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RESEARCH

Evidence of a Susceptible Allele Inverting the Dominance of Rust Resistance in Soybean

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ABSTRACT

Soybean rust (SBR) is the most threatening fungal disease in the major soybean [*Glycine max* (L.) Merr.] production areas around the world. In spite of the availability of chemical control with fungicides, the increase of production costs as well as operational difficulties associated with fungicide use for disease management has prompted the search for resistant genes in the soybean germplasm. Five genes (*Rpp1*, *Rpp2*, *Rpp3*, *Rpp4*, and *Rpp5*) have been reported as capable of conferring SBR resistance in soybean. Here we report the genetic segregation of the SBR resistance in two populations derived from crossing the SBR resistance source PI 594760B with the breeding lines TMG06_0012 (Population 1 [POP-1]) and TMG06_0011 (Population 2 [POP-2]). In both populations the resistance segregated as a single gene, but the resistance gene was dominant in POP-1 and recessive in POP-2. Molecular mapping of the phenotype placed it in the vicinity of the *Rpp1* locus in both POP-1 and POP-2. When the breeding lines TMG06_0011 and TMG06_0012 where crossed with several other sources of resistance at the *Rpp1*, *Rpp2*, *Rpp4*, and *Rpp5* loci or other putative loci, the inversion of gene action type observed with PI 594760B occurred only for the putative *Rpp1* genotypes. We propose that a dominant susceptible allele, present in TMG06_0011, is causing the inversion of dominance observed in each of these crosses.

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Abbreviations: BSA, bulked segregant analysis; dsRNA, double-stranded RNA; HR, homozygous resistant; HS, homozygous susceptible; MG, maturity group; MLG, molecular linkage group; POP-1, Population 1; POP-2, Population 2; R, resistant; RB, reddish-brown; SBR, soybean rust; S, susceptible; SSR, simple sequence repeat; TAN, tan; TMG, Tropical Melhoramento e Genética Ltda., Cambé-PR, Brazil.

Since being reported in Africa (Akinsanmi and Ladipo, 2001; Pretorious et al., 2001), South America (Yorinori et al., 2005; Rossi, 2003), and North America (Schneider et al., 2005), soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi* Syd. & P. Syd., has become the most threatening fungal disease of soybean [*Glycine max* (L.) Merr.]. Although yield losses associated with SBR in experimental plots in the United States are usually lower than 35% (Mueller et al., 2008), yield losses in tropical and subtropical areas that account for approximately 50% of the current world soybean production can be 40 to 80% and even up to 100% (Yorinori et al., 2005). In Brazil, estimates suggest that SBR has caused approximately US$13 billion in economic loss since its first appearance in 2001 (Consórcio antiferrugem, 2009). Therefore, it is easy to understand why the occurrence and rapid spread of SBR in the Western Hemisphere was a turning point in both soybean production and research.

Fungicide application has been the primary tool used to manage SBR (Patil and Anahosur, 1998; Miles et al., 2007). Nevertheless, several negative issues related to chemical control have

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been raised. Increased production costs, environmental and social risks associated with widespread use of fungicides, difficulties in applying fungicide during rainy periods, and difficulties in targeting fungicide to the lower leaves where the disease generally starts are the common concerns for producers. Another serious concern is the possibility that the fungus may be developing tolerance to fungicides, particularly to triazole chemistries, leading researchers as well as agrochemical companies to stop recommending the use of fungicide application as a curative tactic for managing rust.

Attempts to develop cultivars that are resistant to SBR have been undertaken around the world [e.g. India (Singh et al., 1975), Taiwan (Wang and Hartman, 1992), and Nigeria (Twizeyimana et al., 2008)] and SBR-resistant cultivars with specific genes have been released in Uganda (Oloka et al., 2008). To date, however, no resistant cultivars appear to have been cultivated on a large scale in these countries. The largest breeding program using resistance genes for SBR control is probably in Brazil, where two soybean cultivars carrying single SBR resistant genes were released and cultivated on approximately 100,000 ha in the 2009/2010 soybean growing season (authors, unpublished data, 2010).

