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ORIGINAL PAPER

Genetic analysis and molecular mapping of resistance gene to *Phakopsora pachyrhizi* in soybean germplasm SX6907

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Abstract

Key message **In this study, *Rpp6907,* a novel resistance gene/allele to *Phakopsora pachyrhizi* in soybean, was mapped in a 111.9-kb region, including three NBS-LRR type predicted genes, on chromosome 18.**

Abstract Soybean rust caused by *Phakopsora pachyrhizi* Sydow has been reported in numerous soybean-growing regions worldwide. The development of rust-resistant varieties is the most economical and environmentally safe method to control the disease. The Chinese soybean germplasm SX6907 is resistant to *P. pachyrhizi* and exhibits immune reaction compared with the known *Rpp* genes. These characteristics suggest that SX6907 may carry at least one novel *Rpp* gene/allele. Three F_2 populations from the crosses of SX6907 (resistant) and Tianlong 1, Zhongdou40, and Pudou11 (susceptible) were used to map the *Rpp* gene. Three resistance responses (immune, red-brown, and tan-colored lesion) were observed from the F_2 individuals. The segregation follows a ratio of 1(resistance):2(heterozygous):1(susceptible), indicating that the resistance in SX6907 is controlled by a single incomplete dominant gene (designated as *Rpp6907*). Results showed that *Rpp6907* was mapped on soybean chromosome 18 (molecular linkage group G, MLG G) flanked by simple sequence repeat (SSR) markers SSR24 and SSR40 at a distance of 111.9 kb. Among the ten genes marked within this 111.9-kb region between the two markers, three genes (*Glyma18g51930*, *Glyma18g51950*, and *Glyma18g51960*) are nucleotide-binding site and leucine-rich repeat-type genes. These genes may be involved in recognizing the presence of pathogens and ultimately conferring resistance. Based on resistance spectrum analysis and mapping results, we inferred that *Rpp6907* is a novel gene different from *Rpp1* in PI 200492, PI 561356, PI 587880A, PI 587886, and PI 594538A, or a new *Rpp1-b* allele.

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Introduction

Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Sydow is a severe destructive foliar disease in soybean (*Glycine max* (L.) Merr.) (Miles et al. 2003). The disease is disseminated through urediniospores carried by the wind and can rapidly develop, thus causing leaf premature senescence and severe reduction in grain yield. SBR was first reported in 1902 in Japan (Hennings 1903) and was then described in other parts of Asia and Australia in 1934 (Kochman 1977), India in 1951 (Sharma and Mehta. 1996), Hawaii in 1994 (Killgore and Heu. 1994), and Africa in 1996 (Akinsanmi et al. 2001). This disease was

also reported in Paraguay (Paiva and Yorinori. 2002) and Brazil (Yorinori et al. 2005), Argentina, Bolivia, Colombia (Rossi 2003), and the United States (Schneider et al. 2005) in recent years. SBR has been reported in 24 provinces from south to central China (Tan et al. 1983). Most soybean cultivars in China are highly susceptible to SBR, which are conductive to disease development and lead to epidemic (Tan 1994; Tan et al. 1997; Shan et al. 2000, 2012). *P. pachyrhizi* can infect more than 150 species of plants from more than 53 genera, including soybean, related *Glycine* species, and other hosts in the *Fabaceae* family (Hartman et al. 2011). This broad host range is unusual among rust pathogens and may have resulted from genes that contribute to a diverse and complex virulence pattern (Hartman et al. 2005). Considering the explosive nature of the disease and the high potential yield losses (10–80 %), soybean rust has long been viewed as a serious threat to soybean production worldwide because of the lack of resistance resource.

The use of molecular markers is an effective tool for gene identification and transfers (Tanskley 1983; Tanskley and McCouch 1997) and can expedite the development of soybean cultivars carrying single or multiple resistance genes. Soybean has a reasonably dense molecular marker linkage map (Song et al. 2004, 2010), and the association of markers to known genes has been studied by several groups. Molecular markers linked to *Rpp* genes in soybean have already been determined in different linkage populations. To date, SBR resistance alleles at six loci have been identified and mapped. *Rpp1* from PI 200492 (Hyten et al. 2007), *Rpp1-b* from PI 594538A (Chakraborty et al. 2009), and the *Rpp1* allelic genes from PI 587886, PI 587880A (Ray et al. 2009), and PI 561356 (Kim et al. 2012) were mapped to the same region on soybean chromosome 18 (linkage group (MLG G). *Rpp2* (Silva et al. 2008) was mapped on chromosome 16 (MLG J), and *Rpp3* (Hyten et al. 2009) and *Rpp*? (Hyuuga) (Monteros et al. 2007) were mapped on chromosome 6 (MLG C2). *Rpp4* (Silva et al. 2008) and *Rpp6* (Li et al. 2012) were mapped to different regions other than *Rpp1* on chromosome 18 (MLG G), and *Rpp5* (Garcia et al. 2008) was mapped on chromosome 3 (MLG N). *Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4* have already been defeated in the fields of China because of the high virulence variability of the *P. pachyrhizi* isolates (Luo et al. 2006; Shan et al. 2012). These findings show that the SBR resistance genes are not durable, and additional resistance genes in soybean must be discovered.

