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ORIGINAL PAPER

Molecular mapping of soybean rust resistance in soybean accession PI 561356 and SNP haplotype analysis of the *Rpp1* region in diverse germplasm

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Abstract Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Sydow, is one of the most economically important and destructive diseases of soybean [*Glycine max* (L.) Merr.] and the discovery of novel SBR resistance genes is needed because of virulence diversity in the pathogen. The objectives of this research were to map SBR resistance in plant introduction (PI) 561356 and to identify single nucleotide polymorphism (SNP) haplotypes within the region on soybean chromosome 18 where the SBR resistance gene *Rpp1* maps. One-hundred $F_{2:3}$ lines derived from a cross between PI 561356 and the susceptible experimental line LD02-4485 were genotyped with genetic markers and phenotyped for resistance to *P. pachyrhizi* isolate ZM01-1. The segregation ratio of reddish brown versus tan lesion type in the population supported that resistance was controlled by a single dominant gene. The gene was mapped to a 1-cM region on soybean chromosome 18 corresponding to the same interval as *Rpp1*. A haplotype analysis of diverse germplasm across a 213-kb interval that included *Rpp1* revealed 21 distinct haplotypes of which 4 were present among 5 SBR resistance sources that have a resistance gene in the *Rpp1* region. Four major North American soybean ancestors belong to the same SNP haplotype as PI 561356 and seven belong to the same haplotype as PI 594538A, the *Rpp1-b* source. There were no North American soybean ancestors belonging to the SNP haplotypes found in PI 200492, the source of *Rpp1*, or PI 587886 and PI 587880A, additional sources with SBR resistance mapping to the *Rpp1* region.

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Introduction

Soybean rust (SBR) is caused by the fungus *Phakopsora pachyrhizi* Sydow and is one of the most economically important soybean diseases worldwide. SBR was first identified in Japan in 1902 (Hennings 1903), Hawaii in 1994 (Killgore and Heu 1994), and Brazil in 2001 (Yorinori et al. 2005). After SBR was first discovered in the continental USA in plots at the Louisiana State University research station in 2004 (Schneider et al. 2005), the disease spread to 20 US states, to Ontario in Canada, and to 9 states in

Mexico (Isard et al. 2005; Hershman et al. 2011). Most soybean cultivars grown in the USA are highly susceptible to SBR, which could lead to epidemics if weather conditions are conducive to disease development (Miles et al. 2003).

P. pachyrhizi infects more than 150 species of plants from more than 53 genera including soybean, related *Glycine* species, and other hosts in the Fabaceae (Hartman et al. 2011). This broad host range is unusual among rust pathogens and may be the result of genes that contribute to a diverse and complex virulence pattern (Hartman et al. 2005). The ideal conditions triggering infection are 10 h of moisture (rain, dew, or irrigation) on the leaf surface and day temperatures ranging from 15 to 28 °C (optimal 22–23 °C) (Ribeiro et al. 2007). Disease development is suppressed when temperatures exceed 30 °C (Bromfield 1984). As the disease progresses, the leaf tissue around the infected regions become pale brown (TAN reaction) in susceptible genotypes or reddish brown (RB reaction) in incompletely resistant genotypes (Miles et al. 2011). In the case of *Rpp1* from plant introduction (PI) 200492, no lesions develop, and this resistance is referred to as an immune (IM) response in the presence of certain isolates (Miles et al. 2011).

Soybean yield losses up to 80 % in experimental trials have been reported in Asia (Hartman et al. 1991) and 63 % have been reported in Brazil during 2003, 60 % in Paraguay during 2001 (Yorinori et al. 2005), up to 100 % in South Africa (Caldwell and McLaren 2004), and up to 55 % in the USA (Mueller et al. 2009). Because commercial soybean cultivars resistant to SBR are not available in the USA, fungicide applications are the only method currently available to control the disease. Fungicide applications result in significant production cost increases and environmental contamination. The cost of an individual fungicide application is estimated to be from $37 to $50 per ha and two or three applications may be needed over the course of a growing season (Born and Diver 2005).

The development and production of SBR-resistant cultivars could reduce losses caused by the disease without the expense and negative environmental impact of fungicide applications. Over the last decade, there has been a significant effort to find sources of resistance to SBR. More than 16,000 accessions from the USDA Soybean Germplasm Collection have been screened for SBR resistance with a mixture of *P. pachyrhizi* isolates in greenhouse tests (Miles et al. 2006). No US commercial cultivars evaluated were found to have SBR resistance in these tests; however, 805 accessions were identified with resistance and needing further characterization.

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 SBR resistance genes from PI 587886 and PI 587880A (Ray et al. 2009) were mapped to the same region on soybean chromosome 18 [linkage group (LG) G]. *Rpp2* (Silva et al. 2008) was mapped on chromosome 16 (LG J), *Rpp3* (Hyten et al. 2009) and *Rpp*?(Hyuuga) (Monteros et al. 2007) were mapped on chromosome 6 (LG C2), *Rpp4* (Silva et al. 2008) and *Rpp6* (Li et al. 2012) were mapped to different regions than *Rpp1* on chromosome 18 (LG G), and *Rpp5* (Garcia et al. 2008) was mapped on chromosome 3 (LG N). Due to the high virulence variability of *P. pachyrhizi* isolates, *Rpp1*, *Rpp1-b*, and *Rpp3* already have been defeated in the field in Brazil (Ribeiro et al. 2007; Yorinori et al. 2005). This shows that SBR resistance genes are not durable and it is important to discover additional resistance genes in soybean.

Marker-assisted selection (MAS) can result in increased genetic gains in breeding programs through the indirect selection of gene or genes with genetic markers (Pathan and Sleper 2008). Linkage disequilibrium (LD) between genetic markers and target genes provides a basic principle of MAS that marker alleles are not randomly associated with target gene alleles (Utomo and Linscombe 2009). Single nucleotide polymorphisms (SNPs) are a useful tool to quantify LD, and the analysis of SNP haplotypes has been the focus of recent studies (Zhu et al. 2003). There are several advantages of SNP markers over other genetic marker types. These advantages include: SNPs are the most abundant form of genetic variation within genomes and a wide array of technologies have been developed for high throughput SNP analysis (Zhu et al. 2003; Fan et al. 2006). A SNP haplotype refers to a distinct combination of SNPs that are tightly linked in a region of a chromosome (Shastry 2004) or a distinct combination of SNPs within LD block which tend to be inherited as an entire unit from a parent to its progeny. Information provided by SNPs is most useful when several closely spaced SNPs completely define haplotypes in the region being examined (Johnson et al. 2001). SNPs that can differentiate haplotypes have been called 'haplotype tags' (Johnson et al. 2001) and can be used as important genetic markers for MAS and genetic mapping.

The soybean genome has a relatively high LD compared to other plant species. The estimated average distance at which LD decays to half of its maximum value in cultivated soybean is approximately 150 kb and in wild soybean (*Glycine soja* Sieb. and Zucc) 75 kb (Lam et al. 2010). In contrast, similar levels of LD decay were estimated to occur at <1 kb in maize (*Zea mays* L.) and wild and cultivated rice (*Oryza sativa* L.) (Gore et al. 2009; Zhu et al. 2007). The percentage and total long LD (>150 kb) in cultivated soybean (1.5 %, total length 57.7 Mb) were higher than in wild soybeans (0.6 %, total length 35.7 Mb) and the longest LD block in cultivated soybean was ~1 Mb, whereas the longest LD block in wild soybeans was ~500 kb (Lam et al. 2010). The high LD in soybean is likely the result of

domestication bottlenecks, the inbreeding nature of the crop, and selective breeding (Hyten et al. 2006). In the presence of high LD, a small subset of SNP haplotype tags may be sufficient to define the haplotypes completely (Rafalski 2002).

The first objective of this study was to determine the mode of inheritance and map the location of SBR resistance gene or genes in soybean PI 561356. The second objective was to identify SNP haplotypes within the *Rpp1* region where resistance from PI 561356 maps. This genetic mapping and SNP haplotype analysis will be useful for determining genetic variation in the *Rpp1* region on soybean chromosome 18, for identifying SSR and SNP markers closely linked to the resistance genes, and for studying the association between SNP haplotypes and SBR resistance in the *Rpp1* region in soybean.

