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ORIGINAL PAPER

Genetic analysis and mapping of genes for resistance to multiple strains of Soybean mosaic virus in a single resistant soybean accession PI 96983

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Abstract Soybean mosaic virus (SMV) is one of the most broadly distributed soybean (*Glycine max* (L.) Merr.) diseases and causes severe yield loss and seed quality deficiency. Multiple studies have proved that a single dominant gene can confer resistance to several SMV strains. Plant introduction (PI) 96983 has been reported to contain SMV resistance genes (e.g., *Rsv1* and *Rsc14*) on chromosome 13. The objective of this study was to delineate the genetics of resistance to SMV in PI 96983 and determine whether one gene can control resistance to more than one Chinese SMV strain. In this study, PI 96983 was identified as resistant and Nannong 1138-2 was identified as susceptible to four SMV strains SC3, SC6, SC7, and SC17. Genetic maps based on 783 F_2 individuals from the cross of PI 96983 × Nannong 1138-2 showed that the gene(s) conferring resistance to SC3, SC6, and SC17 were between SSR markers BARCSOYSSR_13_1114 and BARCSOYSSR_13_1136, whereas SC7 was between markers BARCSOYSSR_13_1140 and BARCSOYSSR_13_1185. The physical map based on 58 recombinant lines confirmed these results. The resistance gene for SC7 was positioned between BARCSOYSSR_13_1140 and BARCSOYSSR_13_1155, while the resistance gene(s) for SC3, SC6, and SC17 were between BARCSOYSSR_13_1128 and BARCSOYSSR_13_1136. We concluded that, there were two dominant resistance genes flanking *Rsv1* or one of them at the reported genomic location of *Rsv1*. One of them (designated as "*Rsc-pm*") conditions resistance for SC3, SC6, and SC17 and another (designated as "*Rsc-ps*") confers resistance for SC7. The two tightly linked genes identified in this study would be helpful to cloning of resistance genes and breeding of multiple resistances soybean cultivars to SMV through marker-assisted selection (MAS).

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Introduction

Soybean mosaic virus (SMV) is one of the most destructive viral pathogens in soybeans and causes significant yield loss and heavy deficiencies in seed quality. This has led to considerable research. In the USA, SMV was classified into seven strains (G1–G7) (Cho and Goodman 1979, 1982). This classification has recently been updated (Chen and Choi 2007). In China, SMV has now been grouped into 21 strains (SC1–SC21) (Wang et al. 2003; Guo et al. 2005; Li et al. 2010).

Three independent single dominant SMV resistance gene loci were first identified in PI 96983(*Rsv1*), L29 (*Rsv3)* and V94-5152 (*Rsv4*), and mapped to soybean chromosomes 13, 14, and 2, respectively (Yu et al. 1994; Hayes et al. 2000; Hayes and Saghai Maroof 2000; Gore

et al. 2002; Jeong and Saghai Maroof 2004; Saghai Maroof et al. 2010). In China, although most resistance genes to different SMV strains have been positioned on soybean chromosomes 2, 13, and 14, the gene for resistance to SC15 was mapped to chromosome 6 (Yang et al. 2010). Researchers found that a cluster of disease resistance (R) genes existed in the vicinity of the *Rsv1* locus (Jeong et al. 2002) and several candidate R genes belonging to the nucleotide binding site/leucine-rich repeat family might confer resistance to most of the seven SMV strains (G1–G7) (Hayes et al. 2004).

Breeding for resistance to SMV by traditional methods is a time consuming process because of the requirement to inoculate plants and assess disease response phenotypes. Molecular marker-assisted selection has proven to be a highly efficient strategy for selecting resistant lines (Mudge et al. 1997; Cregan et al. 1999; Meng et al. 2003). Recently, high density simple sequence repeat (SSR) markers were developed based on the whole-genome sequence of soybeans (Song et al. 2010). Some of these markers that are tightly linked to R genes have been used to develop soybean lines resistant to SMV (Ma et al. 2010).

Plant introduction (PI) 96983 was described as being susceptible to strain G7, but resistant to all other North American strains of SMV that had been described (Cho and Goodman 1982). Kiihl and Hartwig (1979) observed that PI 96983 contained a single dominant gene *Rsv* (now *Rsv1*) that regulated SMV resistance. Yu et al. (1994) identified three markers, SM176, pA186, and pK644a, which were closely linked to *Rsv1*, with a genetic distance of 0.5, 1.5, and 2.1 cM, respectively, in a population derived from a cross between the resistant parent PI 96983, and the susceptible parent Lee 68 Gore et al. (2002) mapped more than 20 markers to a 6.8 cM region around *Rsv1* in a PI 96983 × Lee 68 population. Li et al. (2006) identified a gene *Rsc14* conferring resistance to Chinese SMV strain SC14 and found five SSR markers in chromosome 13 were closely linked to *Rsc14* in PI 96983.