The use of resistance genes may control soybean diseases, but their "race specific" nature may pose problems (Yamaoka et al. 2002; Bonde et al. 2006). Therefore, discovering new resistance genes is necessary to further improve the SBR resistance and develop SBR-resistant cultivars with multiple resistance genes in soybean. The development and use of SBR-resistant cultivars can reduce losses caused by the disease without the negative environmental effects of fungicide applications. Over the past 40 years, considerable effort has been made to find resistance sources for SBR. More than 14,000 accessions from China have been screened for SBR resistance, and more than 100 accessions of germplasm have been identified with resistances that need further characterization (Tan 1994; Tan et al. 1997; Shan et al. 2000, 2012).

SX6907 is a highly resistant germplasm and has an immune response against *P. pachyrhizi* isolate SS4 (Shan et al. 2012). SX6907 exhibits higher resistance than other known *Rpp1–Rpp4* resistance accessions of PI 200492, PI 230970, PI 462312, and PI 459025 (Shan et al. 2012). In the present study, three F_2 populations were used for SBR resistance genetic analysis and molecular mapping. The purpose was to determine the mode of resistance inheritance and map the SBR resistance gene in SX6907.

Materials and methods

Plant material

Three F_2 populations were obtained from crosses between Zhongdou 40 × SX6907, Tianlong 1 × SX6907, and Pudou11 × SX6907. SX6907 is a landrace obtained from Hubei Province. SX6907 had been identified an immune response to SS4, a *P. pachyrhizi* isolate from Fujian (Shan et al. 2012). Zhongdou40, Tianlong 1 [high-yield varieties developed by Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, (OCRI)], and Pudou11 (a high-yield variety developed by Guangxi Agriculture Academy) were used as the susceptible parents in this study. F_1 hybrid seeds were confirmed using molecular markers and resistance evaluation. Some of F_1 seeds were planted and allowed to set seeds. Plants derived from one F_1 plant were used as one population.

We chose three populations for primary mapping. A total of 116 F_2 plants from Zhongdou 40 × SX6907, 198 F_2 plants from Tianlong 1 × SX6907, and 275 F_2 plants from Pudou11 × SX6907 were used. Additional 800 F_2 individuals from Tianlong 1 × SX6907 were further used for fine mapping. Each cross was sown in the greenhouse along with the parents and remaining F_1 seeds. The experiments were performed in the spring of 2013 (F_2 populations) and autumn of 2013 ($F_{2:3}$ progeny test) inside the greenhouse in OCRI.

The phenotypic data from F_1, F_2, and $F_{2:3}$ plants were obtained by a detached leaf assay. F_1, F_2, and $F_{2:3}$ seeds were sown in small paper cups (one seed per cup Φ 4.0 × 7 cm). Seedlings were grown at 24–26 °C, 18/6 h photoperiod. After 14 days, the fully expanded primary leaves of the seedlings were collected, and each leaf was

used for inoculation as one replicate. Thus, two primary leaves from each single plant were used as two replicates. The parents were used as check lines, and leaves from the ten plants of each line were used. Urediniospores were routinely multiplied on Tianlong 1 leaves and collected into a tube. The urediniospore suspension was adjusted to 10^5 urediniospores per ml using Tween 20 (0.01 % v/v). The detached leaves were placed in a plate (Φ 15.0 × 2.0 cm) padded with a piece of wet filter paper. The upper surface of the leaf was in contact with the paper. Five to six leaves were placed in each plate. Each leaf was inoculated with four drops of urediniospore suspension at 5 μl per drop or two drops at 10 μl per drop. The leaves were stored in the dark at 24 °C at the first night after inoculation and then transferred to a growth chamber at 24 °C and 70 % RH under a 12/12-h photoperiod. Approximately 1–2 ml of water was added daily to keep the filter paper completely wet. Two weeks after inoculation, the leaves were scored for the presence of resistance response symptoms, as described by Garcia et al. (2008). The SBR reactions were classified according to lesion type: IM type, no visible lesion; RB type, appearance of infected lesions 6–8 days after inoculation and absence of sporulation 14 days after inoculation; TAN type, appearance of infected lesions 4–5 days after inoculation and presence of sporulation 10–15 days after inoculation. The data were based on the genotypes (homozygous dominant, recessive or heterozygous for the resistance) of the F_2 individuals using the $F_{2:3}$ test. Leaves were scored as resistant if they exhibited IM response in both replications and as incomplete resistance if they exhibited RB lesions in two replications or RB in one replication and IM in the other replication. Leaves were scored as susceptible if they exhibited TAN lesions in either of both replications.

After detaching leaves for inoculation, the plants were transferred to the greenhouse with conventional management and allowed to set seeds. Progeny ($F_{2:3}$) test was performed to confirm the phenotype and assign the genotype of the F_2 individuals. Chi square (χ^2) test was performed to verify whether the genetic segregation fits any expected model. Molecular analysis was only conducted for the tested progeny of the F_2 plants. As three populations had the same genetic pattern, Tianlong 1 × SX6907 population was selected for further fine mapping. After preliminary genetic mapping, additional F_2 plants from Tianlong 1 × SX6907 were used for genotyping. Resistance assay in $F_{2:3}$ was carried out only for the recombinants.