Materials and methods

Plant material

A population of 100 $F_{2:3}$ lines derived from a cross between PI 561356 and LD02-4485 was used for genetic mapping of SBR resistance. PI 561356 is a maturity group (MG) V soybean accession originating from Zhejiang, China (USDA-ARS 2012). PI 561356 showed a mixed lesion type (RB and TAN) to a mixture of *P. pachyrhizi* isolates from Thailand (TH01-1), Brazil (BZ01-1), Paraguay (PG01-2), and Zimbabwe (ZM01-1) (Miles et al. 2006). LD02-4485 is a high-yielding MG II experimental line developed by the University of Illinois that is susceptible to SBR, but resistant to soybean cyst nematode (*Heterodera glycines* Ichinohe).

Four soybean accessions, PI 200492 (*Rpp1*), PI 594538A (*Rpp1-b*), PI 587886 and PI 587880A, with SBR resistance genes that mapped to the *Rpp1* region on chromosome 18 (Hyten et al. 2007; Chakraborty et al. 2009; Ray et al. 2009), PI 561356, the cultivar Williams 82 as well as 33 major North American soybean ancestors that contribute at least 95 % of the alleles in North America cultivars released from 1947 and 1988 (Gizlice et al. 1994) were used for SNP haplotype analysis in a 213-kb interval surrounding *Rpp1* (Table 1). Seeds of the accessions were obtained from the USDA Soybean Germplasm Collection (Urbana, IL, USA).

P. pachyrhizi isolate evaluation

The 100 $F_{2:3}$ lines were tested for SBR resistance at the USDA-ARS Foreign Disease–Weed Science Research Unit (FDWSRU), Plant Pathogen Containment Facility at Fort Detrick, MD (Melching et al. 1983), under the appropriate permit from the USDA Animal Plant Health Inspection Service. The population was arranged in a randomized complete block design with ten replicates. The experiments included the following known resistant and susceptible checks: PI 200492 (*Rpp1*), L85-2378 (*Rpp1*), PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), PI 459025 (*Rpp4*), G01-PR33 [which carries the SBR resistance gene *Rpp*?(Hyuuga)], and the cultivar Williams (susceptible). The test was initiated by sowing two seeds per cell in flats (6 × 12 cells, 27 × 52 cm) filled with Sunshine LC_1 mix (Sun Grow Horticultural Products, Belleview, WA).

The lines and checks were inoculated with the *P. pachyrhizi* isolate ZM01-1 collected in Zimbabwe during 2001. This isolate was used as the inoculum source to map resistance from PI 561356, because the PI gave a strong RB response to the isolate. Spores of the isolate were routinely increased on "Williams 82" and stored under liquid nitrogen. Inoculum preparation and plant inoculations were conducted as described by Hyten et al. (2007). After inoculation, plants were incubated for 24 h at 20 °C in a dew chamber and then moved to a greenhouse maintained at 20 °C for 14 days until symptoms were ready to be scored. Two leaflets from the first trifoliate of each inoculated plant were rated for resistant RB type or susceptible TAN type. Disease severity based on symptom and lesion development was rated on a scale of 1 (no visible symptom) to 5 (prolific lesions) as described by Miles et al. (2006). The relative percentage of sporulation was also rated on a single plant basis using a scale of 1 (no sporulation) to 5 (76–100 % of the lesions sporulating) as described by Chakraborty et al. (2009). All TAN lesions were sporulating and given a sporulation rating of 5.

PI 200492, PI 594538A, PI 587886, PI 587880A, PI 561356, Williams 82, and 11 North American soybean ancestors belonging to SNP haplotype 1 or 9 (Tables 1, 2) were evaluated for resistance to the *P. pachyrhizi* isolates FL07-1 collected at Quincy, Florida during 2007 and ZM01-1 to test for an association between SNP haplotypes and SBR resistance (Table 2). The test with FL07-1 was conducted at the USDA-ARS Plant Pathogen Containment Facility at Urbana, IL, and the test with ZM01-1 was conducted at the USDA-ARS FDWSRU Plant Pathogen Containment Facility. For the FL07-1 test, at least 12 plants of each PI and Williams 82 were grown in an 11-cm diameter pot in a non-replicated experiment and inoculations were conducted when the first trifoliolate was fully expanded (V1; Fehr et al. 1971). Urediniospores collected from leaves of Williams 82 were suspended in sterile distilled water containing 0.01 % Tween 20 (sodium monolaurate) and inoculated plants were incubated inside a dew chamber set at 20 °C for 24 h (Pham et al. 2009). For the ZM01-1 test, two replications of 12 plants of each PI and Williams 82 were tested and inoculations were conducted as

Table 1 SNP haplotypes and SSR marker alleles for five soybean rust resistance soybean accessions, the cultivar Williams 82, and 33 major North American soybean ancestors in a 213-kb interval including the *Rpp1* region on soybean chromosome 18