The objective of this research was to study the inheritance of resistance to four Chinese SMV strains, and to

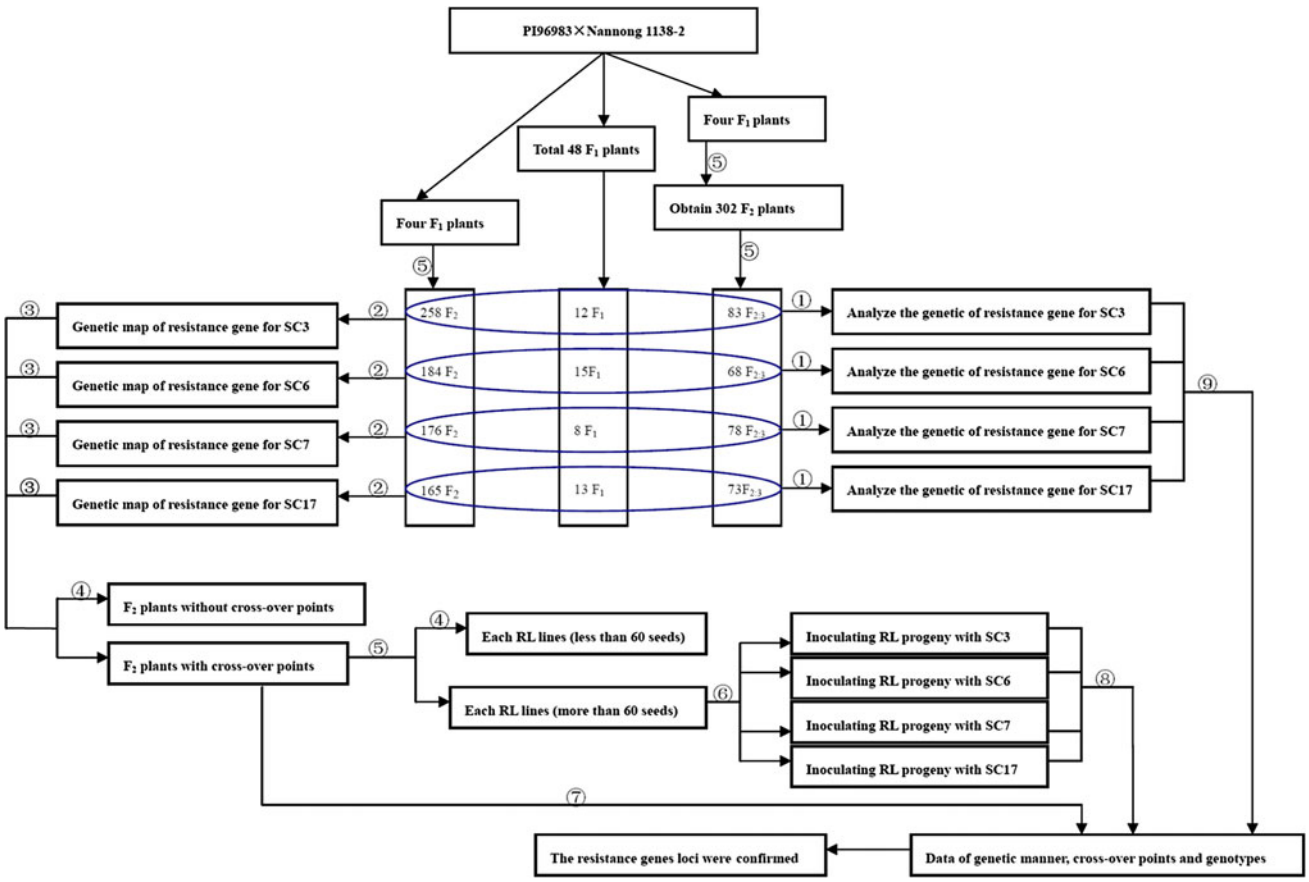


Fig. 1 The flow chart of source of materials and procedure of mapping the resistance genes to Soybean mosaic virus (SMV) strains SC3, SC6, SC7, and SC17. ① Plants were grown in aphid-greenhouse/net-house and inoculated with specific SMV strain ② Scoring six SSR markers on the F_2, ③ Screening of cross-over points with the six SSR marker. ④ Remove. ⑤ Self-breeding and harvested individually. ⑥ each $F_{2:3}$ line was divided into four parts. ⑦ Screening with 23 SSR markers. ⑧ Genotypes of the resistance genes were determined by inoculating $F_{2:3}$ progeny with the specific SMV strains. ⑨ The genetic manner of the resistance genes was determined

map the resistance genes in PI 96983 that confer resistance to the four strains in a PI 96983 × Nannong 1138-2 population. The results showed that two tightly linked loci in PI 96983 control the resistance to the four Chinese SMV strains.