To confirm the consistency of detached leaf assay and plant spray inoculation, we compared plant spray inoculation and detached leaf assay in two F_2 populations derived from Tianlong 1 × SX6907 (78 and 146 individuals, respectively). Approximately 14 days after sowing, two leaflets from each seedling were collected for detached leaf assay. Inoculation method and leaf resistance rating were as same as mentioned above. The plants were used for spray inoculation, and the urediniospore suspension was adjusted to 10^5 urediniospores per ml using Tween 20 (0.01 % v/v). The suspension was sprayed on the plants with a small watering can. Following inoculation, the plants were incubated for approximately 12 h at 24 °C in a dew chamber and later moved to a greenhouse maintained at 20–26 °C and 60 % RH under a 12/12-h photoperiod for 14 days until symptoms were ready for rating. Plant response to rust pathogen was scored as resistant if all inoculated leaves exhibited IM response and as incomplete resistance if all inoculated leaves exhibited RB lesions or RB in some leaves and IM in the other leaves. Plants were scored as susceptible if any of inoculated leaves exhibited TAN lesions.

When a *Rpps* gene was located in a known Rpps region, a comparison of resistance response between SX6907 and the known Rpps carriers was conducted. We compared resistance response to *P. pachyrhizi* isolate SS4 between SX6907and PI 200492, PI 594538A, PI 587886, PI 587880A, and PI 561356 using detached leaf assay.

DNA isolation and molecular markers

Leaf samples were collected from individual plants of the three F_2 populations and $F_{2:3}$ families. Genomic DNA was extracted using the CTAB method (Keim et al. 1988). Simple sequence repeat (SSR) molecular markers were selected based on their distribution throughout the integrated molecular linkage map (Song et al. 2004, 2010). Each SSR marker was tested for polymorphism between the parental lines.

SSR primers were synthesized by the Integrated DNA Technologies Inc (Wuhan, China). Polymerase chain reaction (PCR) was conducted in 96-well microplates with a final volume of 20 μl. Each reaction included 50 ng of genomic DNA, 0.2 μM primer, 0.2 mM dNTP, 2.0 mM $MgCl_2$, and 0.2 U Taq DNA polymerase. The PCR reaction was performed at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 47–62 °C for 30 s, and 72 °C for 45 s, with a final extension of 10 min at 72 °C. The PCR products were analyzed by electrophoresis using 6 % polyacrylamide gels, and DNA fragments were visualized by silver staining (Bassam et al. 1991).

Linkage analysis

The linkage of a SSR marker to the resistance trait in each F_2 population was initially obtained through bulked segregant analysis (BSA), according to the methods of Michelmore et al. (1991). Two different bulk groups were formed for each population. The bulk groups were obtained by

pooling an equal amount of DNA from 15 different plants. A resistant bulk group was formed by pooling an equal amount of DNA from 15 plants with IM reactions and a susceptible bulk group was formed by pooling DNA from 15 plants with TAN reactions, which were homozygous for either resistance (Bulk R) or susceptibility (Bulk S) to SBR. The SSR markers that were polymorphic between the parents were tested. Markers that showed a polymorphic pattern between the R and S bulks were considered to be potentially linked to the resistance gene and were further evaluated within the individual F_2 plants from the corresponding bulk groups. When the marker allele and the phenotype of the individual F_2 plants were consistent with the results of the bulk analysis, additional markers from the same genomic region were included to test the whole population.

After the preliminary genetic mapping of Rpps gene region, additional SSR markers were obtained from Song et al. (2010) and new markers were developed to fine mapping of the gene. We developed new SSR markers between BARCSOYSSR_18_1856 and BARCSOYSSR_18_1864 based on Williams 82 sequence (Song et al. 2010). The additional SSRs in this region were identified using MISA (Thiel et al. 2003; Song et al. 2010). A total of 500 bases of the sequence flanking the individually identified SSR were extracted from the Glyma1.01 soybean genome sequence. SSRs with repeat units from 6 to 35, 5 to 35, and 5 to 35 for the di-, tri-, and tetranucleotide SSR motifs, respectively, were retained. Primers were designed for the final set of the extracted SSR-containing sequences using standalone Primer 3 software (http://primer3.sourceforge.net/releases.php). The targeted PCR product length ranged from 80 to 400 base pairs, the annealing temperature ranged from 53 °C to 62 °C, and the primer length ranged from 18 to 27 nucleotides (Song et al. 2010). SSR markers, BARCSOYSSR_18_1856, BARCSOYSSR_18_1861, and BARCSOYSSR_18_1864, were used for genotyping the recombinants from the progeny of Tianlong 1 × SX6907.

Data analysis and molecular mapping

Chi square tests for the SBR lesion type (RB or TAN) and molecular markers were performed to test the goodness of fit of the observed segregation between the F_2 and $F_{2:3}$ lines. The genetic linkage map was constructed using Joinmap 3.0 (Van Ooijen and Voorrips 2001) with Kosambi mapping function. Segregation distortion was performed using the Chi squared test ($P < 0.05$) with the JoinMap software under the 'locus genotype frequency' function. The LOD grouping thresholds were ≥3.0. Linkage groups were assigned according to Song et al. (2004) and the composite maps at the SoyBase website. The genomic region(s) associated with rust resistance was mapped as quantitative trait loci using the composite interval mapping functions of WinQTL cartographer 2.5 software (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The threshold for the identification of a significant locus ($P < 0.05$) was estimated through permutation test with 1,000 repetitions.