PI number	SBR Resistance gene	Origin	Contribution (%)[a]	SNP Haplotype	Marker positions[b] (bp) on soybean chromosome 18																							
					60,404,182	60,408,112	60,423,746	60,427,879	60,431,734	60,441,713	60,456,270	60,460,936	60,476,584	60,488,421	60,492,783	60,511,774	60,516,754	SSR50[c]	SSR66	60,537,689	60,577,930	60,590,718	60,594,034	60,598,485	60,605,215	60,607,758	60,616,971	SSR1859
PI 200492	*Rpp1*	Japan (Shikoku)		16	C	T	C	**G**[d]	G	A	T	T	G	C	C	G	C	1	1	G	C	**T**	C	G	A	T	A	1
PI 594538A	*Rpp1-b*	China (Fujian)		1	T	G	C	A	T	G	G	T	G	T	C	A	C	2	2	G	T	C	C	**A**	A	G	A	2
PI 587886	*Rpp1-?*	China (Zhejiang)		19	C	T	**A**	A	G	A	T	C	A	C	**T**	G	**N**[e]	3	2	G	C	C	C	G	**C**	T	A	2
PI 587880A	*Rpp1-?*	China (Zhejiang)		19	C	T	**A**	A	G	A	T	C	A	C	**T**	G	**N**	3	2	G	C	C	C	G	**C**	T	A	2
PI 561356	*Rpp1-?*	China (Zhejiang)		9	T	G	C	A	G	G	T	C	A	T	C	G	C	2	2	**A**	C	C	C	G	A	G	**C**	2
Williams82	Susceptible check	China		2	T	G	C	A	T	G	G	T	G	T	C	A	C	4	1	G	T	C	C	G	A	G	A	4
PI number	Cultivar name																											
PI 548406	Richland	China (Jilin)	11.3	1	T	G	C	A	T	G	G	T	G	T	C	A	C	2	3	G	T	C	C	A	A	G	A	3
PI 548488	S-100	China (Heilongjiang)	1.8	1	T	G	C	A	T	G	G	T	G	T	C	A	C	4	1	G	T	C	C	A	A	G	A	3
PI 548298	AK(Harrow)	China	6.9	1	T	G	C	A	T	G	G	T	G	T	C	A	C	4	1	G	T	C	C	A	A	G	A	3
PI 548318	Dunfield	China (Jilin)	3.5	1	T	G	C	A	T	G	G	T	G	T	C	A	C	4	3	G	T	C	C	A	A	G	A	3
PI 548484	Ralsoy	N. Korea (Pyongyang)	0.1	1	T	G	C	A	T	G	G	T	G	T	C	A	C	5	4	G	T	C	C	A	A	G	A	3
PI 548438	Arksoy	N. Korea (Pyongyang)	0.0	1	T	G	C	A	T	G	G	T	G	T	C	A	C	5	4	G	T	C	C	A	A	G	A	3
PI 240664	Bilomi No.3	Philippines (Luzon)	0.0	1	T	G	C	A	T	G	G	T	G	T	C	A	C	4	4	G	T	C	C	A	A	G	A	3
PI 548360	Korean	N. Korea	0.8	2	T	G	C	A	T	G	G	T	G	T	C	A	C	1	5	G	T	C	C	G	A	G	A	3
PI 548311	Capital	China (Heilongjiang)	2.4	2	T	G	C	A	T	G	G	T	G	T	C	A	C	2	5	G	T	C	C	G	A	G	A	3
PI 548348	Illini	China	3.1	3	T	G	C	A	T	G	G	T	G	T	C	A	C	1	1	G	T	C	C	A	A	N	A	3
PI 548362	Lincoln	China (Heilongjiang)	24.2	4	T	G	C	A	T	G	G	T	G	T	N	A	C	1	1	G	T	C	C	A	A	N	A	3
PI 548379	Mandarin (Ottawa)	China (Heilongjiang)	17.2	5	T	G	C	A	T	G	G	T	G	T	N	A	C	2	5	G	T	C	C	G	A	N	A	3
PI 438471	Fiskeby III	Sweden (Ostergotland)	0.7	6	T	G	C	A	T	G	G	T	G	T	C	N	C	5	6	A	C	C	C	G	C	G	A	4
PI 548456	Haberlandt	N. Korea (Pyongyang)	0.1	6	T	G	C	A	T	G	G	T	G	T	C	G	N	3	3	A	C	C	C	G	C	G	A	5
PI 438477	840-7-3	Sweden (Ostergotland)	1.1	7	T	G	C	A	T	G	G	T	A	T	T	N	C	5	6	A	C	C	C	G	N	T	A	4
PI 088788	PI 88788	China (Liaoning)	0.4	8	T	G	C	A	T	G	T	T	A	T	C	N	C	6	7	G	T	C	C	G	C	G	C	3
PI 548485	Roanoke	China (Jiangsu)	0.2	8	T	G	C	A	T	G	T	T	A	T	C	N	C	6	7	G	T	C	C	G	C	G	C	3
PI 548477	Ogden	China, Japan	4.3	9	T	G	C	A	G	G	T	C	A	T	C	G	C	3	3	A	C	C	C	G	A	G	C	3
PI 548302	Bansei	Japan (Hokkaido)	1.1	9	T	G	C	A	G	G	T	C	A	T	C	G	C	5	6	A	C	C	C	G	A	G	C	3
PI 548356	Kanro	N. Korea (Pyongyang)	1.0	9	T	G	C	A	G	G	T	C	A	T	C	G	C	5	6	A	C	C	C	G	A	G	C	3
PI 548352	Jogun	N. Korea (Hamgyong)	0.8	9	T	G	C	A	G	G	T	C	A	T	C	G	C	3	3	A	C	C	C	G	A	G	C	3
FC031745	FC 31745	Unknown	0.0	10	T	T	N	A	T	A	T	T	A	T	T	A	A	4	4	A	T	T	A	A	A	T	A	6
PI 548445	CNS	China (Jiangsu)	3.0	11	C	T	C	G	G	G	G	T	A	C	N	G	C	7	3	G	T	N	C	A	A	N	A	3
PI 360955A	Fiskeby V	Sweden (Ostergotland)	0.5	12	C	T	C	G	G	G	G	T	A	C	C	G	C	7	7	G	C	C	C	G	A	G	A	3
PI 548391	Mukden	China (Liaoning)	4.9	12	C	T	C	G	G	G	G	T	A	C	C	G	C	7	7	G	C	C	C	G	A	G	A	3
PI 180501	PI 180501	China	0.8	12	C	T	C	G	G	G	G	T	A	C	C	G	C	7	7	G	C	C	C	G	A	G	A	3
PI 548402	Peking	China (Beijing)	0.1	13	C	T	C	G	G	A	T	C	A	C	C	G	N	7	8	G	C	C	C	G	A	T	A	6
PI 548461	Improved Pelican	China (Fujian)	0.0	14	C	T	C	G	G	A	T	C	A	C	C	G	A	1	1	N	C	C	C	G	A	G	C	3
PI 548382	Manitoba Brown	Canada	1.5	15	C	T	C	G	G	G	T	C	A	C	C	G	C	2	7	G	C	C	C	G	A	G	A	3
PI 071506	PI 71506	China (Jiangsu)	0.1	17	C	T	C	G	G	A	T	C	A	T	C	G	A	.[f]	.	N	T	T	C	G	C	T	A	6
PI 548657	Jackson	China, Japan	0.2	18	C	T	C	G	G	A	T	C	A	T	N	G	A	.	.	N	T	T	C	G	C	T	A	6
PI 080837	PI 80837	Japan (Akita)	0.0	20	C	T	A	A	G	A	T	C	A	C	T	G	N	.	.	N	C	T	A	A	C	T	C	3
PI 548603	Perry	China, Japan	2.1	21	N	T	C	G	G	G	T	T	A	C	T	G	C	.	1	N	C	T	A	A	C	T	C	3

[a] Percentage of contribution of each PI to the northern soybean varieties from Gizlice et al. (1994)

[b] Physical position of the markers based on the *G. max* genome (assembly version 1.01) available at http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01/. The base pair (bp) positions of the SNP markers correspond to the locations of each SNP on soybean chromosome 18

[c] SSR markers used for genetic mapping of the SBR resistance gene in PI 561356. Seven different allele sizes were present for SSR50, eight for SSR66, and six for SSR1859 in the tested 39 soybean accessions

[d] Boldface indicates SNP markers that can differentiate each haplotype from the other three haplotypes among *Rpp1* sources (haplotype tag)

[e] N indicates that no genotype could be assigned

[f] The accession had no PCR product

described by Hyten et al. (2007). The responses to isolates FL07-1 and ZM01-1 (IM, RB or TAN) were evaluated 15 days after inoculation.

Genetic mapping of the SBR resistance in PI 561356

Genomic DNA from the population was extracted using young trifoliolate leaf tissue pooled from at least ten plants from each line using the CTAB method (hexadecyltrimethylammonium bromide) method described by Keim et al. (1988). To find the position(s) of the resistance gene(s) in PI 561356, bulked segregant analysis (BSA) was used (Michelmore et al. 1991). A resistant bulk was formed by pooling an equal amount of DNA from ten lines with RB reactions and a susceptible bulk was formed by pooling DNA from ten lines with TAN reactions. The two parents, resistant bulk and susceptible bulk, were first screened with simple sequence repeat (SSR) markers that mapped near *Rpp1*, *2*, *3*, *4*, and *5*. Once the potential locations of resistance genes were identified, the lines in the population were tested with additional markers from these locations. After genetic mapping of the population, DNA from lines (line 9, 64, 71, 96, and 100; Fig. 1) with recombination events near the gene was extracted from at least 20 plants to confirm the initial genotyping results. Primer sequences of the SSR markers were obtained from SoyBase (http://soybase.org/resources/ssr.php) and Song et al. (2010).

Table 2 Reactions of 17 soybean accessions to two different *Phakopsora pachyrhizi* isolates and their SNP haplotypes and alleles for three SSR markers

PI number	Resistance gene	SNP haplotype[a]	SSR 50[b]	SSR 66[b]	SSR 1859[b]	*P. pachyrhizi* isolate	
						FL07-1	ZM01-1
PI 200492	*Rpp1*	16	1	1	1	IM	TAN
PI 594538A	*Rpp1-b*	1	2	2	2	TAN	RB
PI 587886	*Rpp1*-?	19	3	2	2	TAN	RB
PI 587880A	*Rpp1*-?	19	3	2	2	TAN	RB
PI 561356	*Rpp1*-?	9	2	2	2	TAN	RB
Williams 82	Susceptible check	2	4	1	3	TAN	TAN
PI number	Cultivar name						
PI 548406	Richland	1	2	3	3	TAN	TAN
PI 548488	S-100	1	4	1	3	TAN	TAN
PI 548298	AK(Harrow)	1	4	1	3	TAN	TAN
PI 548318	Dunfield	1	4	3	3	TAN	TAN
PI 548484	Ralsoy	1	5	4	3	TAN	TAN
PI 548438	Arksoy	1	5	4	3	TAN	TAN
PI 240664	Bilomi No.3	1	4	4	3	TAN	TAN
PI 548477	Ogden	9	3	3	3	TAN	TAN
PI 548302	Bansei	9	5	6	3	TAN	TAN
PI 548356	Kanro	9	5	6	3	TAN	TAN
PI 548352	Jogun	9	3	3	3	TAN	TAN