The development of durable genetic resistance to soybean rust will depend on our understanding of the genetic and molecular bases of the resistance response. At least five different SBR resistant genes (*Rpp* genes) have been identified in cultivated soybean: *Rpp1* (McLean and Byth, 1980), *Rpp2* (Bromfield and Hartwig, 1980), *Rpp3* (Bromfield and Melching, 1982), *Rpp4* (Hartwig, 1986), and *Rpp5* (Garcia et al., 2008). Efforts to understand the resistance response at the molecular level have also been reported. Global gene expression analyses with *Rpp2*- and *Rpp3*-mediated resistant genotypes showed hundreds of differentially expressed genes and a biphasic nature of the disease resistance response (Van de Mortel et al., 2007; Panthee et al., 2007; Panthee et al., 2009). A candidate gene for *Rpp4*-mediated resistance to SBR encodes a protein belonging to the CC-NBS-LRR family of disease resistance genes (Meyer et al., 2009). As has been shown for several resistant genes in other pathosystems, at least two other homologs are present in the vicinity of the *Rpp4* candidate gene.

Allelic variations have been reported for the known *Rpp* genes. These variations include different dominant and incompletely dominant alleles for the *Rpp1* locus (Chakraborty et al., 2009; Ray et al., 2009) and recessive (Calvo et al., 2008) and dominant alleles for the *Rpp2* and *Rpp5* loci (Garcia et al., 2008). To fully understand the genetic basis of soybean rust resistance, it is important to know how these different loci and/or alleles interact among themselves as well as with other unidentified genes in the soybean genome. Here we report an allele at either the *Rpp1* locus or at closely linked locus that confers a dominant susceptible phenotype to SBR.

MATERIALS AND METHODS

Plant Material

As part of a general strategy to discover new *Rpp* genes and to breed new SBR-resistant soybean cultivars at TMG (Tropical Melhoramento e Genética Ltda., Cambé-PR, Brazil, 23°15'04" S and 51°14'54" W), the Chinese maturity group (MG) IX soybean accession PI 594760B (Germplasm Resources Information Network, 2008), previously described as highly resistant to soybean rust (Miles et al., 2006, 2008), was crossed with the high-yielding soybean breeding lines TMG06_0012 and TMG06_0011 (by TMG). Both lines are susceptible to soybean rust and are adapted to Brazilian growing conditions. TMG06_0012 is a MG VIII genotype that is resistant to stem canker (*Diaporthe phaseolorum* var. *meridionalis*), soybean cyst nematode (*Heterodera glycines*), races 1 and 3, and root knot nematode (*Meloidogyne* ssp.). The line is derived from the cross 'MG/BR-46 Conquista'$^{(2)}$ × ('BRS MT Pintado' × Roundup Ready). MG/BR-46 Conquista and BRS MT Pintado are cultivars developed by Fundação MT (Fundação de Apoio a Pesquisa Agropecuária de Mato Grosso, Rondonópolis-MT, Brazil). TMG06_0011 is a MG VI genotype resistant to steam canker. This breeding line is derived from the cross 'Embrapa 48'$^{(2)}$ × 'IAC-12'. Embrapa 48 is a cultivar from EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, Londrina-PR, Brazil) and IAC-12 is a cultivar developed by IAC (Instituto Agronômico de Campinas, Campinas-SP, Brazil). Two populations named Population 1 (POP-1; PI 594760B × TMG06_0012) and Population 2 (POP-2; PI 594760B × TMG06_0011) were produced and, in October 2008, 160 F_2 seeds from POP-1 and 105 F_2 seeds from POP-2 were planted. Plants were grown to maturity, and 20 seeds from each individual F_2 plant were bulked and planted in February 2009 to conduct a $F_{2:3}$ progeny test. Final $F_{2:3}$ data were obtained from 156 families for POP-1 and 104 families for POP-2. The F_1, F_2, and $F_{2:3}$ generations and the parents were evaluated for their SBR responses in greenhouse screenings as described below.

Additional crosses were also made between the breeding lines TMG06_0012 and TMG06_0011 and the soybean accessions PI 200456, PI 200487, PI 200526, PI 224270, PI 471904, PI 561356, PI 587905, CG 84058-18, and CG 84058-21, which each possess a single SBR resistant gene (Garcia et al., 2008; Calvo et al., 2008; Miles et al., 2008; Camargo et al., 2009; authors, unpublished data, 2009). For these crosses, only the F_1 generation was scored for SBR response, except for the crosses involving PI 561356. This PI, a Chinese MG V accession, has a single dominant resistant gene close to the *Rpp1* genomic region and is effective against the *P. pachyrhizi* field populations currently present in Brazil (Camargo et al., 2009), in spite of the ineffectiveness of the original source of *Rpp1* (PI 200492) (Yorinori et al., 2005). The 96 F_2 plants from the cross PI 561356 × TMG06_0011 were screened for SBR resistance. Five F_2 plants that were resistant were backcrossed with TMG06_0011 and were also crossed with the SBR susceptible cultivar CD205 (Coodetec, Cooperativa Central de Pesquisa Agrícola, Cascavel-PR, Brazil). The F_1 plants, 262 F_2 plants from the TMG06_0011 backcross, and 552 F_2 plants from the CD205 cross were rated for SBR resistance.