Results

Inheritance of rust resistance

We investigated rust resistance in F_2 populations and observed three phenotypes in the segregated plants, namely IM, RB, and TAN (Fig. 1). All F_1 plants exhibited RB-type response. The sporulation did not occur in the lines in F_2 population with IM-type response, but occurred in the lines with TAN-type response. No resistance response segregation occurred in $F_{2:3}$ lines of plants with IM- or TAN-type response. The plants with RB-type lesions had resistance segregation in the $F_{2:3}$ progenies. The segregation ratio of the resistance response (IM, RB, or TAN) in F_2 individuals and $F_{2:3}$ lines fitted well with the Mendelian 1(IM):2(RB):1(TAN) and 1R (resistance homozygous):2H (heterozygous):1S (susceptible homozygous) ratio, respectively (Table 1). The plant spray inoculation result was consistent with the detached leaf assay result (Table 2, Supplementary Table S1, S2, Fig. S1). The resistance to SX6907 is presumably controlled by a single incomplete dominant gene.

Mapping *Rpp6907* with SSR markers

The BSA method showed that the SSR markers near *Rpp1* were associated with the lesion type. Four SSR markers, namely BARCSOYSSR_18_1856, BARCSOYSSR_18_1858, BARCSOYSSR_18_1861, and BARCSOYSSR_18_1864, exhibited polymorphisms between the resistant and susceptible parents, as well as between the resistant and susceptible bulk groups. Linkage analysis results revealed that *Rpp* in SX6907 was linked to these markers and was located between BARCSOYSSR_18_1856 and BARCSOYSSR_18_1864. The resistance locus was mapped on the same marker interval of the three F_2 populations (Fig. 2). The results showed only one single peak with a LOD score of 40.84, 38.6, and 38.4, which accounts for 99.13, 98.4, and 98.0 % of the phenotypic variance, respectively. To identify additional markers in the *Rpp1* region that are polymorphic between Tianlong 1 and SX6907, 54 SSR markers were developed on the marker interval of BARCSOYSSR_18_1856 and 1864, and 11 SSR markers were polymorphic (Table 3). Additional 800 F_2 individuals were subsequently used for genotyping. Sixteen recombinants were identified by the

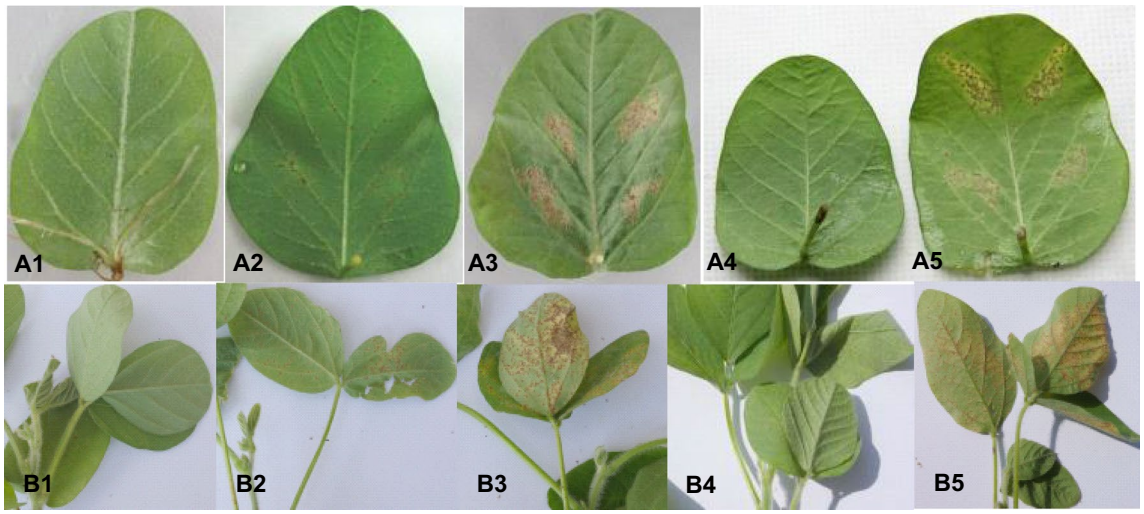

Fig. 1 Three types of resistance response in F_2 individuals and parents 14 days after inoculation. IM response give no visible lesion in leaf (**a1**) and plant (**b1**); RB response give less *red-brown* lesion with no sporulation in leaf (**a2**) and plant (**b2**); and TAN gave *tan-colored* lesion with sporulation in leaf (**a3**) and plant (**b3**). SX6907 (**a4**, **b4**) and Tianlong 1 (**a5**, **b5**) were used as resistance and susceptible check, respectively

Table 1 Phenotype and genotype segregation of populations derived by crossing different susceptible parents to SX6907