IM immune response (no visible symptoms; resistant reaction), *RB* reddish brown-colored lesions (resistant reaction), *TAN* pale brown-colored lesions (susceptible reaction)

[a] Haplotypes based on 21 SNP markers within the 213-kb interval including the *Rpp1* region on soybean chromosome 18

[b] SSR markers used for genetic mapping of the SBR resistance gene in PI 561356. Seven different allele sizes were present for SSR50, eight for SSR66, and six for SSR1859 in the tested 39 soybean accessions (Table 1)

Polymerase chain reaction (PCR) and evaluation of PCR products were carried out as previously described by Wang et al. (2003). PCR consisted of 36 cycles of denaturation at 94 °C for 25–30 s, annealing at 46–62 °C for 25–30 s, and extension at 68 °C for 25–30 s with a PTC 100 Programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA). The PCR products were analyzed by electrophoresis in both 3 % agarose gels (BMA, Rockland, ME, USA) and 6 % nondenaturing polyacrylamide gels (Wang et al. 2003). The polymorphic information content (PIC) values for SSR50, SSR66, and SSR1859 were calculated by using the formula PIC = $1 - \sum(Pi)^2$, where *Pi* represents the proportion of the soybean genotypes carrying the i^{th} allele (Botstein et al. 1980).

Statistical analysis

The Chi-square tests for SBR lesion type (RB or TAN) and molecular markers were performed to test the goodness of fit of the observed segregation among $F_{2:3}$ lines. Linkage analysis was performed to map the location of a gene controlling SBR lesion type (RB or TAN) with JoinMap 3.0 (Van Ooijen and Voorrips 2001). A logarithm (base 10) of the odds (LOD) score of 5.0 was used as a threshold to group markers into a linkage group. Genomic region(s) associated with disease severity was mapped as quantitative trait loci (QTL) using the interval mapping (IM) functions in MapQTL® 4.0 (Van Ooijen et al. 2002). The LOD score threshold for declaring a putative locus as significant was determined by 1,000 permutations in MapQTL® 4.0. The gene position was defined as the point of maximum LOD score. Analysis of variance in PROC GLM in SAS 9.2 (SAS Institute 2002) was used to test the significance of the association between lesion type and disease severity. Disease severity and sporulation were also analyzed by PROC GLM in SAS 9.2 (SAS Institute 2002). The means for disease severity and sporulation were separated using the least significant difference (LSD) at $P = 0.05$.

SNP haplotype analysis

The SoySNP50 Illumina Infinium chip (Song et al. 2012) was used to genotype the 5 SBR resistance soybean accessions and 33 major North American soybean ancestors in the 213-kb interval including *Rpp1* region

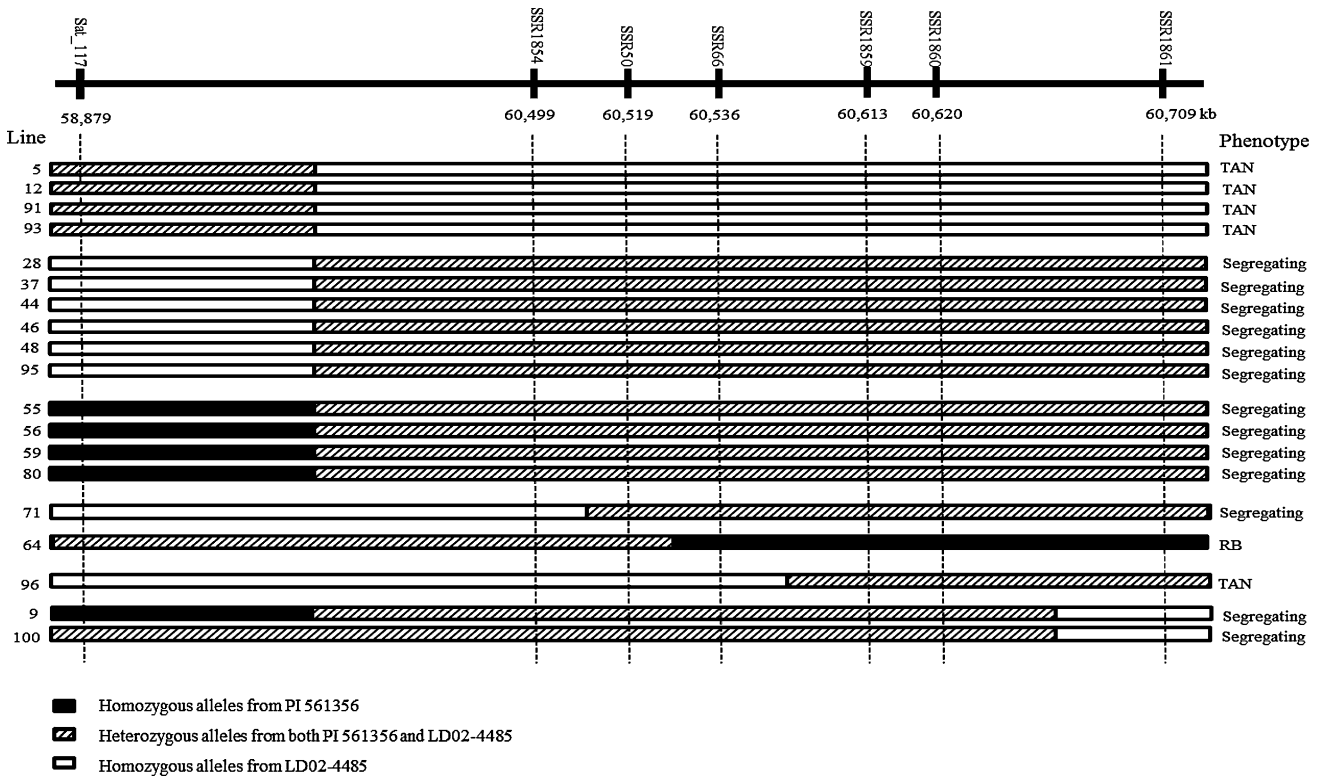


Fig. 1 Graphical genotypes of recombinant lines from PI 561356 × LD02-4485 population in the soybean rust resistance gene interval on soybean chromosome 18. Phenotypes indicate the type of reaction to the *P. pachyrhizi* isolate ZM01-1. RB is the resistant and TAN the susceptible reaction. The lines with segregating phenotype consisted of progeny with RB or TAN reaction. The physical positions of the markers (kb) are based on soybean chromosome 18 sequence of the *G. max* genome (assembly version 1.01) available at http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01/

on chromosome 18. The SoySNP50 Infinium chip contains a total of 52,041 SNPs and the Infinium assay (Song et al. 2012) was used as per the manufacturer's instructions and analyzed using Illumina GenomeStudioV2010.2 software (Illumina, San Diego, CA). The SNP haplotype analysis was conducted using MEGA version 4 (Tamura et al. 2007). The distance between any pair of accessions was calculated based on the percentage of the SNPs carrying different alleles. If a SNP call was missing in one accession, the SNP was only deleted from the comparisons of the SNP in that one accession with its paired comparison with the other accessions and all other paired comparisons for that SNP were included. The neighbor-joining method was used for the construction of trees and a bootstrap with 1,000 replicates was used to measure confidence in the branch.