In all the crosses the susceptible parents TMG06_0012 and TMG06_0011 were used as the female parents and we always used the pollen from a single resistant plant for each combination. Also,

seeds from a single F_1 plant were used to produce the F_2 population in all the crosses. Seeds were sown in 8-L plastic pots filled with a mixture of soil, manure, and sand (5:3:2 by volume). In the F_1 generation only one seed was planted in each pot. For the F_2 generation four seeds were sown in a pot, and all the seeds from the same $F_{2:3}$ family were planted together in the same pot. All the populations were planted and were evaluated along with the parental genotypes and with remnant seeds from the previous generation(s).

Soybean Rust Screening

The SBR resistance screening was conducted using a greenhouse-maintained population of *P. pachyrhizi* according to the procedures described by Garcia et al. (2008) and Calvo et al. (2008). The greenhouse was equipped with filtered ventilation and a double door entry system that minimized introduction of contaminating rust spores from outside. The response to SBR was evaluated in the V2 growth stage (Fehr et al., 1971) and was confirmed in the V4 stage. Each plant was scored for lesion type: susceptible (TAN), resistant [reddish-brown (RB) or immune], or mixed (TAN and RB lesions on the same leaf) (Bromfield, 1984). For the progeny test, only $F_{2:3}$ families with 11 or more individuals were rated. Families were classified as homozygous resistant (HR), homozygous susceptible (HS), or segregating based on the presence of RB and/or immune, TAN, or both lesion types, respectively. Phenotypic (F_2) and genotypic ($F_{2:3}$) segregation hypotheses were tested with a chi-square (χ^2) test.

Because we used a possibly heterogeneous *P. pachyrhizi* population for screenings, we also conducted a validation test to confirm the results of the SBR evaluation. Single-lesion isolates were obtained by pipeting 10 μL of a solution of water and Tween 20 (0.01% v/v) onto a TAN lesion from a infected leaf of the cultivar BRS Bacuri (by EMBRAPA) that is used to maintain our *P. pachyrhizi* population (Garcia et al., 2008). The urediniospore suspension was then diluted in a ratio of 1:20, and 10 μL of the diluted suspension was used to inoculate healthy detached leaves from BRS Bacuri plants that had been grown in a growth chamber. Leaves were cultured in Petri dishes as described by Twizeyimana et al. (2007). After 2 wk of incubation, well-developed rust lesions were identified and the isolation procedure was repeated. Following the third round of culture, all of the urediniospores obtained from a single lesion were used to inoculate healthy leaves to obtain a larger amount of spores for screening purposes. These urediniospores were used to repeat the SBR screening with the remaining seeds of the soybean parental genotypes (PI 594760B, TMG06_0011, and TMG06_0012), the F_1 from POP-1 and POP-2, and with 10 randomly chosen $F_{2:3}$ progenies from each cross.

Molecular Analysis

Molecular mapping was performed in the POP-1 (PI 594760B × TMG06_0012) and POP-2 (PI 594760B × TMG06_0011) populations. DNA isolation, polymerase chain reaction (PCR), and marker detection procedures were conducted as described by Garcia et al. (2008). Public simple sequence repeat (SSR) molecular markers mapped in the vicinity of the known *Rpp* resistance genes were used for mapping analysis. Sequences from each molecular marker were retrieved from Soybase (2009) and primers were synthesized by Invitrogen (Carlsbad, CA).

The possible genomic regions associated with the SBR resistance trait in each F_2 population were initially identified through bulked segregant analysis (BSA; Michelmore et al., 1991). Four bulks were formed by pooling an equal amount of DNA from eight different homozygous plants that were either resistant (resistant bulks) or susceptible (susceptible bulks) to SBR. The polymorphic markers in that region were then used to screen each F_2 plant from POP-1 and POP-2. All polymorphic SSR markers used were codominant, and the 1:2:1 expected segregation was tested with the chi-square (χ^2) test. The F_2 SBR reactions were converted to data suitable for mapping based on the genotypes (homozygous dominant, recessive, or heterozygous for the resistance) of the F_2 individuals surmised from the $F_{2:3}$ tests. JoinMap version 3.0 (Van Ooijen and Voorrips, 2001) was used for map constructions. A likelihood of odds (LOD) score threshold of 2.0 and Kosambi's mapping function were used for linkage confirmation and distance calculations. Control maps were constructed by removing either the *Rpp* gene data or each SSR marker used on an individual basis to check the mapping data. The maps obtained were compared to the consensus soybean linkage map of Song et al. (2004).