Materials and methods

SMV strains, plant materials, and strategy of mapping

SMV strains SC3, SC6, SC7, SC17, and soybean accessions PI 96983 and cultivar Nannong 1138-2 were provided by the National Center for Soybean Improvement, Nanjing Agricultural University. SC3, SC7, and SC17 are prevalent in the Huang-Huai and Changjiang Valleys, while SC6 is prevalent in the north of China (Guo et al. 2005; Wang et al. 2005; Li et al. 2010).

The flow chart of source of materials and procedure of mapping the resistance genes to SMV strains SC3, SC6, SC7, and SC17 are illustrated in Fig. 1. A total of 48 F_1, 783 F_2 plants, and 302 $F_{2:3}$ lines derived from the cross of PI 969863 × Nannong 1138-2 was divided into four parts and used in genetic analysis and mapping of resistance genes for SMV strains SC3, SC6, SC7, and SC17, respectively.

Fifty-eight F_2 plants with cross-over events between two of the six markers flanking *Rsv1* and *Rsc14* were identified from the 783 F_2 plants and then self-bred to form 58 $F_{2:3}$ recombinant lines (RLs). Seedlings from each RL were divided into four groups and each was inoculated with one of the four SMV strains, respectively. If any RLs had identical cross-over points and showed similar reactions to the four SMV strains, they were classified into the same RL.

Resistance identification and genetic analysis

PI 969863, Nannong 1138-2 and their F_1, F_2, and $F_{2:3}$ lines were planted in an aphid-free greenhouse and inoculated

Table 1 Genetic analysis of resistance to SMV strains SC3[a], SC6, SC7, and SC17

Strains	Parent or progeny	No. of plant (lines)			Total	Expected ratio	χ_c^{2b}	*P*
		Resistant	Segregate	Susceptible				
SC3	PI 96983 (P_1)	23		0	23			
	F_1	12 (10R + 2 N)		0	12			
	F_2	186 (161R + 25 N)		72	258	3 (R + N):1S	1.013	0.314
	$F_{2:3}$	22 [20 (all R) + 2 (R + N)]	43 {38 [3R:1S] + 5 [3 (R + N):1S]}	18	83	1 (R + N):2[3 (R + N):1S]:1S	0.494	0.781
	Nannong 1138-2 (P_2)	0		18	18			
SC6	PI 96983 (P_1)	15		0	15			
	F_1	15		0	15			
	F_2	142		42	184	3R:1S	0.355	0.551
	$F_{2:3}$	14	41	13	68	1R:2Heterozygous:1S	2.912	0.233
	Nannong 1138-2 (P_2)	0		22	22			
SC7	PI 96983 (P_1)	18		0	18			
	F_1	8		0	8			
	F_2	129		47	176	3R:1S	0.189	0.663
	$F_{2:3}$	15	47	16	78	1R:2Heterozygous:1S	3.308	0.191
	Nannong 1138-2 (P_2)	0		12	12			
SC17	PI 96983 (P_1)	25		0	25			
	F_1	13		0	13			
	F_2	120		45	165	3R:1S	0.341	0.559
	$F_{2:3}$	20	38	15	73	1R:2Heterozygous:1S	0.808	0.668
	Nannong 1138-2 (P_2)	0		21	21			

[a] SMV strains SC3, SC6, SC7, and SC17 were identified by Li et al. (2010)

[b] The Yates correction factor c = 1/2

with SMV when the unifoliolate leaves began to unfold. SMV inoculum for each strain and F_2 population was homogenized in a sterile, ice-chilled mortar and pestle in 3–5 ml of 0.01 M phosphate buffer (mixture of sodium phosphate and potassium phosphate, pH 7.4). Plants inoculated with 0.01 M phosphate buffer were used as negative controls. For each F_2 population inoculated with a specific SMV strain, the observations were made 1 week after the inoculations, and then reactions to each SMV strain were evaluated at 3-day interval for 3 weeks. Plant reactions to the strains were classified into three categories (Chen et al. 1991; Liao et al. 2011). Resistant (*R*): no symptoms or local necrotic lesions on inoculated leaves; susceptible (*S*): typical mosaic; and necrotic (*N*): systemic necrosis or stem tip necrosis. In Chi-squared (χ^2) test, necrotic was deemed as resistant. χ^2 analyses according to Hatch and Lazaraton (1991) were performed to test for goodness-of-fit of the observed vs. expected segregation ratios. $\chi^2_c = \sum [(|E_O - E| - 0.5)/E]^2$, *Eo* and *E* are the observed and expected frequencies, respectively. When the degree of freedom was 1, the Yates correction factor (Yates 1934) was applied in the χ^2 calculation. "Pymetrozine" was diluted 2,500 times and sprayed to avoid cross infection by aphids. The leaves were collected and the plants were individually harvested for subsequent resistance identification and analyses.