Results

The *P. pachyrhizi* isolate ZM01-1 produced TAN lesions on PI 200492 (*Rpp1*), PI 462312 (*Rpp3*), G01-PR33 [*Rpp*?(Hyuuga)] and Williams, while it did not overcome the resistance of PI 230970 (*Rpp2*) and PI 459025B (*Rpp4*) (Table 3). The responses of these soybean accessions were consistent with those previously observed by Chakraborty et al. (2009) after inoculation with the same isolate. The parents of the population, PI 561356 and LD02-4485, produced RB and TAN lesions to the ZM01-1 isolate, respectively. There were significant differences for disease severity among the SBR resistance sources. PI 561356 had significantly ($P = 0.05$) less disease severity than all of the resistance sources except PI 200492 (*Rpp1*), and also had significantly less sporulation than all of the other resistance sources (Table 3). PI 230970 (*Rpp2*) and PI 459025B (*Rpp4*), which both gave RB reaction, had less sporulation than the other resistance sources with the TAN lesions (Table 3). These results show that SBR resistance in PI 561356 is more effective in controlling ZM01-1 than genotypes with *Rpp2* and *Rpp4*.

The segregation of SBR lesion types (RB or TAN) for the 100 $F_{2:3}$ lines fit a 3 resistant:1 susceptible segregation ratio ($\chi^2 = 0.12$, $P = 0.73$) when the homozygous resistant and segregating lines were combined into a single class

Table 3 Reaction, disease severity, and sporulation of lines in the PI 561356 × LD02-4485 population, and parents and checks after inoculation with the *Phakopsora pachyrhizi* isolate ZM01-1

Genotype	Reaction	Disease severity[a]	Sporulation[b]
Checks and parents			
PI 200492 (*Rpp1*)	TAN	2.6	5.0
L85-2378 (*Rpp1*)	TAN	3.3	5.0
PI 230970 (*Rpp2*)	RB	3.3	2.9
PI 462312 (*Rpp3*)	TAN	3.1	5.0
PI 459025B (*Rpp4*)	RB	2.8	3.0
G01-PR33 [(*Rpp?*(Hyuuga)]	TAN	2.9	5.0
Williams (susceptible)	TAN	2.8	5.0
PI 561356(*Rpp1*-?)	RB	2.4	1.2
LD02-4485 (susceptible)	TAN	3.0	5.0
LSD ($\alpha = 0.05$)	–	0.4	0.3
Population			
Population mean ($n = 100$)	RB/TAN	2.7	3.1
Homozygous RB line mean ($n = 13$)	RB	2.2	1.1
Segregating line mean ($n = 61$)	RB/TAN	2.7	2.7
Homozygous TAN line mean ($n = 26$)	TAN	3.1	5.0

RB reddish brown-colored lesions (resistant reaction), *TAN* pale brown-colored lesions (susceptible reaction)

[a] Disease severity on a scale of 1 (no visible lesions), 2 (light infection with few lesions present), 3 (light to moderate infection), 4 (moderate to severe infection), and 5 (prolific lesions)

[b] Amount of uredinia sporulation within RB or TAN lesions. Sporulation on a scale of 1 (no sporulation), 2 (<25 % of the lesions sporulating), 3 (26–50 % of the lesions sporulating), 4 (51–75 % of the lesions sporulating), and 5 (76–100 % of the lesions sporulating). All TAN lesions were rated 5 for sporulation

(Table 4). However, when the homozygous resistant and segregating lines are separated, the segregation among the lines does not fit a 1:2:1 segregation ($\chi^2 = 8.22$, $P = 0.02$) (Table 4). The segregation of individual resistant and susceptible plants in the segregating lines also fit to 3 resistant:1 susceptible ratio ($\chi^2 = 1.04$, $P = 0.59$), indicating that resistance from PI 561356 is controlled by a single, dominant gene.

Lines selected with RB and TAN lesions were used in BSA with markers linked to known SBR resistance genes. In the BSA, SSR markers near *Rpp1* and *Rpp4* were associated with the SBR lesion type. All lines in the population were then genotyped with all available polymorphic SSR markers near the two genes, and the SSR markers Satt503, Satt288, and Sat_117 near *Rpp1* were genetically linked to lesion type while markers linked to *Rpp4* were not associated with resistance. This shows that PI 561356 has a major SBR resistance gene in the *Rpp1* region and that the PI does not have a major allele at *Rpp4* or linked to *Rpp4*. Unfortunately, the SSR markers, Sct_187, Sat_064, and Sat_372, which were used in previous mapping of *Rpp1* and *Rpp1-b*, did not show polymorphisms between the two parents, PI 561356 and LD02-4485. To identify additional markers in the *Rpp1* region that are polymorphic between our two parents, seven BARCSOYSSR markers located in this region (Song et al. 2010) as well as the several SSR markers designed based on super contig 112 of the Williams 82 sequence but not listed in Song et al. (2010) were tested. A total of six SSR markers, BARCSOYSSR_18_1854 (SSR1854), SSR50, SSR66, BARCSOYSSR_18_1859 (SSR1859), BARCSOYSSR_18_1860 (SSR1860) and BARCSOYSSR_18_1861 (SSR1861), were identified as polymorphic in this screening and all lines in the population were then genotyped with these SSR markers (Table 5).

All SSR markers used to map the resistance in the population fit a 3:1 segregation when those lines homozygous for the allele from the resistant parent and the segregating lines were combined in one class and compared to lines homozygous for the allele from the susceptible parent (Table 4). The segregation of Satt503, Satt288, and Sat_117 in the population fit a 1 resistant:2 segregating:1 susceptible ratio, but the SBR lesion type and the other markers did not due to the presence of fewer than expected homozygous resistant lines and more than expected heterozygous lines in the population (Table 4).

SBR lesion type in the population was mapped as a qualitative trait with the data from the population in which the homozygous resistant, segregating, and homozygous susceptible lines were in separate classes using JoinMap 3.0. This mapping placed the resistance gene from PI 561356 into a 1-cM region on soybean chromosome 18, flanked on one side by the marker SSR50 and on the other

Table 4 Inheritance of soybean rust resistance (RB or TAN reaction) to the *Phakopsora pachyrhizi* isolate ZM01-1 and the SSR markers used to map the SBR resistance gene in the population of 100 $F_{2:3}$ lines from the cross PI 561356 × LD02-4485

Locus	Expected ratio	Observed ratio	Chi-square	Probability
Satt503	3:1[a]	72:28	0.34	0.56
Satt288	3:1	76:24	0.01	0.94
Sat_117	3:1	72:28	0.34	0.56
SSR1854	3:1	73:27	0.21	0.64
SSR50	3:1	74:26	0.12	0.73
SSR66	3:1	74:26	0.12	0.73
SBR resistance	3:1	74:26	0.12	0.73
SSR1859	3:1	75:25	0.00	1.00
SSR1860	3:1	75:25	0.00	1.00
SSR1861	3:1	73:27	0.21	0.64
Satt503	1:2:1[b]	21:51:28	1.00	0.61
Satt288	1:2:1	21:55:24	1.18	0.55
Sat_117	1:2:1	17:55:28	3.42	0.18
SSR1854	1:2:1	12:61:27	9.34	0.01
SSR50	1:2:1	12:62:26	9.68	0.01
SSR66	1:2:1	13:61:26	8.22	0.02
SBR resistance	1:2:1	13:61:26	8.22	0.02
SSR1859	1:2:1	13:62:25	8.64	0.01
SSR1860	1:2:1	13:62:25	8.64	0.01
SSR1861	1:2:1	13:60:27	7.92	0.02

[a] 3 homozygous resistant (R) and segregating (H) lines:1 homozygous susceptible (S) lines

[b] 1 Homozygous resistant (R) lines:2 segregating (H) lines:1 homozygous susceptible (S) lines

Table 5 List of new SSR and BARCSOYSSR markers used to map the SBR resistance gene in PI 561356

Name	Sequence ID	Product size[a]	Primer	Sequences	Positions[b]
SSR1854	BARCSOYSSR_18_1854[c]	183	Forward Reverse	TGGTTCTTTCAATTTAATATGC AAGTAGAATGTACGAATTATTG	60,499,775–60,499,957
SSR50	–	226	Forward Reverse	AGCACTAACAACTTTCTTTG GTTCTTAAATCTTACCCTCAC	60,518,978–60,519,203
SSR66	–	279	Forward Reverse	AGATTGGGTGAGAACATAAG GGAGAGCGTAAAAGAAATTC	60,535,339–60,535,617
SSR1859	BARCSOYSSR_18_1859	294	Forward Reverse	CTCAATCGCATCCTTGCATA GCCTTCCAACTCATGTTTCAA	60,613,084–60,613,377
SSR1860	BARCSOYSSR_18_1860	145	Forward Reverse	AGACATTCGTTGCAAAAGCC TTAGCCCTTCCCAAGAAACA	60,619,935–60,620,079
SSR1861	BARCSOYSSR_18_1861	247	Forward Reverse	TGCCACAATGTCCACAACTT CCCTTTTCTTTTGCCTCTCC	60,709,612–60,709,858

