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ORIGINAL PAPER

Identification of a new soybean rust resistance gene in PI 567102B

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Abstract Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. and P. Syd. is one of the most economically important diseases of soybean (*Glycine max* (L.) Merr.). Durable resistance to *P. pachyrhizi* is the most effective long-term strategy to control SBR. The objective of this study was to investigate the genetics of resistance to *P. pachyrhizi* in soybean accession PI 567102B. This accession was previously identified as resistant to SBR in Paraguay and to *P. pachyrhizi* isolates from seven states in the USA (Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas). Analysis of two independent populations, one in which F_2 phenotypes were inferred from F_2-derived F_3 ($F_{2:3}$) families and the other in which F_2 plants had phenotypes measured directly, showed that the resistance in PI 567102B was controlled by a single dominant gene. Two different isolates (MS06-1 and LA04-1) at different locations (Stoneville, MS and Ft. Detrick, MD) were used to independently assay the two populations. Linkage analysis of both populations indicated that the resistance locus was located on chromosome 18 (formerly linkage group G), but at a different location than either *Rpp1* or *Rpp4*, which were previously mapped to this linkage group. Therefore, the SBR resistance in PI 567102B appeared to be conditioned by a previously unreported locus, with an underlying single dominant gene inferred. We propose this gene to be designated *Rpp6*. Incorporating *Rpp6* into improved soybean cultivars may have wide benefits as PI 567102B has been shown to provide resistance to *P. pachyrhizi* isolates from Paraguay and the US.

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Introduction

Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. and P. Syd. is one of the most economically important diseases of soybean (*Glycine max* (L.) Merr.). It was first reported in Japan in 1903 (Hennings 1903). The pathogen has spread from Asia to Africa (Ono et al.1992; Rytter et al. 1984), South America (Morel et al. 2004; Yorinori et al. 2005) and North America (Schneider et al. 2005). The first discovery of *P. pachyrhizi* in the US was in Hawaii in 1994 (Killgore et al. 1994), and the disease was first detected in the continental United States in Louisiana in 2004 (Schneider et al. 2005).

Soybean rust can result in yield losses as high as 80% (Ogle et al. 1979; Yang et al. 1990, 1991; Sinclair and Hartman 1999). Since its arrival in 2004, the impact of SBR on soybean production in North America has been limited to the southern US. Substantial yield losses were reported in some fields in Alabama and Georgia in 2005 and in Louisiana in 2006, as well as in some research plots in Georgia, Louisiana, Florida, and South Carolina (Sikora and Hershman 2007). In 2009, yield losses of between 8 and 25% attributed to SBR were first reported in Mississippi (Mississippi State University Extension Service (http://msucares.com/crops/soybeans/rust/index.html).

In 1991, Yang et al. predicted that yield losses could exceed over 10% in the US soybean growing areas, with losses of up to 50% in the Mississippi Delta and

southeastern coastal states (Yang et al. 1991). Since soybean is susceptible to *P. pachyrhizi* at any stage of development (Melching et al. 1989) and *P. pachyrhizi* can move long distances very quickly via wind-borne urediniospores to infect soybean, it is important to develop disease management strategies to control SBR.

Since no commercial soybean cultivars grown in the US are reported to possess resistance to *P. pachyrhizi*, chemical treatments with fungicides have been used as the first line of defense to minimize the impact of SBR (Levy 2005). While proper fungicide applications can manage SBR effectively, fungicides increase production costs and are not an environmentally friendly approach. Fungicide treatments may also have unforeseen consequences due to activity on non-target beneficial fungi important in keeping soybean pests from reaching damaging levels (Ragsdale et al. 2008). Furthermore, some *P. pachyrhizi* populations have exhibited increased tolerance to certain fungicides (Godoy 2009). Development of high-yielding soybean cultivars with resistance to *P. pachyrhizi* is the most effective long-term strategy to control SBR.