Susceptible parent	F_2 test						$F_{2:3}$ test					
	Numo. of plants						Numo. of lines					
	IM	RB	TAN	Ttotal	Eexpected ratio	χ^2	R	H	S	Ttotal	Eexpected ratio	χ^2
Tianlong 1	48	103	47	198	1:2:1	0.09 NS*	47	100	49	196	1:2:1	0.12 NS
Zhongdou40	29	57	32	116	1:2:1	0.32 NS	29	56	33	116	1:2:1	0.62 NS
PD11	70	136	69	275	1:2:1	0.04 NS	68	134	65	267	1:2:1	0.07 NS

IM leave without visible infect lesions, *RB* leaf with red-brown lesions without sporulation, *TAN* leaf with tan-colored lesions, *R* resistance, *H* heterozygous, *S* susceptible

* NS: non-significance of the Chi square value ($P = 0.05$)

Table 2 Comparison the resistance response in F_2 populations by using plant spray inoculation and detached leaf assay

Population no.	Inoculation method	F_2 test				Expected ratio	χ^2
		Num. of plants					
		IM	RB	TAN	Total		
Population 1	Plant spray inoculation detached leaf assay	23	38	17	78	1:2:1	0.769 NS
		24	37	17	78	1:2:1	1.269 NS
Population 2	Plant spray inoculation detached leaf assay	41	67	38	146	1:2:1	1.11 NS
		38	73	35	146	1:2:1	0.123 NS

IM leaf without visible infect lesions, *RB* leaf with red-brown lesions without sporulation, *TAN* leaf with tan-colored lesions

NS: non-significance of the Chi square value ($P = 0.05$)

two flanking markers, namely BARCSOYSSR_18_1856 and 1864 (Table 4). The recombinants 689 and 690 with RB-type lesions were heterozygous for the marker alleles downstream of the marker SSR24. The recombinant 519

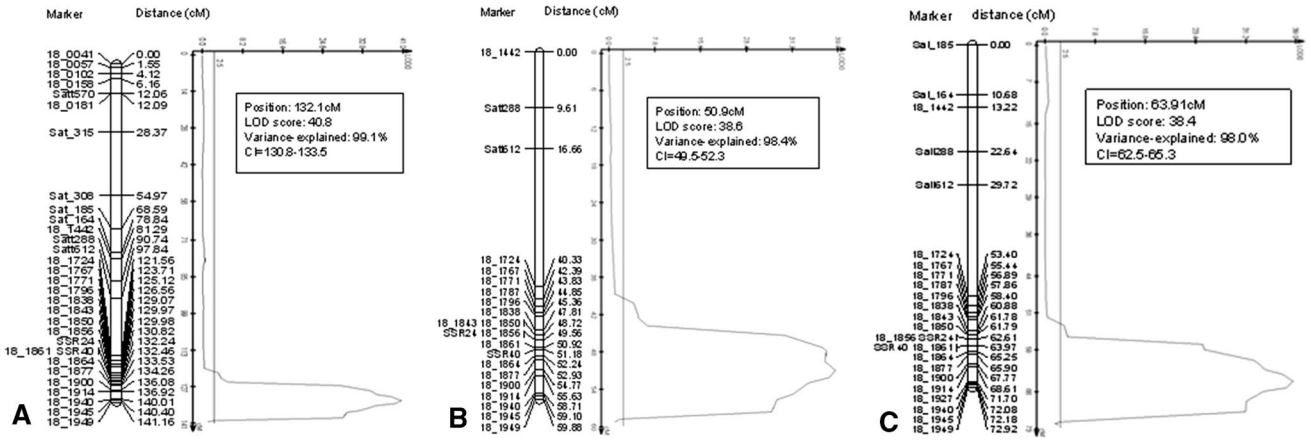

Fig. 2 Genetic mapping of *Rpp6907* on chromosome 18 using three F_2 populations of Zhongdou 40 × SX6907 (**a**), Tianlong 1 × SX6907 (**b**) and PD11 × SX6907 (**C**). *CI* confidential interval

Table 3 SSR markers developed in *Rpp1* locus region

Marker	Position (bp)[a]	Product size[b]	Primer sequence
BARCSOYSSR_18_1856[c]	60,503,838	192	Forward TGGCCATATGCCTAGCTGAT Reverse ATGGTGAGCAAACGTCATTG
SSR16	60,587,749	165	Forward GGTGAATCCGTTTCCATTTG Reverse TTGTGGCTAAAGCTCCACCT
SSR19	60,611,695	108	Forward GGCCTACATTAGCTGTGGGA Reverse ACGTGGACCCTGTCATTCTC
BARCSOYSSR_18_1858	60,612,567	143	Forward TAGCTTTATAATGAGTGTGATAGAT Reverse GTATGCAAGGGATTAATTAAG
SSR21	60,617,483	280	Forward ACCTCCTCCTCTCCCTGAAG Reverse CGGTTCAATCTCAAAGGAGG
SSR22	60,618,014	110	Forward ACCAAACCCGATGATGATGT Reverse CCAGATTCCAAACCCCTTCT
SSR24	60,620,161	276	Forward GAAGAGGGTCTTCAAAATCAATC Reverse TTGTTAATCAGGATCTATAAGACATTG
SSR27	60,633,057	264	Forward TGAATGATCTCAAAGAAAGGAATG Reverse TGGCAGGACCTACCTGCTAT
SSR32	60,681,986	216	Forward TGAACAGTGTGAGGATGGAGA Reverse TTTAAAATGCATTGTGGGCA
SSR36	60,709,160	242	Forward AAGGAAAATGACCTCCAGCA Reverse CCCAAGCCTTGTTGGTTTTA
BARCSOYSSR_18_1861	60,709,740	247	Forward TGCCACAATGTCCACAACTT Reverse CCCTTTTCTTTTGCCTCTCC
SSR37	60,719,092	279	Forward TTTTCTTAGCCTTGTACTTTCCAA Reverse CGATCGAGCGCAATTTTACT
SSR40	60,732,116	397	Forward TTGACTTCTTTACAAACAAATGTTGA Reverse CCAAGTCTAACTTTTTCCCTCAAA
SSR41	60,738,317	200	Forward ATAAAAGGTGGATTTTGGAATTT Reverse CAGATCTTGGGCAATGCTTC
BARCSOYSSR_18_1864	60,867,759-	185	Forward TGAATGATATATGTTTTGCGAAGA Reverse CAATAGAGCCGGATGGATGT