RESULTS

Genetic Segregation

Unusual genetic behavior in crosses involving the SBR-resistant PI 594760B and two of our susceptible breeding lines (TMG06_0012 and TMG06_0011) was identified in our study. On the cross involving TMG06_0012, the F_1 plants were resistant to SBR. Conversely, when we crossed the same PI with TMG_0011, all the F_1 plants were susceptible. Since we used pollen from the same PI 594760B plant for both crosses, we speculated that this contrasting resistant response probably was caused by our breeding lines.

Phenotypic analysis of the F_2 population derived from the cross PI 594760B × TMG06_0012 (POP-1) showed a segregation of 124 plants with the RB resistance lesion type and 36 plants with the TAN susceptible lesion type. Analysis in the $F_{2:3}$ progenies identified 48 lines homozygous for the RB lesion type (HR), 73 lines segregating, and 35 lines homozygous for the TAN lesion type (HS). A chi-square test revealed that the observed segregations fit the 3:1 resistant (R):susceptible (S) ratio and a 13:3 R:S ratio in the F_2 generation but only a 1:2:1 R:segregating:S ratio in the $F_{2:3}$ generation, as expected for a single dominant resistance gene (Table 1). Segregation was further analyzed by randomly choosing some segregating $F_{2:3}$ lines and combining the data from the plants within these $F_{2:3}$ lines. Only a 3:1 R:S segregation ratio was observed for this test (Table 2), supporting the hypothesis of a single dominant gene, as expected, since the $F_{2:3}$ plants were derived from F_2 genotypes heterozygous at the resistance locus.

In the F_2 generation from the population derived from the cross PI 594760B × TMG06_0011 (POP-2), 27 out of 105 plants were scored as having the RB lesion type and 78 as having the TAN lesion type. This phenotypic segregation fits a 1:3 R:S ratio (Table 1), suggesting that a single recessive gene controls the resistance in this cross. The observed segregation also fits the 3:13 R:S ratio that would be expected in

Table 1. Soybean rust (SBR) resistance segregation in F_2 and $F_{2:3}$ populations derived by crossing the SBR-resistant PI 594760B to two different susceptible soybean breeding lines.

Population	F_2 test: No. of plants RB†	F_2 test: No. of plants TAN‡	χ^2 of the expected segregations 3:1 R:S§	χ^2 of the expected segregations 13:1 R:S	$F_{2:3}$ test: No. of families HR¶	Segregating	HS#	χ^2 of the expected segregations	
								1:2:1 R: Segregating: S	1:2:1 R: Segregating: S
Population 1 (PI 594760B × TMG06_0012)	124	36	0.53 p†† = 0.48 NS‡‡	1.48 p = 0.22 NS	48	73	35	2.81 p = 0.25 NS	166.58 p < 0.01**
Population 2 (PI 594760B × TMG06_0011)	27	78	1:3 R:S 0.03 p = 0.86 NS	3:13 R:S 3.34 p = 0.07 NS	27	52	25	1:2:1 R: Segregating: S 0.09 p = 0.96 NS	1:8:7 R: Segregating: S 61.39 p < 0.01**

**Significant at the 0.01 probability level.

†RB, reddish-brown (resistant lesion type).

‡TAN, tan (susceptible lesion type).

§R, resistant; S, susceptible.

¶HR, homozygous resistant.

#HS, homozygous susceptible.

††Probability of significance of the chi-square. Values higher than 0.05 are considered nonsignificant

‡‡NS, nonsignificant.

the F_2 generation if a second gene suppressing the resistance was present (Table 1). Nevertheless, the hypothesis of a suppressor gene was rejected due to the 27 HR, 52 segregating, and 25 HS lines observed in the $F_{2:3}$ progeny test and the ratio of 57 plants with the RB lesion type to 153 plants with the TAN lesion type observed when the data from randomly chosen segregating $F_{2:3}$ lines were analyzed. These segregations fit only 1:2:1 (Table 1) and 1:3 (Table 2) R:S ratios, respectively, strongly supporting the presence of a single recessive gene controlling the resistance in this population.