Four single dominant genes conditioning soybean resistance to *P. pachyrhizi* (*Rpp* genes) were reported in the 1980s. These are *Rpp1* (McLean and Byth 1980), *Rpp2* (Bromfield and Hartwig 1980), *Rpp3* (Bromfield and Melching 1982; Hartwig and Bromfield 1983), and *Rpp4* (Hartwig 1986). Recently, a new locus, *Rpp5*, was reported by Garcia et al. (2008). *Rpp1* and *Rpp4* have been mapped to two different loci on chromosome 18 (formerly linkage group (LG) G; Hyten et al. 2007; Silva et al. 2008), *Rpp2* was mapped to chromosome 16 (LG J) by Silva et al. (2008), *Rpp3* was mapped to chromosome 6 (LG C2) by Hyten et al. (2009), and *Rpp5* was mapped to chromosome 3 (LG N) by Garcia et al. (2008). In addition, Monteros et al. (2007) mapped the *Rpp?* (Hyuuga) resistance allele to the vicinity of the *Rpp3* locus. Recently, it was reported that Hyuuga also carried another resistance gene, which mapped to chromosome 3 based on the additional screening of Hyuuga-derived recombinant inbred lines (Kendrick et al. 2011). Chakraborty et al. (2009) mapped a gene from PI 594538A (*Rpp1-b)* that was distinct from *Rpp1* to the same region as *Rpp1*, and Ray et al. (2009) mapped resistance genes from PI 587880A and PI 587886 that have different specificities from *Rpp1* and *Rpp1b* to the same region of the genome. Three recessive genes conferring resistance to *P. pachyrhizi* have also been reported (Calvo et al. 2008; Ray et al., 2011). Since none of the known *Rpp* genes provides resistance against all isolates of *P. pachyrhizi* (Hartman et al. 2005), mapping of *Rpp* genes offers breeders the opportunity to pyramid two or more *Rpp* genes to obtain broader and/or more durable resistance (Pedersen and Leath 1988). Besides single-gene resistance, partial resistance expressed as delayed and/or reduced SBR growth and sporulation may be controlled by genes with minor effects (Wang and Hartman 1992; Hartman et al. 2005).

The objective of this study was to investigate the genetics of resistance to *P. pachryhizi* in PI 567102B. This soybean accession was previously identified as resistant to three *P. pachyrhizi* isolates from Mississippi (Li 2009a; Li and Young 2009), as well as to isolates from Alabama, Florida, Georgia, Louisiana, South Carolina, and Texas (Li et al. unpublished data 2010, 2011; Walker et al. 2011) and to pathogenic field populations in Paraguay (Miles et al. 2008).

Materials and methods

Plant materials and population development

PI 567102B (Germplasm Resources Information Network (GRIN); http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1501610) is a maturity group (MG) IX soybean accession from Indonesia that was identified as resistant to *P. pachyrhizi* in previous inoculated assays at Ft. Detrick, MD (Miles et al. 2006), Capitán Miranda, Paraguay (Miles et al. 2008), and at Stoneville, MS (Li 2009a). DS-880 (PI 659348) is a MG V soybean breeding line that was released as a germplasm by the USDA-ARS in 2010 because of its resistance to multiple diseases, including soybean cyst nematode (*Heterodera glycines* Ichinohe), and high yield potential in the mid-southern US. More information about DS-880 can be obtained in GRIN (http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=PI+659348). The cross DS-880 × PI 567102B was made at the USDA-ARS facility at Stoneville, MS in 2006. Multiple F_1 plants were grown from December 2006 to April 2007 at the USDA-ARS Tropical Agriculture Research Station at Isabela, Puerto Rico. The F_2 seed from each F_1 plant were planted in separate rows in the field at Stoneville, MS in May 2007 to confirm appropriate F_2 segregation for each F_1-derived row. F_2 plants derived from each F_1 plant were segregating for pod color (brown and tan) and maturity as expected.

Seed from one F_1 plant were planted in the field at Stoneville in April 2009. Leaf tissue was harvested from each F_2 plant for DNA extraction. Individual F_2 plants were harvested at maturity to form F_2-derived F_3 ($F_{2:3}$) families. The $F_{2:3}$ families were screened in 2009 and 2010 at Stoneville for their reaction to a 2006 Mississippi isolate of *P. pachyrhizi* (MS06-1). Seed from a separate and independent F_1 plant were sent to Ft. Detrick, MD in 2011 for F_2 seedling leaf sampling and assays with a 2004 *P. pachyrhizi* isolate from Louisiana (LA04-1). Hence, two separate and independent populations were developed from the same parents and were assayed with different isolates.

Evaluation of $F_{2:3}$ families

A total of 104 $F_{2:3}$ families derived from the cross DS-880 × PI 567102B and the parental lines were evaluated in the USDA-ARS Crop Genetics Research Unit at Stoneville, MS during each winter of 2009 and 2010 (Table 1). One seed of each of the soybean lines was planted in individual Jiffy Poly-Pak™ pots (Hummert, St. Louis, MO) in a flat (27 × 52 cm) that contained 5 × 10 pots. Ten seed from each family and seed of the parental lines were planted each year in a randomized complete block design with two replications where year served as replication. Across years the randomization of families was independent. Each parental line was planted three times each year. Sun Grow Metro Mix™ 360 medium (Sun Grow Horticulture Products, Belleview, WA) was used for filling the pots. The plants were placed in Conviron growth chambers (Model PGR15, Conviron Inc. Pembina, ND) under a 16-h photoperiod with a light intensity of 433 μE m^{-2} s^{-1} at a constant 25 ± 2°C. The plants were watered daily from above.