DNA extraction and SSR marker analysis

DNA was extracted from soybean leaves using the cetyl trimethyl ammonium bromide method (Saghai Maroof et al. 1984). PI 96983 has been reported to contain *Rsv1* and *Rsc14* SMV resistance genes on chromosomes 13. The integrated soybean genetic linkage map (Song et al. 2004) indicated that Sct_033 and Satt334 flank *Rsv1* and *Rsc14* (Cregan et al. 1999; Gore et al. 2002; Li et al. 2006). Thus, 133 soybean SSR markers between Satt334 (BARCSOYSSR_13_1098) and Sct-033 (BARCSOYSSR_13_1230) were selected from the BARCSOYSSR_1.0 database developed by Song et al. (2010), and their polymorphisms between PI 96983 and Nannong 1138-2 were evaluated. Polymorphic markers were further used for genotyping of the progenies. PCR was conducted according to Li et al. (2006) with minor modifications. SSR primers were synthesized by Invitrogen Biotech Ltd. Co. (Shanghai, China).

Linkage analysis and fine mapping resistance genes

The scores of six SSRs markers and the SMV disease reaction of the 783 F_2 plants were used in calculating genetic distances between the markers and the resistance genes by JoinMap3.0 software (Van Ooijen and Voorrips

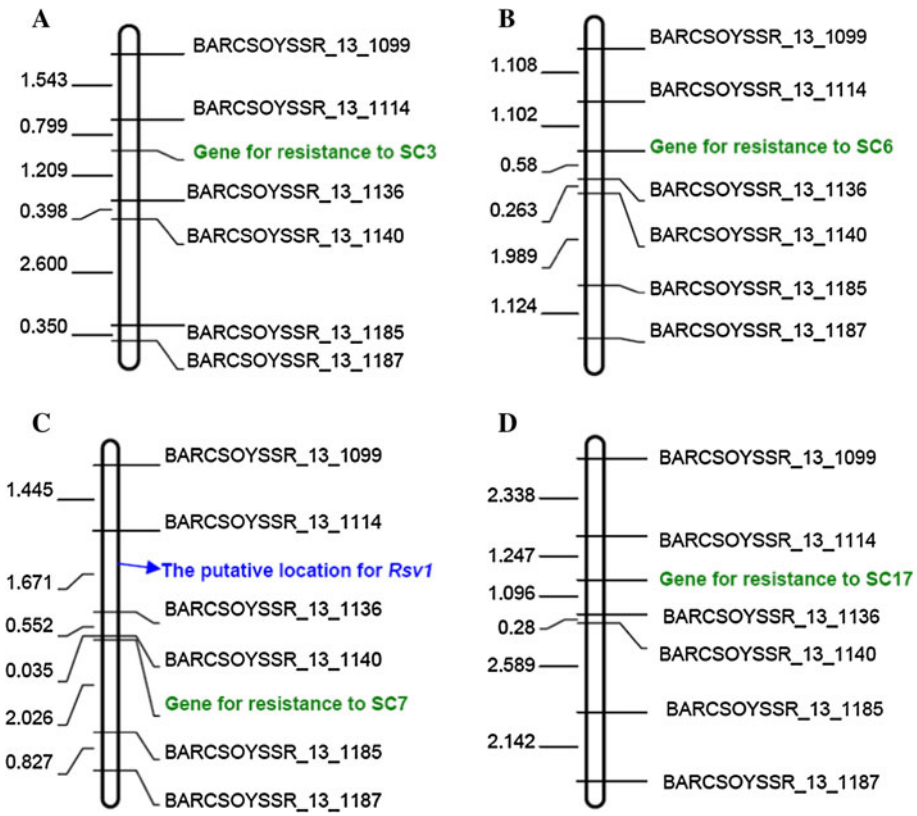


Fig. 2 Genetic linkage map of SSR markers and genes resistant to Soybean mosaic virus (SMV) strains SC3, SC6, SC7, and SC17. **a** Linkage map derived from the 258 F_2 plants inoculated with SMV strain SC3. **b** Linkage map derived from the 184 F_2 plants inoculated with SMV strain SC6. **c** Linkage map derived from the 176 F_2 plants inoculated with SMV strain SC7 and the putative location for *Rsv1* (Gore et al. 2002). **d** Linkage map derived from the 165 F_2 plants inoculated with SMV strain SC17. Genetic distance in centimorgans (cM) was calculated using the Kosambi function

2001) using the Kosambi function (Kosambi 1944). The linkage maps were drawn using Map Draw (Liu and Meng 2003). Twenty-three SSR markers with polymorphisms between the parents were used in identifying the cross-over points in the 58 F_2 individuals which were then used to make the 58 $F_{2:3}$ recombinant lines (RLs). Genotypes of the F_2 plants were determined by corresponding RLs inoculated with the specific SMV strains. After determining the response to specific SMV strains, the resistance gene loci were inferred according to cross-over points and genotypes.