[a] PCR product sizes are based on the *G. max* genome (assembly version 1.01) available http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01/

[b] Physical position of the markers based on the *G. max* genome (assembly version 1.01) available at http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01/. The base pair (bp) positions of the markers correspond to the locations of forward and reverse primer on soybean chromosome 18

[c] BARCSOYSSR sequence ID are based on Song et al. (2010). Forward and reverse primer sequences of the SSR1854 in our study are different from the sequences in Song et al. (2010), although the SSR motifs are the same. The primer pairs used in the present study were first developed based on the sequence of super contig 112 before BARCSOYSSR_18_1854 was released by Song et al. (2010)

side by SSR1859 or SSR1860 (Fig. 1). No recombination was observed between SSR66 and SBR resistance as well as between SSR1859 and SSR1860. The lack of recombination between SSR1859 and SSR1860 is not surprising considering these two markers are only 6.7 kb apart based on the *G. max* genome (assembly version 1.01) (http://soybase.org). Graphical genotypes of the 19 lines with recombination events near the resistance gene also supported its location between SSR50 and SSR1859 (Fig. 1). The physical distance between SSR50 and SSR1859 based on the *G. max* genome (assembly version 1.01) is 94.4 kb (http://soybase.org).

There was a significant ($P < 0.0001$; $R^2 = 0.70$) association between SBR lesion type (RB or TAN) and disease severity in the population with homozygous RB lines having a severity rating of 2.2 and homozygous TAN lines

having a rating of 3.1 (Table 3). Similarly, homozygous RB lines have significantly less sporulation than homozygous TAN lines (Table 3). Quantitative trait loci (QTL) controlling disease severity from PI 561356 were mapped using the interval mapping function in MapQTL 4.0 and the LOD peak for the trait mapped to the same interval as the SBR lesion type. SSR66 has the highest LOD score for disease severity (LOD = 26.2) and the percentage of the variance explained by the segregation for the marker was 70.1. A QTL for sporulation was also mapped to the same interval (data not shown), which was expected because all lines in the population with a TAN reaction were given a sporulation rating of 5.0. This resulted in the sporulation and lesion type ratings being non-independent. The most likely explanation for these results is that the allele from PI 561356 that confers RB lesion type also confers reduced severity and sporulation, although we cannot completely exclude the possibility that these phenotypes are controlled by more than one gene.

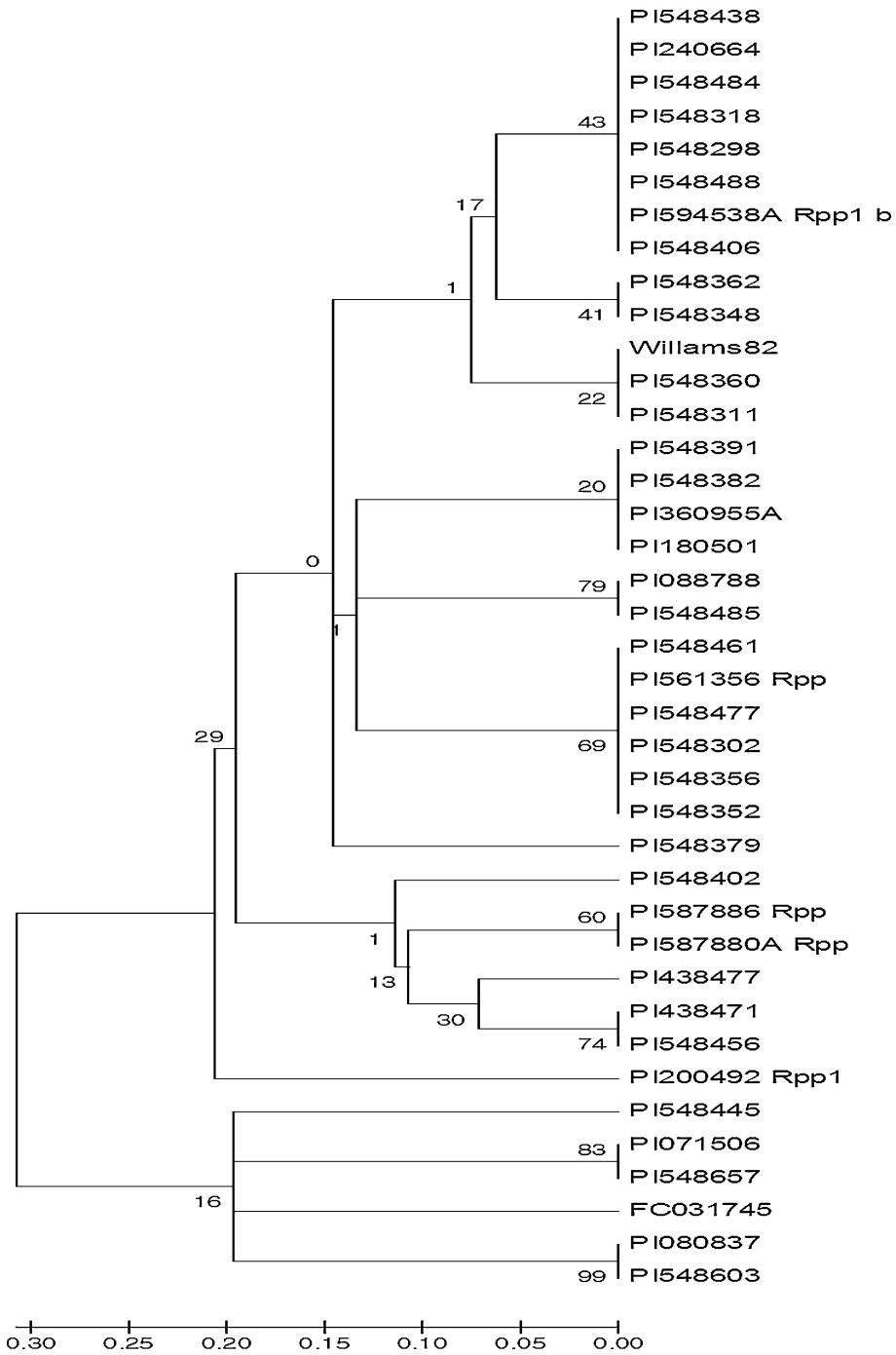
Thirty-nine soybean accessions including the 33 major North American soybean ancestors, Williams 82, and 5 SBR resistance sources with resistance genes mapping to the *Rpp1* region were analyzed to identify SNP haplotypes across a 213-kb interval on chromosome 18 that includes *Rpp1* (Table 1). Twenty-one SNP markers located across this interval formed 21 distinct haplotypes including 4 haplotypes among the 5 SBR-resistant PIs (Table 1). PI 200492 (*Rpp1*) belongs to SNP haplotype 16, PI 594538A (*Rpp1-b*) belongs to haplotype 1, PI 587886 and PI 587880A form haplotype 19, and PI 561356 belongs to haplotype 9. Haplotypes 16 and 19 were unique to these resistant sources, as none of the ancestral soybean ancestors had these haplotypes. In contrast, seven North American soybean ancestors belong to haplotype 1 along with PI 594538A (*Rpp1-b*) and the four ancestors belong to haplotype 9 with PI 561356 (Table 1). Haplotype analysis using only the eight SNP markers located within the 94.4-kb region between SSR50 and SSR1859 where the SBR resistance gene from PI 561356 mapped formed 16 haplotypes (Fig. 2). Although there were changes in the total number of haplotypes compared to the result from the 213-kb region analysis, the five SBR resistance sources formed the same four unique haplotypes. One key difference was that PI 548461 (Improved Pelican) was grouped to the same haplotype with PI 561356 in the 94.4-kb region analysis (Fig. 2). There are a total of nine SNPs (haplotype tags) that are unique for specific haplotypes among the five SBR resistance sources in the 213-kb region and five of them are located in the 94.4-kb region (Table 1). A dendrogram based on the eight SNP markers in the 94.4-kb region surrounding *Rpp1* showed clustering of the resistance sources PI 587886 and PI 587880A, which was expected because they had the same haplotype. The other sources did not cluster suggesting that these resistance alleles are not the result of a recent divergence from an ancestral *Rpp1* allele.