A *P. pachyrhizi* isolate (MS06-1) collected from kudzu leaves in Jefferson County, MS in August 2006, was used to phenotype the $F_{2:3}$ population. The identity of the isolate was confirmed by microscopy, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), as previously described (Li et al. 2007). Urediniospores were increased on a susceptible soybean cultivar, "Williams 82", in the Stoneville Research Quarantine Facility in Mississippi (Li 2009a). The isolate was then purified by picking a single uredinium using a fine needle under an Olympus SZX12 dissecting microscope, suspending the spores in 0.01% (v/v) Tween™ 20 (sodium monolaurate) in sterile distilled water, and then reinoculating a newly developed leaflet of Williams 82. This inoculation–isolation cycle was repeated four times. Urediniospores from this purified culture were harvested using a Cyclone Surface Sampler™ (Burkard Manufacturing Co. Ltd, UK) connected to a vacuum pump, beginning 10–14 days after inoculation (DAI) and continuing at weekly intervals.

Inoculation was performed on 21-day-old seedlings as previously described (Li 2009a, b). Inocula were prepared using freshly collected urediniospores from Williams 82. Spore suspensions were made using sterile distilled water containing 0.01% (v/v) Tween™ 20, mixed, and filtered through a 100-μm cell strainer (BD Biosciences, Bedford, MA) to remove any debris and clumps of urediniospores. Urediniospores were quantified using a hemocytometer and then diluted to a final concentration of 30,000 per mL. Inoculation was at the rate of 1 mL of spore suspension per plant and was applied with a Preval™ sprayer (Yonkers, NY). After inoculation, the plants were placed in a dew chamber in the dark at 22°C overnight (approximately 16 h) and were then moved to Conviron growth chambers, where temperatures were maintained at 23°C during the day and 20°C at night under a 14-h photoperiod with, a light intensity of 280 μE m^{-2} s^{-1}.

Assessments of lesion types were performed at 14 DAI. The reaction phenotypes on each soybean line were recorded and classified as "TAN", "RB", or an immune reaction (IR), as previously described (Bonde et al. 2006; Bromfield 1984). The "TAN" lesion type classification indicated that the lesion color was tan and this was considered to be a susceptible reaction, whereas the "RB" type referred to the reddish-brown lesion color and was considered a resistant reaction. An "IR" type indicated a lack of obvious symptoms. The lesion responses of individual $F_{2:3}$ families were used to infer the phenotype of the corresponding F_2 plant. For families in which greater than 90% of the individuals exhibited TAN lesions, the F_2 phenotype was considered susceptible. For families in which greater than 90% of the individuals had an RB lesion type or IR, the corresponding F_2 phenotype was inferred to be resistant. All other families were considered to be segregating, thereby indicating a heterozygous F_2-phenotype. The 90% breakpoint for the classes (susceptible, heterozygous, or resistant) was considered conservative and

Table 1 The number of individual plants of each parent evaluated and the number of $F_{2:3}$ families of each family type classification (susceptible, segregating or resistant) as determined by the percentages of TAN, RB, and IR plants observed in each family

Genotype	No. of plants or families evaluated[a]	Percentage of plants with each rust phenotype		
		TAN	RB	IR
Parents	No. of plants			
DS-880	43	100	0	0
PI 567102B	49	0	18.4	81.6
Family type	No. of families			
Susceptible	28	98.8	0.0	1.2
Segregating	55	41.0	11.6	47.3
Resistant	21	1.6	8.6	89.8
Total	104			

Percentages are over all plants for each classification. Data are from seedling screens conducted in 2009 and 2010 in Stoneville, MS using a pure isolate MS06-1 collected in Mississippi in 2006. RB and IR plants were combined for the resistant family type

TAN tan-colored lesions, *RB* reddish-brown-colored lesions, *IR* an immune reaction (no lesions)

[a] The number reported are from separate evaluations of $F_{2:3}$ families. $F_{2:3}$ families were evaluated in the winter of 2009 and in the winter of 2010. Across all families, an average of 17.4 plants was phenotyped for each family

appropriate based on a visual inspection of the data distribution.