Results

Inheritance of resistance to SC3, SC6, SC7, and SC17

The results (Table 1) indicated that after inoculation with strains SC3, SC6, SC7, and SC17, the resistant parent (PI 96983) showed asymptomatic and the susceptible parent (Nannong 1138-2) developed a typical mosaic pattern. About 10 % progeny of the cross between PI 96983 and Nannong 1138-2 produced systemic necrosis after inoculation with strain SC3. However, no systemic necrosis was detected in the progeny after inoculation with strains SC6, SC7, and SC17 including 36 F_1 plants. However, two of 12 F_1 plants showed systemic necrosis after inoculation with SC3 but other 10 F_1 plants showed no symptoms. The phenotypic data from F_2 and $F_{2:3}$ plants infected by SC6, SC7, and SC17 showed a satisfactory fit ($P > 0.05$) to segregation ratios of 3R:1S and 1R:2Heterozygous:1S. For the F_2 and $F_{2:3}$ plants inoculated with SC3, the plants exhibiting necrosis were considered resistant and included in the resistant class. For both the F_2 and $F_{2:3}$ plants inoculated with SC3 the data fit ($P > 0.05$) the expected segregation ratios of 3:1 (F_2) and 1:2:1 ($F_{2:3}$). These segregation ratios indicated that the resistance of PI 96983 to each of the four SMV strains was controlled by a dominant gene(s).

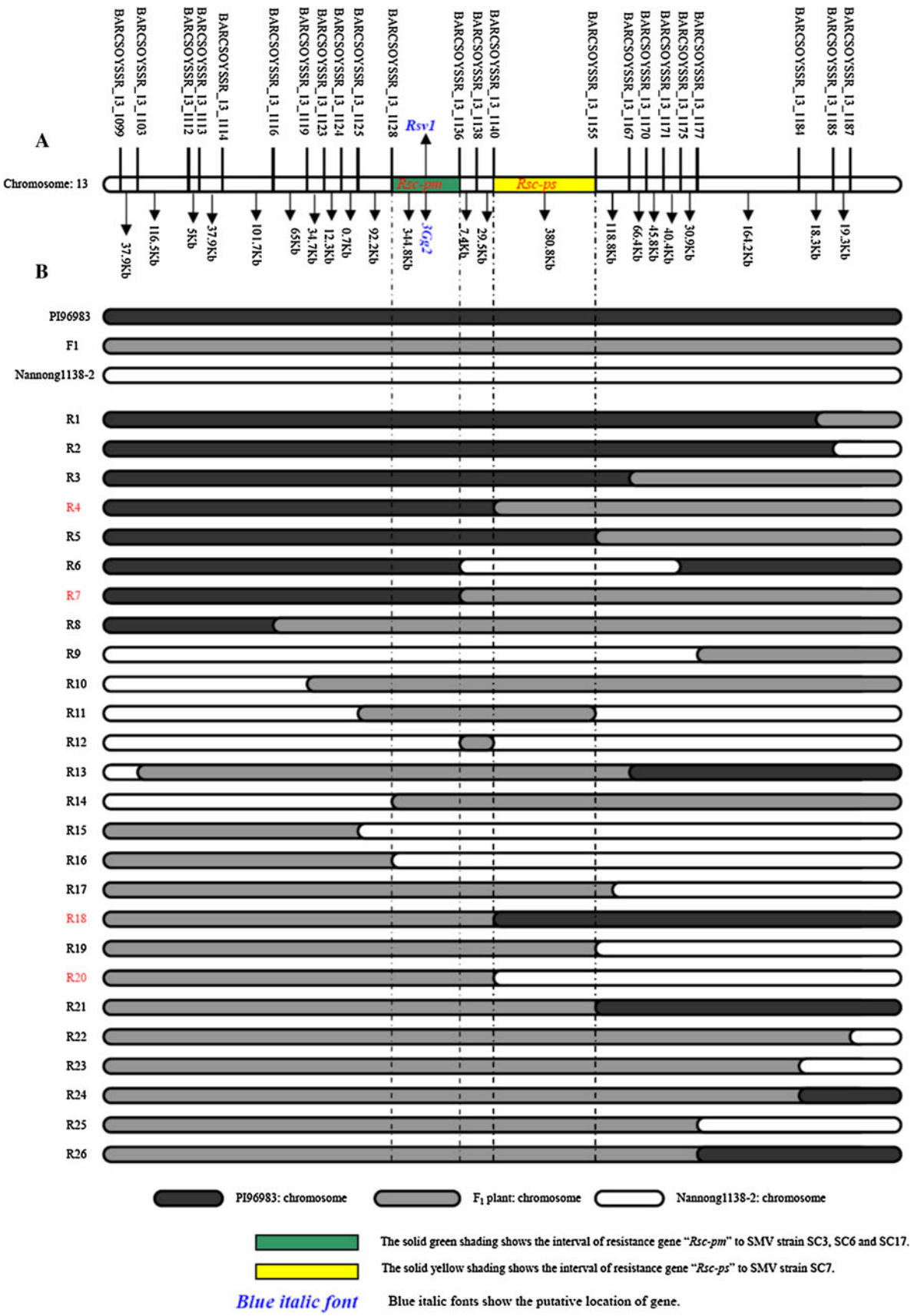
Mapping genes for resistance to the four SMV strains with SSR markers