Molecular Mapping

We used the published SSR markers linked to each of the five known SBR resistance genes to identify genomic regions potentially involved in the SBR resistance found in both segregating populations. In both POP-1 and POP-2 populations, BSA showed that the F_2–inferred SBR phenotypic data were significantly associated only with SSR markers linked to the *Rpp1* locus. Therefore, we concluded that the *Rpp2*, *Rpp3*, *Rpp4*, and *Rpp5* loci were not involved in the resistance segregating in these two populations.

The *Rpp1* locus is located on soybean molecular linkage group (MLG) G (chromosome 18) between the SSR markers Sct_187 and Sat_064 (Hyten et al., 2007). Based on the molecular map of Song et al. (2004), the markers closest to the *Rpp1* locus are Sct_199, Satt472, Satt191, Sat_117, and Sct_182 (all on the centromeric side of the *Rpp1* locus) and Sat_372 and Sat_064 (both on the telomeric side the *Rpp1* locus). For POP-1, Satt472, Satt191, and Sat_117 were the only polymorphic markers. For POP-2, Satt191, Sat_117, and Sct_187 were polymorphic. These markers, along with the phenotypic lesion type were considered qualitative traits and were used for the molecular mapping. All the markers analyzed segregated according to the expected single gene 1:2:1 ratio (Table 3). Moreover, the genetic deviation toward the homozygous resistant genotype observed in POP-1 $F_{2:3}$ segregation (Table 1) was also observed for segregation of the molecular markers (Table 3).

In POP-1 the resistance locus mapped within 6.9 cM of Satt191 and 5.1 cM of Sat_117 (Fig. 1). Although we were not able to flank the SBR resistance locus in this population, this is the same region where the *Rpp1*, *Rpp1b*, and the likely *Rpp1* allele discovered in PI 587886 were previously mapped (Hyten et al., 2007; Chakraborty et al., 2009; Ray et al., 2009). In POP-2, the resistance locus was positioned between Sat_117 and Sct_187 (Fig. 1), in

Table 2. Soybean rust (SBR) resistance segregation data from F_3 plants selected from segregating $F_{2:3}$ families.

Population	No. of F_3 plants inside segregating $F_{2:3}$ families RB†	TAN‡	χ^2 of the expected segregations 3:1 R:S§	13:3 R:S
Population 1 (PI 594760B × TMG06_0012)	255	77	0.58 p¶ = 0.47 NS#	4.30 p = 0.04*
Population 2 (PI 594760B × TMG06_0011)	57	153	1:3 R:S 0.51 p = 0.47 NS	3:13 R:S 9.71 p < 0.01**

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

†RB, reddish-brown (resistant lesion type).

‡TAN, tan (susceptible lesion type).

§R, resistant; S, susceptible.

¶Probability of significance of the chi-square. Values higher than 0.05 are considered nonsignificant.

#NS, nonsignificant.

Table 3. Segregation from the simple sequence repeat (SSR) markers used on the molecular mapping of each F_2 population.

SSR marker	Population							
	Population 1 (PI 594760B × TMG06_0012)†			χ^2 of the expected segregation	Population 2 (PI 594760B × TMG06_0011)			χ^2 of the expected segregation
	S	H	R	1:2:1	S	H	R	1:2:1
Sat_472	29	69	44	3.28 p‡ = 0.19 NS§	–	–	–	–
Satt191	30	69	44	2.92 p = 0.23 NS	21	61	20	3.94 p = 0.14 NS
Sat_117	29	70	44	3.21 p = 0.20 NS	22	59	20	2.94 p = 0.23 NS
Sct_187	–	–	–	–	27	53	22	0.65 p = 0.72 NS

†Individuals showing the SSR allele from the susceptible (S) parent, heterozygous (H) for the SSR allele, or showing the allele from the resistant (R) parent PI 594760B.
‡Probability of significance of the chi-square. Values higher than 0.05 are considered nonsignificant.
§NS, nonsignificant.

the same region as the POP-1 locus and *Rpp1* from other sources and in the same map order as the soybean consensus linkage map (Song et al., 2004).

Together, the genetic and molecular data suggest that a single gene located at or very close to the *Rpp1* locus in both populations is controlling SBR resistance. However, the resistance gene has a dominant nature in POP-1 while this relation is inverted in POP-2, where the resistance gene segregates as recessive.