Evaluation of the F_2 population

A total of 300 F_2 seed, along with the parents PI 567102B and DS-880, and six selected genotypes (Table 2) were planted at the USDA-ARS Foreign Disease-Weed Science Research Unit (FDWSRU) Plant Pathogen Biological Safety Level 3 Plant Pathogen Containment Facility (Melching et al. 1983) from February to May, 2011. The *P. pachyrhizi* isolate Louisiana 04-1 (LA04-1) was obtained in 2004 from Dr. Ray Schneider, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA. This isolate was collected from infected soybean leaves in a field plot near Baton Rouge, LA (Schneider et al. 2005). The infected leaves were shipped to the FDWSRU Plant Pathogen Biological Safety Level 3 Plant Pathogen Containment Facility under the appropriate USDA Animal Plant Health Inspection Service permit. Single-pustule purification and urediniospore increases on the soybean cultivar Williams 82 were described previously (Pham et al. 2009). Urediniospores from a well-separated pustule were collected in 10 mL of 0.01% (v/v) Tween 20/water using a Pipetman™ P20 (Gilson, Inc., Middleton, WI), transferred to a 1.7 mL microfuge tube (LabSource, Chicago, IL) containing 1.0 mL of 0.01% (v/v) Tween 20/water, and mixed by inverting several times. The spore suspension was sprayed onto 3–4 week-old Williams 82 plants, placed into a dew chamber at 20°C for 18 h, after which they were transferred to a greenhouse at 25°C. Supplemental illumination was provided by 1,000 W Metalarc™ lights (Sylvania, Danvers, MA) suspended 0.6 m apart above the bench. Approximately 2 weeks after inoculation, this process was repeated from another well-separated pustule. After the fourth round of single-pustule purification, urediniospores were collected from infected leaves using a mechanical harvester (Cherry and Peet 1966) and stored in 2.0 mL cryovials (Sarstedt, Newton, NC) in liquid nitrogen.

Due to the large number of plants that needed to be inoculated and the limited space available within the Plant Pathogen Containment Facility at the FDWSRU, the experiment was divided into four separate inoculations on different days. The seed for each line and the population were randomly divided by four and planted on separate dates and inoculated ~4 weeks after seeding. Two seed of from either the population, parental lines or the control lines (checks) were planted in 9 cm square × 8 cm deep plastic pots (catalog #400-SVT-500; T.O. Plastics, Inc., Clearwater, MN) containing Sunshine™ LC1 mix (Sun Gro Horticulture Products, Belleview, WA) on 8 February 2011, 22 February 2011, 25 March 2011 and 8 April 2011. The parental lines and checks were included in each planting date. The plants were grown in a conventional, non-containment greenhouse at the FDWSRU at 25°C. When the second trifoliolate was fully expanded, approximately 4 weeks after seeding, tissue samples were collected and stored at −20°C. Once tissue was collected from the plants it was shipped on dry ice to Stoneville, MS for DNA extraction and molecular marker analysis. After the tissue samples were taken, the plants were transferred to the Plant Pathogen Containment Facility at FDWSRU where they were inoculated on 9 March 2011, 23 March 2011, 20 April 2011 and 4 May 2011. The reaction phenotypes were rated 2 weeks after inoculation.

For inoculation, approximately 100 mg of urediniospores of LA04-1 were removed from liquid nitrogen storage; heat shocked at 40°C for 5 min, and maintained in a humid environment overnight as previously described (Bonde et al. 2006). Urediniospores were suspended in 0.01% (v/v) Tween 20 solution and adjusted to a concentration of approximately 25,000 urediniospores per mL using a hemocytometer. Approximately 5 mL of the urediniospore suspension was sprayed onto a fully expanded trifoliolate of each plant using an atomizer at 1.4×10^5 Pa. After inoculation, the pots were placed randomly in a dew chamber at 20°C for 18 h, and then transferred to a greenhouse and randomly positioned on the bench. The

Table 2 The number of plants with each lesion type observed for selected genotypes, the parents of an F_2 population and an F_2 population inoculated with *Phakopsora pachyrhizi* isolate LA04-1 in 2011 at the Plant Pathogen Containment Facility at Ft. Detrick, MD

Genotype/population	Number of plants with each rust phenotype		
	TAN	RB	IR
PI 200492 (*Rpp1*)[a]	0	0	98
PI 459025B (*Rpp4*)[b]	0	86	0
PI 567099A (*rpp3*)[c]	0	87	0
PI 587886 (*Rpp1c?*)[d]	92	0	0
PI 594538A (*Rpp1b*)[e]	87	0	0
Williams 82	83	0	0
Parents[f]			
DS-880	95	1	0
PI 567102B	0	97	0
F_2 population	64	209	0

TAN tan-colored lesions, *RB* reddish-brown-colored lesions, *IR* an immune reaction (no lesions)

[a] McLean and Byth (1980)

[b] Hartwig (1986)

[c] Ray et al. (2011)

[d] Ray et al. (2009)

[e] Chakraborty et al. (2009)

[f] Parental lines of the F_2 population screened at Ft. Detrick

greenhouse was maintained at 25°C and supplemental illumination was provided by 1,000 W Metalarc lights (Sylvania, Danvers, MA) suspended 0.6 m apart above the bench. The pots were placed in metal trays and watered from the bottom. The reaction phenotypes were rated as either IR, RB, or TAN.