Four F_2 populations with 258, 184, 176, and 165 plants were used for the mapping of the resistance gene(s). Of the 23 SSR markers polymorphic between PI 96983 and Nannong 1138-2, six markers, i.e., BARCSOYSSR_13_1099, BARCSOYSSR_13_1114, BARCSOYSSR_13_1136, BARCSOYSSR_13_1140, BARCSOYSSR_13_1185 and BARCSOYSSR_13_1187 that flanked *Rsv1* and *Rsc14* were used to examine for their linkage with the gene(s) for resistance to the four SMV strains. The results showed that the six SSR markers were linked to gene(s) resistant to SC3 (Fig. 2a),

Table 2 The disease reaction of R1–R26 to four SMV strains

Strain	Control		Disease reaction of RLs																							
	P_1	F_2	P_2	R1	R2	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26
SC3	R	Seg	S	R	R	R	R	R	R	Seg	R	Seg	S	S	Seg	Seg	S	S	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg
SC6	R	Seg	S	R	R	R	R	R	R	Seg	R	Seg	S	S	Seg	Seg	S	S	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg
SC7	R	Seg	S	R	R	Seg	R	R	Seg	Seg	R	Seg	S	S	Seg	Seg	S	S	Seg	R	Seg	S	Seg	Seg	Seg	Seg	Seg	Seg
SC17	R	Seg	S	R	R	R	R	R	R	Seg	R	Seg	S	S	Seg	Seg	S	S	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg	Seg

P_1 PI 96983, *P_2* Nannong 1138-2, *R*, *S*, and *Seg* homozygous resistant, homozygous susceptible, and heterozygous resistant, respectively


A
Chromosome: 13
BARCSOYSSR_13_1099
BARCSOYSSR_13_1103
BARCSOYSSR_13_1112
BARCSOYSSR_13_1113
BARCSOYSSR_13_1114
BARCSOYSSR_13_1116
BARCSOYSSR_13_1119
BARCSOYSSR_13_1123
BARCSOYSSR_13_1124
BARCSOYSSR_13_1125
BARCSOYSSR_13_1128
BARCSOYSSR_13_1136
BARCSOYSSR_13_1138
BARCSOYSSR_13_1140
BARCSOYSSR_13_1155
BARCSOYSSR_13_1167
BARCSOYSSR_13_1170
BARCSOYSSR_13_1171
BARCSOYSSR_13_1175
BARCSOYSSR_13_1177
BARCSOYSSR_13_1184
BARCSOYSSR_13_1185
BARCSOYSSR_13_1187
Rsv1
Rsc-pm
Rsc-ps
37.9Kb
116.5Kb
5Kb
37.9Kb
101.7Kb
65Kb
34.7Kb
12.3Kb
0.7Kb
92.2Kb
344.8Kb
3Gp2
7.4Kb
29.5Kb
380.8Kb
118.8Kb
66.4Kb
45.8Kb
40.4Kb
30.9Kb
164.2Kb
18.3Kb
19.3Kb
B
PI96983
F1
Nannong1138-2
R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
PI96983: chromosome
F1 plant: chromosome
Nannong1138-2: chromosome
The solid green shading shows the interval of resistance gene "Rsc-pm" to SMV strain SC3, SC6 and SC17.
The solid yellow shading shows the interval of resistance gene "Rsc-ps" to SMV strain SC7.
Blue italic font
Blue italic fonts show the putative location of gene.