[a] Physical position of the markers based on the *G.max* genome(assembly version 1.01) available http://soybase.org/gbrowse/cgi-bin/gbrowse.gmax 1.01/. The base pair (bp) position of the markers correspond to the locations of simple sequence repeats on the soybean chromosome 18

[b] PCR product sizes are based on the *G.max* genome(assembly version 1.01) available http://soybase.org/gbrowse/cgi-bin/gbrowse.gmax 1.01

[c] BARCSOYSSR sequence ID is based on Song et al. (2010)

Table 4 Phenotypes and genotypes of recombinants from F_2 population and $F_{2:3}$ families

Recombinant[a]	Genotype in F_2 individual															Phenotype in $F_{2:3}$ family
	18_1856	SSR16	SSR19	18_1858	SSR21	SSR22	SSR24	SSR27	SSR32	SSR36	18_1861	SSR37	SSR40	SSR41	18_1864	
179	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Susceptible
135	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Susceptible
26	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	Susceptible
76	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Susceptible
148	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	Susceptible
153	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	Susceptible
54	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Susceptible
178	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Susceptible
173	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Susceptible
689	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	Segregating
690	A	A	A	H	A	A	A	H	H	H	H	H	H	H	H	Segregating
519	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	Segregating
1	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H	Segregating
116	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H	Resistant
119	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H	Resistant
82	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H	Resistant

A: Genotypes of recombinant individuals, homozygous as susceptible parent Tianlong 1, B: homozygous as resistant parent SX6907, H: heterozygous

[a] Progeny of recombinants

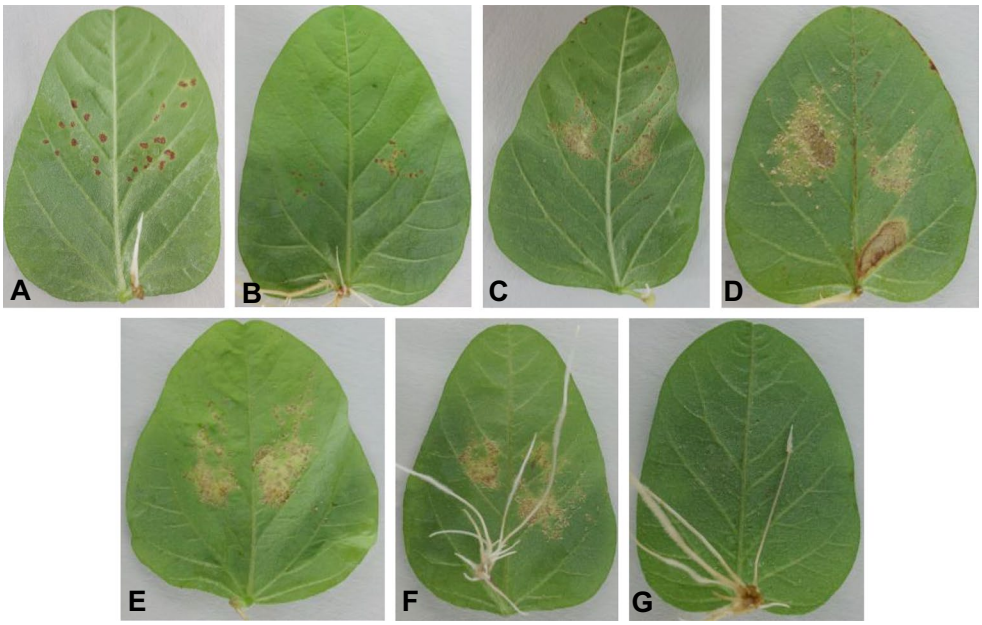

Fig. 3 Resistance response of seven accessions to SS4 14 days after inoculation. PI 587886 (**a**) and PI 587880A (**b**) exhibited RB response; PI 594538A (**c**), PI 561356 (**d**) and PI 200492 (**e**) exhibited TAN response; Tianlong 1 (**f**) and SX6907 (**g**) were used as susceptible and resistant check, respectively

with RB-type lesions was heterozygous for the marker alleles upstream of the marker SSR40. By combining the recombinant break point and phenotypes of the corresponding progeny families, the resistance gene from SX6907 was mapped on chromosome 18 flanked by SSR24 and SSR40. The physical distance between SSR24 and SSR40 based on the *G. max* genome (assembly version 1.01) is 111.9 kb (http://www.soybase.org).