The three SSR markers (SSR50, SSR66, and SSR1859) that flank or co-segregate with the SBR resistance gene in PI 561356 could distinguish the SBR resistance sources and SSR66 and SSR1859 could distinguish the SBR resistance sources from the ancestral accessions better than the SNP markers (Table 1; Fig. 3). The SSR marker SSR50 separated the five SBR resistance sources into three groups, and SSR66 and SSR1859 separated these resistance sources into two groups (Table 1; Fig. 3). SSR1859 was the only marker that separated the resistance sources into groups that included none of the ancestral sources. A pattern similar to that observed for the SNP haplotypes was found with three markers for PI 200492. This was the only accession with resistance to FL07-1 and it had a unique allele for the markers compared to the other resistance sources (Table 2; Fig. 3). SSR66 was also predictive of resistance to ZM01-1, as this marker gave unique alleles to PI 594538A, PI 587886, PI 587880A and PI 561356, which are the only accessions with resistance to this *P. pachyrhizi* isolate (Table 2; Fig. 3). The PIC values of SSR50, SSR66, and SSR1859 were 0.83, 0.85, and 0.50, respectively.

The 5 SBR resistance sources, Williams 82, and 11 North American soybean ancestors belonging to SNP haplotype 1 or 9, which were chosen because resistance sources had these haplotypes, were evaluated with the *P. pachyrhizi* isolates, FL07-1 and ZM01-1, to test for an association between SBR resistance and SNP haplotypes or SSR marker genotypes (Table 2). The only evidence of an association between SNP haplotypes and resistant reactions was that PI 200492 (*Rpp1*, haplotype 16) had a unique haplotype and was the only accession that showed a resistant reaction to FL07-1 (Table 2). There was no association between haplotypes and reactions to ZM01-1, as the SBR-resistant sources PI 594538A, PI 587886, PI 587880A, and PI 561356 showed RB reactions, while all other accessions, including accessions with the same haplotype as PI 594538A and PI 561356, gave TAN reactions (Table 2).

Discussion

The SBR resistance gene controlling lesion type (RB or TAN), disease severity, and sporulation from PI 561356 was mapped onto soybean chromosome 18 in this study. SBR resistance alleles from the other four soybean accessions were previously mapped to the same region on chromosome 18 (Hyten et al. 2007; Chakraborty et al. 2009; Ray et al. 2009). Although we could not use the same markers as in the previous studies, the physical locations of these markers on the *G. max* genome (assembly version 1.01) (http://soybase.org) could be directly compared.



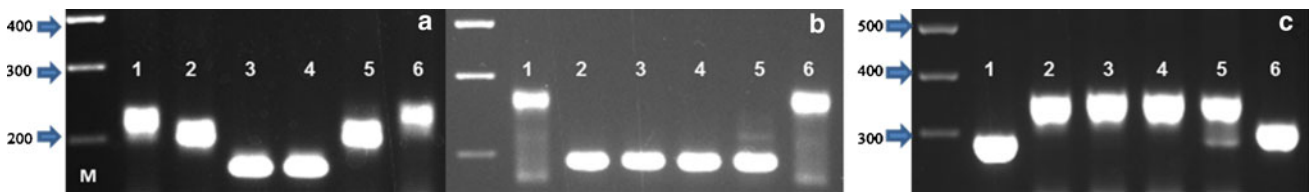


Fig. 2 Dendrogram showing similarity coefficients and genetic relationships among 39 soybean genotypes analyzed by eight SNPs in a 94.4-kb region between SSR50 and SSR1859 on chromosome 18. The digits at nodes are the percent occurrence in 1,000 bootstrap replications

Fig. 3 Agarose gel electrophoresis of SSR50 (**a**), SSR66 (**b**), and SSR1859 (**c**) for the five soybean rust-resistant soybean accessions and Williams 82. *M* molecular size marker. Size markers are the 200, 300, 400, and 500 bp fragments of a 1-kb DNA ladder. *Lane 1* PI 200492 (*Rpp1*), *lane 2* PI 594538A (*Rpp1-b*), *lane 3* PI 587886, *lane 4* PI 587880A, *lane 5* PI 561356, and *lane 6* Williams82

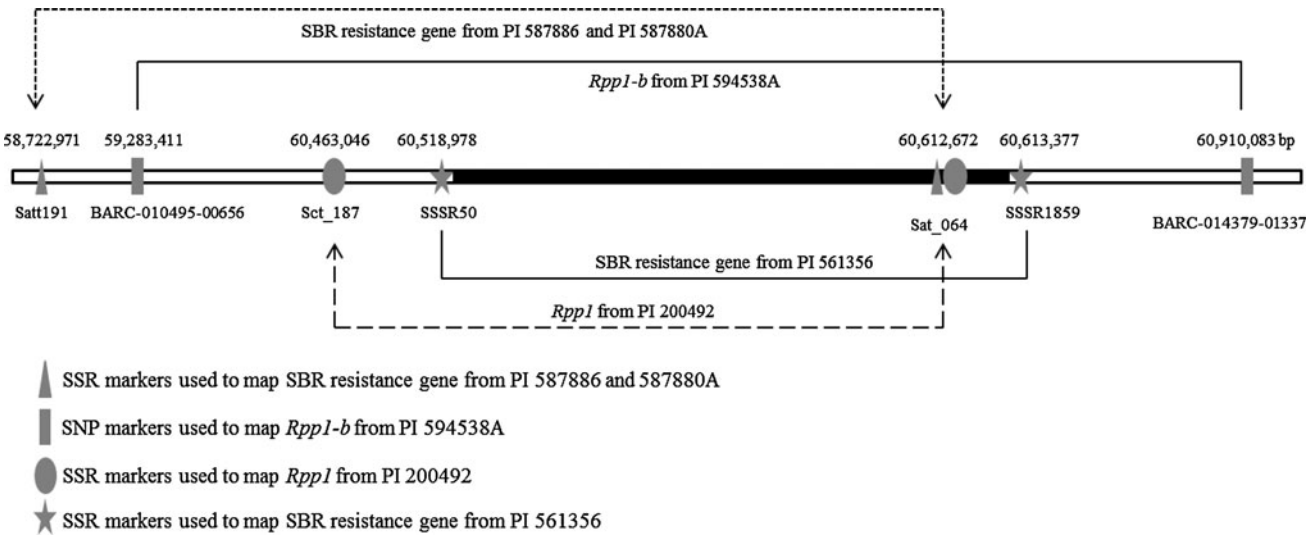


Fig. 4 Physical positions of the soybean rust resistance gene regions from five soybean accessions with the genes that map to the *Rpp1* region on chromosome 18. Physical positions of the flanking SSR or SNP markers used to genetically map *Rpp1* from PI 200492 (Hyten et al. 2007), *Rpp1-b* from PI 594538A (Chakraborty et al. 2009), the SBR resistance gene from PI 587886 and PI 587880A (Ray et al. 2009), and the SBR resistance gene from PI 561356 are based on soybean chromosome 18 sequence of the *G. max* genome (assembly version 1.01) available at http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1

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 mapped to a 1,626.7-kb interval between BARC-010495-00656 and BARC-014379-01337, and Ray et al. (2009) mapped alleles from two PIs to a 1,889.7-kb interval between Satt191 and Sat_064 (Fig. 4). We mapped the SBR resistance gene from PI 561356 to a 94.4-kb interval between SSR50 (60,518,978) and SSR1859 (60,613,311) (Figs. 1; 4). A comparison of the interval that resistance genes were mapped across the five sources reveals an overlapping 93.6-kb interval between SSR50 and Sat_064 that resistance from each source maps. Therefore, it is possible that all five sources have a resistance allele at the same locus in this interval (Fig. 4). It is still possible, however, that resistance alleles from these sources could be at different tightly linked loci. Fine mapping or cloning of the resistance genes from these sources will be required to separate these two possibilities.