◄ **Fig. 3** Physical map and cross-over points of the 58 recombinant lines (RLs). **a** Physical positions of the *Rsc-pm* and *Rsc-ps* loci and SSR markers along Gm13, *Rsc-pm* and *Rsc-ps* were resistant to strains SC3, SC6, SC17, and SC7, respectively. The physical distances were calculated based on the positions of the SSR markers, which were developed by Song et al. (2010). **b** Schematic diagram showing the possible 26 cross-over events occurring in the 58RLs

SC6 (Fig. 2b), SC7 (Fig. 2c), and SC17 (Fig. 2d). Resistance gene(s) to SMV strains SC3, SC6, and SC17 were mapped between BARCSOYSSR_13_1114 and BARCSOYSSR_13_1136 (Fig. 2a, b, d), whereas the resistance gene to SC7 mapped lower on the chromosome between BARCSOYSSR_13_1140 and BARCSOYSSR_13_1185 (Fig. 2c). The genetic maps suggested that gene(s) that controlled the resistance to the SMV strains SC3, SC6, and SC17 might be clustered or might be a single gene, and there was likely another gene controlling the resistance to SMV strain SC7. As the sample sizes of four populations were not large enough for fine mapping, the results needed further confirmation. Hence, 58 F_2 plants which had cross-over events between the SSR markers BARCSOYSSR_13_1099 and BARCSOYSSR_13_1187 were selected from an F_2 population and self-pollinated to harvest recombinant lines (RLs) for further genetic analysis and physical fine mapping.

Fine mapping of genes conferring resistance to four SMV strains

The 58 F_2 plants with a cross-over event were identified using 23 SSRs flanking the resistance genes. The $F_{2:3}$ lines derived from the F_2 plants were inoculated with SC3, SC6, SC7, and SC17. Based on the SSR marker data and the phenotypic data, the 58 RLs were classified into 26 categories based on similarity and designated as R1–R26 (Fig. 3 and Table 2). The results showed that R4 and R7 were homozygous resistant to SC3, SC6, and SC17, but heterozygous resistant to SC7 (Fig. 3 and Table 2). Additionally, R18 was homozygous resistant to SC7 and R20 was homozygous susceptible to SC7, but both R18 and R20 were heterozygous resistant to SC3, SC6, and SC17 (Fig. 3 and Table 2). The genetic analysis of these four RLs indicated that resistance to SC7 was not allelic with resistance to SC3, SC6, and SC17. The marker data and resistance phenotypes of R5, R19, R20, and R21 suggested that the gene for resistance to SC7 was between BARCSOYSSR_13_1140 and BARCSOYSSR_13_1155, which verified the genetic map in Fig. 2c. Similarly, R7, R15, and R16 strongly suggested that the locus of resistance to SC3, SC6, and SC17 was between BARCSOYSSR_13_1128 and BARCSOYSSR_13_1136. In the 58 RLs, resistance to SC3, SC6, and SC17 co-segregated. Genetic and molecular marker analysis of F_1, F_2, and $F_{2:3}$ RL plants and populations indicated that there were two genes near *Rsv1*, one controlling resistance to SMV strains SC3, SC6, and SC17 which we designated as *"Rsc-pm"* and the other controlling the resistance to SC7 which we designated as *"Rsc-ps"*. Where "*R*" refers to resistance, "*sc*" refers to SMV, "*p*" indicates PI 96983, and "*m*" and "*s*" refer to resistance to multiple strains (SC3, SC6, and SC17) or a single strain (SC7), respectively. The designations simplify reference and further discussion.

Other genes affecting SMV resistance

Results of the resistance indentation (Table 2) showed all R6 and R9 plants were resistant to the four SMV strains, which indicated that their corresponding F_2 were homozygously resistant at the mapped loci *Rsc-pm* and *Rsc-ps*. However, marker data (Fig. 3b) revealed that the corresponding F_2 of R6 was homozygous susceptible at the *Rsc-ps* locus and the corresponding F_2 of R9 was homozygous susceptible at the two loci. Similarly, the corresponding F_2 of R11 was heterozygous for both the *Rsc-pm* and *Rsc-ps* loci, but all R11 plants were susceptible to all four SMV strains. The phenotypes of R6, R9 and R11 seemed to contradict their corresponding F_2 marker data, which indicated that there may be other genes affecting SMV resistance.