As the *P. pachyrhizi* isolate SS4 in the present study might be different from FL07-1 or ZM01-1 (Kim et al. 2012), the SX6907 resistance response was compared with other known *Rpp1* carriers. PI 200492, PI 561356, and PI 594538A exhibited susceptible response with TAN-type lesions. However, PI 587886 and PI 587880A exhibited incomplete resistance with RB-type lesions (Fig. 3), and SSR24 and SSR40 produced different size PCR products between these two PIs and SX6907 (Table 5). Thus, we inferred that SS4 was a different isolate from FL07-1 and ZM01-1. We have known that *Rpp1* in PI200492 was mapped to the region of Gm18:60463046-60612672 (Hyten et al. 2007); *Rpp1?* in PI 561356 was mapped to the region of Gm 18:60518978-60613377 (Kim et al. 2012); and *Rpp1?* in PI 587880A and PI 587886 was mapped to the region of Gm 18:58722971-60612672 (Chakraborty et al. 2009). By combining molecular marker analysis and resistance assay results in the present study, we inferred that SX6907 hosted a novel *Rpp* gene different from PI 561356, PI 200492, PI 587886, and PI 587880A, or a novel allele of *Rpp1-b* in PI 594538A, which we temporarily designated as *Rpp6907*.

Table 5 Reactions of seven soybean accessions to *Phakopsora pachyrhizi* isolate SS4 and their alleles for two SSR markers

Genotype	Resistance gene	SSR24	SSR40	*P. pachyrhizi* isolate SS4
SX6907	*Rpp6907*	1[a]	1	IM
Tianlong 1	–	2	2	TAN
PI 200492	*Rpp1*	3	3	TAN
PI 594538A	*Rpp1-b*	1	2	TAN
PI 587886	*Rpp1-?*	1	2	RB
PI 587880A	*Rpp1-?*	4	2	RB
PI 561356	*Rpp1-?*	1	1	TAN

[a] Mean different size of PCR products

Candidate gene prediction

Sequence analysis results of the soybean genome showed that the physical distance of the region between markers SSR24 and SSR40, which were at nucleotide positions Gm18:60,620161 and 60,732116, respectively, was approximately 111.9 kb. According to the soybean gene annotation database accessible at *G. max* genome (assembly version 1.01) (http://www.soybase.org), ten predicted genes exist in this region (Table 6). Among these predicted genes, three

Table 6 Predicted candidate genes in the mapping region

Gene name	Chromosome location	Gene annotations
Glyma18g51880	60,632,452–60,634,378	Dirigent-like protein
Glyma18g51890	60,639,221–60,652,488	PHD finger protein
Glyma18g51900	60,656,814–60,674,825	Nuclear transport receptor RANBP7/RANBP8
Glyma18g51911	60,667,870–60,670,284	RNA recognition motif
Glyma18g51920	60,676,142–60,684,489	Eukaryotic aspartyl protease
Glyma18g51930	60,685,177–60,687,775	NBS-LRR disease-resistance protein
Glyma18g51950	60,693,444–60,696,291	NBS-LRR disease-resistance protein
Glyma18g51960	60,704,363–60,706,532	NBS-LRR disease-resistance protein
Glyma18g51970	60,708,698–60,714,044	Serine/threonine protein phosphatase
Glyma18g51980	60,720,285–60,723,728	Predicted chitinase
Glyma18g51990	60,725,655–60,728,111	P21-Rho-binding domain

(*Glyma18g51930, Glyma18g51950*, and *Glyma18g51960*) are NBS-LRR-type genes. These three genes may be good candidates for *Rpp6907* because most common R proteins contain a nucleotide-binding site and a leucine-rich repeat (NBS-LRR) domain (Dangl and Jones 2001).

Discussion

In this study, *Rpp6907* (flanked by markers SSR24 and SSR40 on Gm18: 60,620,161–60,732,116) was identified. SBR resistance alleles from the other five soybean accessions were previously mapped to the same region on chromosome 18 (Hyten et al. 2007; Chakraborty et al. 2009; Ray et al. 2009; Kim et al. 2012). Although we could not use the same markers utilized in previous studies, the physical locations of these markers on the *G. max* genome (assembly version 1.01) (http://soybase.org) could be directly compared. Hyten et al. (2007) reported that *Rpp1* from PI 200492 was mapped to a 149.6-kb interval between Sct_187 and Sat_064. Chakraborty et al. (2009) reported that *Rpp1-b* from PI 594538A was mapped to a 1,626.7-kb interval between BARC-010495-00656 and BARC-014379-01337. Ray et al. (2009) mapped alleles from two PIs PI 587880A and PI 587886 to a 1,889.7-kb interval between Satt191 and Sat_064. Kim et al. (2012) finely mapped the SBR resistance gene from PI 561356 to a 94.4-kb interval between SSR50 (60,518,978) and SSR1859 (60,613,311). A comparison of the intervals with resistance genes mapped across the previous five sources revealed an overlapping 93.6-kb interval between SSR50 and Sat_064 that exhibited resistance in each source maps. These five sources possibly had a resistance allele at the same locus in the overlapping interval. However, there is no overlapping between the mapping regions of SX6907 (Gm18: 60,620,161–60,732,116) and PI 200492, PI 587886, PI 587880A and PI 561356. This result indicates that SX6907 hosted a novel locus different from that in PI 200492, PI 587886, PI 587880A, and PI 561356. As SX6907 has a 111.9-kb overlap with PI 594538A, SX6907 and PI 594538A has different resistance response to SS4, *Rpp6907* should be considered as a new locus/allele different from *Rpp1-b*.