Schmutz et al. (2010) reported that the average ratio of genetic-to-physical distance is 1 cM per 197 kb in euchromatic regions and 1 cM per 3.5 Mb in heterochromatic regions in soybean. Ninety-three percent of the recombination occurs in repeat-poor, gene-rich euchromatic genomic regions that only accounts for 43 % of the genome. The *Rpp1* region is located in a gene-rich interval outside the pericentromeric region on chromosome 18 (http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01). In our study, the SBR resistance gene from PI 561356 was genetically mapped to a 1-cM interval which corresponds to a 94.4-kb physical distance on Williams 82 chromosome 18 (http://soybase.org). The ratio of genetic-to-physical distances of the *Rpp1* region in PI 200492 was 187 kb cM^{-1}, *Rpp1-b* in PI 594538A was 290 kb cM^{-1}, and the SBR resistance gene regions in PI 587886 and PI 857880A was 116 kb cM^{-1}. Differences in the genetic-to-physical distances in these regions are likely the result of the small genetic distances sampled and relatively large errors in these genetic distance estimates caused by the small populations used in these studies. The relatively high recombination rate in the *Rpp1* interval suggests that this would be a good candidate region for positional cloning.

The SBR resistance gene from PI 594538A was given the allelic designation *Rpp1-b* because, although it mapped to the *Rpp1* region, the gene from PI 594538A gave an RB response to the *P. pachyrhizi* isolate ZM01-1, while *Rpp1* produced a TAN reaction (Chakraborty et al. 2009). The resistance reactions of PI 561356 were the same as PI 594538A for ZM01-1, FL07-1, and the four isolate mixture tested by Miles et al. (2006). This consistency of resistance reactions and mapping of resistance genes to the same interval suggests that these two sources may have the same resistance allele. The SBR resistance genes in the *Rpp1* region from PI 587886 and PI 587880A were found to have a similar resistance pattern as PI 594538A for FL07-1 and ZM01-1 in our test, and for six additional *P. pachyrhizi* isolates in a test reported by Ray et al. (2009). There is some evidence suggesting that PI 587886 and PI 587880A may not have *Rpp1-b*, as one isolate tested by Ray et al. (2009) gave a mixed IM/RB reaction for PI 587886 and PI 587880A, while PI 594538A gave a RB reaction. Miles

et al. (2006) showed that both PI 561356 and PI 594538A gave mixed reactions to a mixture of four *P. pachyrhizi* isolates, while PI 587886 and PI 587880A gave RB reactions.

The relative genetic positions of the markers used to map the SBR resistance gene in PI 561356 were consistent with their genetic and physical locations on the *G. max* consensus map 4.0 and the *G. max* genome (assembly version 1.01) (http://soybase.org; Figs. 1; 4). The current gene annotation of the 94.4-kb region between SSR50 and SSR1859 on the *G. max* genome (assembly version 1.01) predicts the presence of nine high-confidence genes (verified 16 August 2011). Of the predicted genes, Glyma18g51700 and Glyma18g51750 are nucleotide-binding site (NBS)—leucine-rich repeat (LRR) genes encoding a leucine-rich transmembrane protein. Nucleotide and peptide similarity between the two genes are 93 and 88 %, respectively (http://soybase.org; http://www.phytozome.net). Their protein sequences share homology with BED finger NBS-LRR resistance protein in black cottonwood (*Populus trichocarpa* Torr. & Gray) (*E* value: 2e−118) and putative disease resistance protein RPM1 in castor bean (*Ricinus communis* L.) (*E* value: 4e−100) (http://www.ncbi.nlm.nih.gov/). There also is evidence that SBR resistance genes in soybean might be in the NBS-LRR gene family, as Meyer et al. (2009) identified an NBS-LRR as a single candidate gene for *Rpp4* in PI 459025B. Monteros et al. (2010) narrowed the *Rpp*?(Hyuuga) interval down to a 371-kb region and reported that this region also included NBS-LRR and LRR genes. Although Glyma18g51700 and Glyma18g51750 are good candidate genes for SBR resistance in PI 561356, the gene annotation is from Williams 82, which does not have resistance to SBR. Therefore, this gene and other candidate genes in the region need to be cloned from their resistance sources and complemented in a susceptible background to identify the coding sequence for *Rpp1*.

SNP haplotype analyses were previously conducted across intervals containing *Rpp*?(Hyuuga) and *Rpp3*, which both map to the same region on chromosome 6. Monteros et al. (2010) tested a 800-kb region where *Rpp*?(Hyuuga) maps with five SNP markers and Hyten et al. (2009) tested a 897-kb interval which includes *Rpp3* from PI 462312 with 275 SNPs. In both studies, there was no haplotype tag that could differentiate *Rpp*?(Hyuuga) from *Rpp3*, which was previously mapped to the same region on chromosome 6 by Hyten et al. (2009). In our study, 21 SNP markers mapping within the *Rpp1* region produced 4 distinct SNP haplotypes among the 5 *Rpp1* sources, as well as nine haplotype tags, were identified (Table 1).

There was no clear association between the SNP haplotypes in the *Rpp1* region, the origin of the accessions, or SBR resistance reactions to two *P. pachyrhizi* isolates (Tables 1, 2). PI 200492 originated from Shikoku, Japan, while PI 594538A was collected from Fujian, China, and PI 587886, PI 587880A, and PI 561356 were from Zhejiang, China. Haplotype 1 included PI 594538A and seven North American soybean ancestors and these were collected from China, North Korea, or the Philippines. Four North American ancestral genotypes and PI 561356 belonged to haplotype 9 and these were collected from China, Japan, or North Korea (Table 1). Although these 11 major North American soybean ancestors belong to the same SNP haplotypes with PI 594538A or PI 561356, they produced TAN lesions to both *P. pachyrhizi* isolates, while PI 594538A and PI 561356 produced RB lesions to ZM01-1 (Table 2).

There were no SNP markers or haplotypes that could distinguish between the 5 SBR-resistant accessions and the 33 susceptible ancestral accessions. In contrast, the SSR markers were more informative than the SNP markers and SSR1859 produced PCR products unique for only SBR resistance sources (Table 1; Fig. 3). SSR1859 produced two differently sized PCR products among the five resistance sources tested and these were different from those produced by any of the susceptible accessions. The marker SSR66 could differentiate the resistance sources, except PI 200492 (*Rpp1*), from the 33 major North American soybean ancestors (Table 1). The allele from PI 200492 is shared with Williams 82 and many other susceptible accessions. There were no SNP and SSR markers in the *Rpp1* region that could differentiate between PI 587886 and PI 587880A, suggesting that these two PIs may have the same resistance allele in the *Rpp1* region (Table 1; Fig. 3). These results suggest that SSR66 and SSR1859 could be useful in predicting whether SBR-resistant accessions with unknown resistance genes have the same resistance allele in the *Rpp1* region as the five known sources used in the current study.

PI 200492 has a unique SNP haplotype, SSR genotype, and resistance response compared to the other four resistance sources with resistance in the *Rpp1* region. This indicates that the *Rpp1* region from PI 200492 has undergone a separate evolutionary path compared to the other sources with resistance genes mapping to the *Rpp1* region.

Although single SBR resistance genes such as *Rpp1*, *1-b*, *2*, *3*, *4*, *5*, and *6* can be introduced into North American elite cultivars by backcrossing in a relatively short time, *P. pachyrhizi* isolates have overcome or will likely overcome any single gene resistance in the future. Therefore, it is important to continue screenings to identify novel SBR resistance sources. Breeding the right combination of resistance alleles at *Rpp1* and other loci including alleles for partial resistance using the flanking markers may lead to increased durability of resistance to SBR and could be effectively used in the other soybean breeding programs.

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