DNA isolation and molecular markers

For the $F_{2:3}$ families screened at Stoneville, MS, leaf samples were collected from the respective individual F_2 plants and freeze dried in a Model 2400 freeze dryer (The Freeze Dry Company, Nisswa, MN). Similarly, leaf tissue from individual $F_{2:3}$ plants from nine homozygous resistant (immune lesions) and ten homozygous susceptible (TAN lesions) $F_{2:3}$ families were collected and freeze dried. The freeze-dried tissue was then ground to a fine powder using a tissue pulverizer (Garcia Manufacturing, Visalia, CA). For the F_2 population inoculated at Ft. Detrick, MD, 10–15 9.5-mm diameter leaf punches collected from each F_2 plant prior to transferring the plants into the Plant Pathogen Containment Facility, were placed in screw cap 1.5 mL tubes, frozen in liquid N, and the tubes were stored at −80°C. The tubes were shipped on dry ice to Stoneville, MS for DNA extraction and subsequent marker analysis. Just before DNA extraction, the frozen tissue was pulverized in a Geno Grinder™ 2000 (Spex CetiPrep, Metuchen, NJ). DNA was isolated from the pulverized freeze dried tissue and the pulverized frozen tissue using a Maxwell 16™ automated DNA isolation machine (Promega, Madison, WI) following manufacturer's protocols.

Simple sequence repeat (SSR) molecular markers were selected based on their reported genomic locations and their primer sequences were obtained from SoyBase (http://soybase.org/resources/ssr.php). Primers were manufactured with either a hexachlorofluorescein (HEX) or 6-carboxy-fluorescein (FAM) 5′-fluorescent label (Integrated DNA Technologies, Coralville, IA). PCR amplification was performed on a MJ Research PTC 225 (Biorad, Hercules, CA) using conditions of 95° for 120 s; 30 cycles of 94° for 30 s, 46° for 30 s, 72° for 30 s; and one cycle of 72° for 300 s followed by maintenance at 4°C until detection. Amplicons were detected on an ABI 3730 (Applied Biosystems, Foster City, CA) at the USDA-ARS Midsouth Area Genomics Facility at Stoneville, MS. The products were analyzed using GeneMapper™ 3.7 (Applied Biosystems, Foster City, CA). All polymorphic SSR markers identified in this study were co-dominant.

To help fill in a gap in the SSR marker map, SNP primers were designed based on the supplemental data provided by Choi et al. (2007), and were tested for polymorphisms using a KASP reaction following manufacturer's protocols (K-Bioscience, Hoddesdon Herts, UK). Reaction results were read on a Roche LightCycler™ 480 (Roche Applied Science, Indianapolis, IN) following protocols from K-Bioscience. One useful SNP (BARC-013237-00459) was identified and included in the analysis.

Statistical analysis

By evaluating $F_{2:3}$ families, the phenotype of the respective F_2 source plant was inferred (resistant, segregating, or susceptible) for the population evaluated at Stoneville, MS. This allowed the identification of heterozygotes in the F_2 generation and allowed the testing against a 1:2:1 expected segregation ratio for a single gene. Phenotyping of the F_2 population at the FDWSRU did not allow the determination of heterozygotes and therefore segregation was tested against a 3:1 ratio. The expected segregation ratios were compared to observed ratios using a χ^2 goodness-of-fit test (Sokal and Rohlf 1995). Once the qualitative single gene nature of the observed SBR resistance in this study had been confirmed, SSR markers and SBR phenotypes were analyzed for putative linkage using a two-way contingency table to analyze allele frequencies. The probabilities of deviations from expected values were made using the Fisher's exact test (Sokal and Rohlf 1995) as computed using SAS ver. 9.2 (SAS Institute, Cary, NC). JoinMap 4.0 (Van Ooijen 2006) was used to further evaluate linkage, order molecular markers and create linkage maps for each population. In addition, correlation analysis (PROC CORR, SAS ver. 9.2) was used to evaluate the relationship between selected molecular marker alleles and the lesion phenotype (TAN or IM) for individual $F_{2:3}$ plants phenotyped at Stoneville, MS. A total of 164 individual $F_{2:3}$ plants were evaluated. The plants were randomly selected from 10 $F_{2:3}$ families phenotyped as homozygous TAN and nine $F_{2:3}$ families phenotyped as homozygous immune. These data provided additional evidence in support of the results obtained with the two independent F_2 populations in a manner similar to that reported by Ray et al. (2009, 2011).

Results

The results of rust resistance segregation for the $F_{2:3}$ families tested at Stoneville, MS are shown in Table 1. For the susceptible parent (DS-880), 43 individual plants were phenotyped and all showed only TAN lesions. For the resistant parent (PI 567102B), 49 plants were phenotyped and no TAN lesions were observed on any plant, but plants with RB lesions or no lesions (IR) were observed (18.4% RB and 81.6% IR, Table 1). That all plants of the susceptible parent had TAN lesions indicates the uniformity of the rust pressure applied. Twenty seeds from each of 104