We finely mapped the *Rpp6907* locus in SX6907 that confers resistance to *P. pachyrhizi* isolate SS4. In this study, three resistance phenotypes in the F_2 population were observed for the first time, and the phenotype segregation ratio fitted the expected model of the genotype segregation (1R:2H:1S) in both plant spray inoculation and detached leave assay. The results of $F_{2:3}$ line resistance responses confirmed that the phenotype segregation agreed with the genotype segregation. Three phenotypes enabled the identification of the genotype for each F_2 individual directly, thus providing convenience to the researcher and reducing time spent for fine mapping. IM-type lesion was observed when PI 200492 interacted with the India 73-1 isolate, but only two lesion types (IM and TAN) have been reported in the population of PI 200492 × Williams 82 (Hyten et al. 2007). RB-type lesion has been assumed as a resistance response lesion type in most studies (Garcia et al. 2008; Chakraborty et al. 2009; Ray et al. 2009; Kim et al. 2012; Li et al. 2012). However, in the present study, this type represents a heterozygous genotype. This difference could be attributed to the resistance mechanism of SX6907, which needs further investigation. Although the plant spray inoculation and detached leaf assay are routine methods in SBR resistance evaluation (Bromfield et al. 1980; Shan et al. 2008; Twizeyimana et al. 2008; Vittal et al. 2014), the inoculum's quantity and environment in the detached leaf assay could be easily controlled.

In this study, we also observed that the SSR markers were more informative in distinguishing the SBR-resistant accessions, which has been reported by Kim et al. (2012). SSR24 and SSR40 have different PCR product sizes compared with SX6907 and the other five resistance accessions. PI 587880A and PI 587886 have been assumed to

have the same resistance allele because of the same resistance response and the absence of polymorphic molecular markers. In this study, SSR24 and SSR40 produced polymorphic PCR products between the two PIs. We, therefore, predicted that these two PIs may possess different alleles in the mapped region. These results suggest that SSR24 and SSR40 could be useful in predicting whether the SBR resistant accessions with unknown resistance genes have the same resistance allele in the *Rpp1* region.

The current gene annotation of the 111.9-kb region between SSR24 and SSR40 on the *G. max* genome (assembly version 1.01) predicted the presence of ten high-confidence genes. *Rpp6907* was mapped to the R gene-rich regions at the end of chromosome 18, where *Rpp1-b* from PI 594538A (Chakraborty et al. 2009), and *Rps4/6* (Demirbas et al. 2001) and *RpsJS* (Sun et al. 2014) were also mapped. Among the predicted genes, *Glyma18g51930, Glyma18g51950,* and *Glyma18g51960* are NBS-LRR genes that encode a leucine-rich protein. The nucleotide similarity between the three genes is 94 %, whereas the peptide similarities among *Glyma18g51930, Glyma18g51950*, and *Glymal18g51960* are 79, 44, and 37 %, respectively. Evidence shows that SBR resistance genes in soybean may be from the NBS-LRR gene family, as Meyer et al. (2009) identified NBS-LRR as a single candidate gene for *Rpp4* in PI 459025B. Monteros et al. (2010) narrowed the *Rpp*? (Hyuuga) interval to a 371-kb region and reported that this region also includes NBS-LRR and LRR genes. Although *Glyma18g51930, Glyma18g51950*, and *Glyma18g51960* are good candidate genes for SBR resistance in SX6907, the gene annotation is from Williams82, which is susceptible to SBR. Therefore, the candidate genes in the region need to be isolated from their resistance sources and complemented in a susceptible background to identify the coding sequence for *Rpp6907.*

New races of *P. pachyrhizi* appear with the release of resistant cultivars, and thus the use of genetic resistance still remains the most effective strategy to reduce losses caused by the pathogen. Thus, identifying new *Rpp* genes and developing molecular markers to the genes of interest for marker-assisted selection (MAS) are important. SX6907 may be useful as a source of resistance for breeding new cultivars planted in parts of China or other countries where SBR is a problem. The markers SSR22 and SSR40, co-segregated with *Rpp6907*, can be used as convenient tools for the MAS of *Rpp6907* in breeding programs.

Author contribution statement Haifeng Chen and Sheng Zhao performed QTL mapping, data analysis and drafted the manuscript. Zhonglu Yang performed phenotypic analysis and QTL mapping. Aihua Sha provided advice on experiment design. Qiao Wan, Chanjuan Zhang, Limiao Chen, and Songli Yuan prepared samples and performed SSR analysis. Dezhen Qiu performed greenhouse experiments measurement. Shuilian Chen prepared reagents. Zhihui Shan designed research, performed population construction and phenotypic assay, analyzed the data, and reviewed the manuscript. Xin-an Zhou supervised the project, designed research, and reviewed the manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of China.

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