001). Babu et al. (2008) reported LRR protein kinase shared defense and stress response in plants to single-stranded, positive-sense RNA virus infections.

Glyma02g13400 is a transcription factor of the MADS box protein family with a possible coiled-coil (CC) structure (Lupas et al. 1991). The CC domains play an important role in R-*Avr* specific recognition to activate downstream defense signaling (Shirasu and Schulze Lefert 2003). Ade et al.(2007) confirmed that the CC domain was required for activation of *RPS5* in transgenic tobacco. In the present study, the gene was significantly up-regulated at 1 and 2 hpi in the resistant parent. Therefore, we suggest the gene can regulate plant response to SMV in a short period of time.

Glyma02g13460 and Glyma02g13470 are serine/threonine protein kinase genes, which are related to signal transduction. Serine/threonine protein kinases play key roles in cell to cell recognition processes during defense against pathogens (Martin et al. 1993). In previous studies, Xa21 and Xa26 were characterized as two serine/threonine protein kinases with a specific ligand-binding domain LRR at the N-terminus, which conferred resistance to *Xanthomonas oryzae* pv. *oryzae* (Song et al. 1995; Sun et al. 2004). In the present work, the level of expression in the two genes for the resistant parent was down-regulated at 8 hpi; it is may be that down-regulating gene expression transfers a viral invasion signal that activates resistance.

Disease resistance mechanisms are very complex processes controlled by a network of genes. Therefore, the function of the five candidate genes identified in this study and the processes of host-pathogen interactions requires further investigation.

We identified SNP and genomic-SSR markers tightly linked with R_{SC8}. R_{SC8} was narrowed down to an interval on chromosome 2, which spanned 200 kb. The bioinformatics analysis and QRT-PCR indicated that five candidate genes are likely correlated with activating plant defense pathways in response to SMV infection. These findings will provide important information for map-based cloning of R_{SC8}.

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