$F_{2:3}$ families were sown. However, germination was less that 100%, and in a few cases, the phenotype of individual plants was not clear. These individual plants were eliminated from the analysis. In total, across all $F_{2:3}$ families, the phenotypes of 1,813 plants were determined (on average 17.4 plants per family). Each $F_{2:3}$ family was classified as susceptible (>90% TAN lesions), resistant (>90% RB lesions or IR), or segregating (a mixture of TAN and RB lesions and IR) on the basis of the number of plants with each lesion type in each family. For the $F_{2:3}$ families, 28 were classified as susceptible, 55 as segregating and 21 as resistant (Table 1). The classification of each family was used to infer the respective F_2 phenotype (homozygous susceptible, heterozygous, or homozygous resistant). When tested against the 1:2:1 ratio (susceptible:heterozygous:resistant) expected for a single gene in the F_2 generation when the heterozygotes are known, the data fit this ratio very well ($\chi^2 = 1.29$, $P = 0.5251$). This result indicates that the SBR resistance in PI 567102B is controlled by a single gene.

An independent F_2 population from the same cross (DS-880 × PI 567102B) was inoculated in the FDWSRU Plant Pathogen Containment Facility at Ft. Detrick, MD using an isolate (LA04-1) collected in Louisiana (Table 2). For the susceptible parent (DS-880), 96 plants were inoculated of which all but one developed TAN lesions. However, for the resistant parent (PI 567102B), 97 plants were inoculated and all developed the resistant RB lesion type (Table 2). For the F_2 population, 273 plants had phenotypes determined, of which 64 exhibited TAN lesions and 209 had RB lesions. When tested against a 3:1 ratio (RB:TAN) expected for a single gene in the F_2 generation when the heterozygotes are not known, the data fit the ratio very well ($\chi^2 = 0.35$, $P = 0.5525$). These results provide additional evidence that the SBR resistance in PI 567102B is controlled by a single gene and indicate that the resistance is dominant.

Between the parental lines used in this study, a polymorphic SSR molecular marker was identified on either side of the genomic location of each of the five known *Rpp* loci. These markers were applied to both F_2 populations and the results are shown in Table 3. The data indicated that the resistance gene in PI 567102B is not likely located at or near the location of *Rpp1*, *Rpp2*, *Rpp3*, or *Rpp5*. However, it did indicate the possibility that the resistance locus was located near *Rpp4* on chromosome 18. A significant association of both markers flanking *Rpp4* in both F_2 populations was identified by single marker analysis. However, the significance of the association was much weaker in the F_2 population screened at Stoneville (Table 3). To resolve the location of the resistance locus, an additional 20 polymorphic markers (19 SSRs and one SNP) were identified on chromosome 18. These markers were applied to each F_2 population, and a linkage map constructed for each population. When compared to each other and to the consensus map of Song et al. (2004), the marker orders in each map were very similar, although not exactly the same (Fig. 1). Nonetheless, the resistance locus in PI 567102B independently mapped to between markers Satt324 (LOD >23.0) and Satt394 (LOD >11.0) in both F_2 populations (Fig. 1). As expected, linkage to other markers near Satt324 and Satt394 on chromosome 18 was detected in both F_2 populations but with decreasing strength as distance increased (data not shown). As indicated in Fig. 1, *Rpp1* and *Rpp4* have been previously mapped to this linkage group. However, the resistance in PI 567102B maps to a different region on chromosome 18 than either of these *Rpp* genes, indicating that the resistance in PI 567102B is controlled by a different locus.

The marker alleles of the two markers (Satt324 and Satt394) flanking the putative location of the resistance locus in PI 567102B were further evaluated in individual plants selected from either homozygous immune (IR) or homozygous TAN $F_{2:3}$ families. In total 164 plants were evaluated (68 from nine homozygous immune families and

Table 3 Molecular marker associations between polymorphic SSR markers at each of the bold five major known resistance loci (*Rpp1*, *Rpp2*, *Rpp3*, *Rpp4*, and *Rpp5*) and the F_2-inferred resistance SBR phenotypic data from Stoneville, MS and the F_2 resistance data from Ft. Detrick, MD

SSR marker and *Rpp* locus	Chromosome no. (molecular linkage group)	Map position[a] cM	F_2—inferred Stoneville Prob.[b]	F_2 Ft. Detrick Prob.
Satt191	18 (G)	96.57	0.8322	0.1923
Rpp1	18 (G)			
Sat_064	18 (G)	108.70	0.7652	0.9165
Satt183	16 (J)	42.51	0.1199	0.3164
Rpp2	16 (J)			
Sct_001	16 (J)	44.68	0.2029	0.2962
Satt658	6 (C2)	113.62	0.2137	0.1764
Rpp3	6 (C2)			
Sat_263	6 (C2)	118.78	0.3761	0.0164
Satt288	18 (G)	76.77	0.0204	0.0001
Rpp4	18 (G)			
Satt612	18 (G)	80.38	0.0253	0.0001
Sat_166	3 (N)	38.59	0.0647	0.9272
Rpp5	3 (N)			
Sat_280	3 (N)	43.45	0.2576	0.5674