Discussion

Systemic necrosis in the progeny of PI 96983

Kiihl and Hartwig (1979) found that segregation occurred in the progeny of necrotic F_2 plants after inoculation with SMV strain SMV-1 and that heterozygous plants produced systemic necrosis. In this study of the four SMV strains, only SC3 induced systemic necrosis in the progeny of PI 96983. However, only two of the 12 F_1 plants evaluated produced systemic necrosis after inoculation with SC3. Additionally, only 25 of 258 F_2 individual plants showed systemic necrotic after the inoculation with SC3. Chen et al. (1991) also found that crosses from PI 96983 and susceptible parents always have a low frequency of necrosis plants, but that crosses from the resistance genotype "Kwanggyo" usually have a high frequency. It appears that systemic necrotic expression can vary among resistance genotypes. In summary, systemic necrosis is a complex phenotype, which can be influenced by SMV strains, resistance genes, and the environment.

The correspondence between SMV resistance genes found in USA and China

SMV had been classified into 21 strains (SC1–SC21) in China. Seven SMV strains (G1–G7) have been identified in

US (Guo et al. 2005; Li et al. 2010; Wang et al. 2003; Cho and Goodman 1979, 1982). Possibly this is a result of differential hosts to SMV being used in China and the US. The relationships between the 21 SMV strains in China and the seven strains in the US have not been studied. American researchers found that resistance in soybean to different SMV strains were conditioned by a single dominant gene, for example, *Rsv1* in PI 96983 was resistant to G1–G6 (Cho and Goodman 1982; Yu et al. 1994); *Rsv3* conferred resistance to the three most virulent strains G5–G7 (Jeong et al. 2002); and the dominant gene in LR2, later designated *Rsv4* by Hayes et al. (2000) conferred resistance to SMV strains G1–G7. In China, many SMV resistance genes which conferred resistance to different Chinese SMV strains were found (Fu et al. 2006; Li et al. 2006; Wang et al. 2011a, b; Ma et al. 2011; Yang and Gai 2010). Most of these have been mapped to chromosomes 2, 13, and 14, respectively. *Rsv*1, *Rsv3*, and *Rsv4* have also been mapped chromosomes 13, 14, and 2, respectively. Some of the resistance genes in China may be identical to *Rsv*1, *Rsv3*, or *Rsv4*; however, this needs further research.

Yu et al. (1994), Hayes et al. (2000), Gore et al. (2002) and Li et al. (2006) used PI 96983 as resistance parent and mapped the resistance genes *Rsv1* and *Rsc14* at two closely linked loci. In the present study, we identified two closely linked SMV resistance genes *Rsc-pm* and *Rsc-ps* on chromosome 13. The physical map of the *Rsc-pm* locus (Fig. 3a) showed that the *Rsc-pm* locus contains the *3gG2* gene which is a strong candidate for *Rsv1* (Hayes et al. 2004). In addition, comparing the map of *Rsc-pm* with the high-resolution map of *Rsv1* (Gore et al. 2002), indicates that it is likely closely linked to *Rsv1*, and that the locus we identified as *Rsc-pm* may be *Rsv1*. As the *Rsc14* locus has not been fine mapped, the correspondence between *Rsc14* and either the *Rsc-pm* or *Rsc-ps* could not be judged.

In summary, the correspondence between *Rsv1* and the two loci (*Rsc-pm* and *Rsc-ps*) identified in this study should be further investigated. Although *Rsv1* had been fine mapped by many researchers, none of the studies can definitively confirm the number of genes comprising *Rsv1*. Possibly, if more than two American strains were used in conducting similar research to this study, multiple SMV resistance loci may have been detected.

Other genes closely linked with the two mapped loci may effect on expression of resistance

Rsv1 was formerly described as a single dominant SMV resistance gene in PI 96983 (Kiihl and Hartwig 1979; Yu et al. 1994). Observations made by Gore et al. (2002) contradicted previous genetic data of *Rsv1*. Their results indicated that "one or more tightly linked genes besides *Rsv1* conditioned resistance to SMV in PI 96983". In addition, several tightly linked genes regulating diseases defense also exist in other plants (Ellis et al. 1995; Meyers et al. 1998; Parniske and Jones 1999; Song et al. 1997). In this study, the phenotypes of R6, R9, and R11 contradicted corresponding F_2 marker data. This was similar to the previous reports (Gore et al. 2002) and implied that other genes closely linked with the two mapped loci affected SMV resistance. In our study, the population of the 23 SSR markers was not large enough to identify other cross-over points and, therefore, the presence of other the genes affecting SMV resistance could not be confirmed. Sequencing this region in all four lines might be able to help us finding the genes affecting SMV resistance.

In this paper, we report that two closely linked genes conferring resistance to different SMV strains were mapped to two narrow, but different closely linked intervals. These results would contribute to cloning and pyramiding the resistance genes for different SMV strains and help to breed new soybean cultivars with multiple resistances to different SMV strains.

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