One polymorphic SSR marker was found on either side of the reported position of each of the five SBR resistance loci. At a locus within a linkage group, all markers were linked to each other (LOD > 3.0, data not shown)

[a] Molecular linkage group and map position as reported by Song et al. (2004)

[b] Fisher's exact test for single marker analysis

96 from 10 homozygous TAN families). For Satt394 there was a significant correlation between the respective resistant or susceptible parental marker allele and the resistant or susceptible phenotype of individual $F_{2:3}$ plants ($r = 0.82$, $P < 0.0001$). Interestingly, the majority of differences between the respective parental marker allele and the phenotype were because the marker was in a heterozygous state. However, for Satt324 there was a very high correlation between the respective parental marker allele and the resistant or susceptible phenotype of the individual plants ($r = 0.95$, $P < 0.0001$). More specifically, the parental allele of Satt324 did not match the phenotype of the $F_{2:3}$ plant in only five of the plants evaluated. The greater correlation of Satt324 may be a reflection of the shorter distance between Satt324 and the putative resistance locus (Fig. 1) as compared to Satt394. These data from individual $F_{2:3}$ plants provide additional evidence that the resistance exhibited by PI 567102B is located near Satt324.

Discussion

Our main SBR research goals are to understand the resistance mechanism to *P. pachyrhizi* and to develop high-yielding soybean cultivars with resistance to US isolates of *P. pachyrhizi*. Known *Rpp* genes condition resistance to only a limited set of *P. pachyrhizi* isolates and have not been durable sources of resistance against SBR (Hartman et al. 2005). Further, the ability of *P. pachyrhizi* to overcome single-gene resistance has been reported (Bromfield 1984; Hartman et al. 2005; Silva et al. 2008). Therefore, extensive research has been conducted worldwide to identify new sources of resistance, new SBR resistance genes and alternative alleles of known genes (Chakraborty et al. 2009; Garcia et al. 2008; Li 2009a; Li and Young 2009; Miles et al. 2006; 2008; Ray et al.2009; 2011). One of the strategies used to identify new sources of resistance to *P. pachyrhizi* in the US was to evaluate soybean lines that were previously identified as resistant to foreign isolates of *P. pachyrhizi* (Li 2009b). PI 567102B was one of the 805 lines identified as putatively resistant to a mixture of four *P. pachyrhizi* isolates from Brazil (BZ01-1), Paraguay (PG01-2), Thailand (TH01-1), and Zimbabwe (ZM01-1) in a seedling screen at Ft. Detrick, MD (Miles et al. 2006) and in an adult-plant field screen conducted in Paraguay (Miles et al. 2008). In addition to the evaluations in Paraguay and Ft. Detrick, PI 567102B and nine other resistant genotypes identified in Paraguay were evaluated in the Stoneville Research Quarantine Facility using three Mississippi isolates of *P. pachyrhizi.* The soybean genotypes evaluated included four known resistance sources for *Rpp1-4* (Li 2009a). PI 567102B was the most resistant line identified in that study. Interestingly, PI 567102B had an RB reaction without sporulation to two of the three Mississippi *P. pachyrhizi* isolates and an IR reaction to the third isolate. In contrast, PI 200492 (*Rpp1*) had an immune reaction, whereas PIs 230970 (*Rpp2*), 462312 (*Rpp3*) and 459025B (*Rpp4*) had RB reactions with different amounts of sporulation to all three isolates. The differences in reaction phenotypes between PI 567102B and the soybean accessions containing the four known *Rpp* genes indicated a potentially different source of resistance. It was hypothesized that PI 567102B might contain a new SBR resistance gene that could be different from reported *Rpp* genes. Moreover, PI 567102B was also identified as resistant to *P. pachyrhizi* isolates from Alabama, Florida, Georgia, Louisiana, South Carolina, and Texas (Li et al. unpublished data, 2011; Walker et al. 2011), as well as Mississippi (Li 2009a, Li and Young 2009), inferring a potential for broad resistance. Hence PI 567102B was used as the resistant parent to construct populations for further studies in the genetics of SBR resistance.

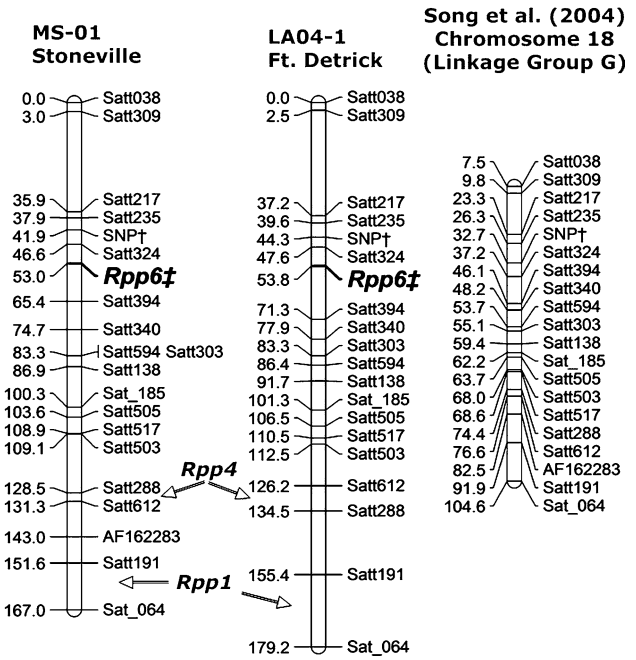

Fig. 1 Genomic location of resistance putatively identified as *Rpp6* from the F_2-inferred SBR phenotypic data from Stoneville, MS and F_2 phenotypic data from Ft. Detrick, MD. The consensus map of chromosome 18 (formerly MLG G; Song et al. 2004) is included for reference. Linkage calculations were made using JoinMap 4.0 (Van Ooijen 2006). †BARC-013237-00459, ‡Putative genomic location of the resistance exhibited by PI 567102B mapped in this study

In this report, we identified a new SBR resistance locus that differs from the location of the five previously reported *Rpp* loci. It was proposed to the Soybean Genetics Committee that this new locus be designated as *Rpp6*. The conclusion that a different locus was detected was based on three observations: (1) analysis of markers flanking *Rpp1*,

Rpp2, *Rpp3*, and *Rpp5* indicated the resistance in PI 567102B was not near those loci; (2) marker analysis indicated that the resistance in PI 567102B mapped to chromosome 18 but in a different position (Fig. 1) from the reported genomic locations of *Rpp1* or *Rpp4* on chromosome 18 (Hyten et al. 2007; Monteros et al. 2007); and (3) the two independent segregating populations were inoculated with different isolates (MS06-1and LA04-1) at different locations (Stoneville, MS and Ft. Detrick, MD), but produced highly similar estimates for the genomic location of the locus affecting resistance, thereby providing confidence for a new proposed *Rpp6* gene in PI 567102B.

The difference in phenotypic reactions between PI 567102B and four known sources of resistance (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4)* to three Mississippi isolates of *P. pachyrhizi* was previously reported (Li 2009a; Walker et al. 2011). These reports provide additional evidence supporting the conclusion that the proposed *Rpp6* gene in PI 567102B is a different gene than the known *Rpp* genes.

Many disease resistance loci in soybean have been found to be on the same chromosome. For example, clusters of resistance gene analogues (RGAs) were identified on chromosome 16; (Kanazin et al. 1996; Graham et al. 2002) and *Rps1*-k on chromosome 3 (Gao and Bhattacharyya 2008). Included in a cluster were resistance to powdery mildew (*Rmd-c*), Phytophthora stem and root rot (*Rps2*), and an infective nodulation gene (*Rj2*; Graham et al. 2002). Likewise, several other disease resistant loci map to chromosome 18. Jackson et al. (2008) mapped the purple seed stain resistance locus (*Rpss1*) to a region on chromosome 18 that contained a quantitative trait loci (QTL) conferring resistance to race 3 of soybean cyst nematode (SCN) (Wang et al. 2001). Yue et al. (2001) identified another QTL on this region of chromosome 18 conferring resistance to SCN races 1, 2, 3, and 5 in soybean. Two QTLs for resistance to soybean sudden death syndrome (*rfs and rfs1*) were also located near this region of chromosome 18 (Meksem et al. 1999; Iqbal et al. 2001; Triwitayakorn et al. 2005; Kassem et al. 2006). Since *Rpp1* and *Rpp4,* and the proposed *Rpp6* gene are all located on chromosome 18. It will be interesting to isolate these three resistance genes and investigate their evolutionary relationships. The identification of two flanking SSR markers linked to the proposed *Rpp6* gene could facilitate the use of marker-assisted selection to incorporate this gene into breeding SBR resistant lines and cultivars. This will have wide application as *Rpp6* has been shown to provide resistance to both Paraguayan and to US isolates of *P. pachyrhizi*.

Conclusion

Analysis of two independent F_2 populations (one for which the F_2 phenotype was inferred from the corresponding $F_{2:3}$ families and one with phenotypes measured directly) indicated that the resistance to *P. pachyrhizi* in PI 567102B as measured in this study is controlled by a single dominant gene. Linkage analyses of both populations indicated that the resistance locus was located on chromosome 18 but at a different location than either *Rpp1* or *Rpp4,* which were previously mapped to this chromosome. Thus, it was concluded that the resistance to *P. pachyrhizi* in PI 567102B is conditioned by a previously unreported gene. We propose that the new locus be designated as *Rpp6*. Incorporating this new resistance gene into improved soybean cultivars may have wide benefits as it has been shown to have resistance to both Paraguayan and US isolates of *P. pachyrhizi*.

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