Role of the TMG06_0011 in Suppressing the Resistance Phenotype

To investigate the potential inversion of dominance caused by our breeding line, we performed several crosses between known sources of SBR resistance genes and our two breeding lines, TMG06_0012 and TMG06_0011 (Table 4). PI 200487, PI 200526, and PI 471904 are sources of dominant alleles in the *Rpp5* genomic region (Garcia et al., 2008). PI 200456 is the original source of the recessive allele of *Rpp5* (Garcia et al., 2008; Calvo et al., 2008). The F_1 plants were all scored as resistant for the first three PIs and susceptible for the cross involving PI 200456. These data agree with what was previously reported for these PIs, indicating that no inversion of dominance occurred at the *Rpp5* locus. Inversion of dominance also did not occur in the crosses involving PI 224270, source of a recessive allele at the *Rpp2* locus (Garcia et al., 2008; Calvo et al., 2008) or CG 84058-18 and CG 84058-21, sources of a dominant allele in the *Rpp4* region (authors, unpublished data, 2009). However, the inversion occurred when we crossed TMG06_0011 with PI 587905, which carries a dominant resistance allele in the *Rpp1* genomic region (authors, unpublished data, 2009), as well as with PI 561356, which was recently reported as also possessing a dominant gene near the *Rpp1* locus (Camargo et al., 2009). Unfortunately, we were not able to test the *Rpp3* locus, since the *P. pachyrhizi* isolated used in our study is able to defeat the resistance conferred by the sources of *Rpp3* resistant alleles.

Genetic Analyses of the PI 561356 × TMG06_0011 Cross

We further evaluated the PI 561356 × TMG06_0011 cross. Several responses to rust have been reported for this PI: it was reported as having a single dominant gene close to the *Rpp1* locus (Camargo et al., 2009), a TAN and/or mixed lesion type was observed for this PI in previously studies (Miles et al., 2006), and under our screening conditions it had an immune phenotype (resistant reaction with no apparent lesion symptoms). All the F_1 plants derived from this cross showed the TAN susceptible lesion type. The F_2 generation segregated 72 plants with the TAN lesion type to 24 immune, which perfectly fits the expected segregation ratio for a single recessive gene (3:1 S:R, $\chi^2 = 0.00$, $p = 0.99$ [nonsignificant]).

With the purpose of confirming the hypothesis of a single locus involved in the resistance, we backcrossed the F_2 resistant plants from the cross PI 561356 × TMG06_0011 (which in theory were homozygous for the single recessive resistance gene) with TMG06_0011. We also crossed these

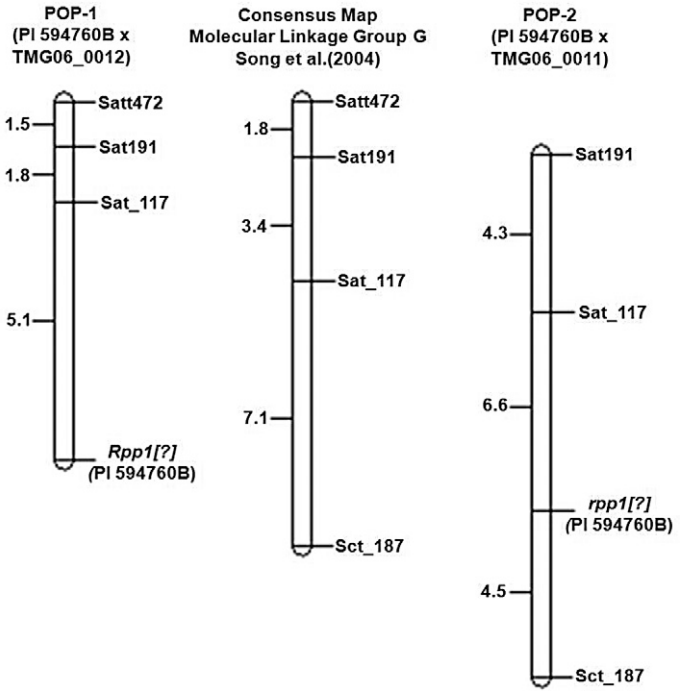

Figure 1. Genetic linkage map location of a putative *Rpp1* locus present in PI 594760B based on the phenotypic data of two F_2 populations and the segregation of public simple sequence repeat (SSR) markers. The molecular linkage group (MLG) G (chromosome 18) from the soybean linkage consensus map (Song et al., 2004) is positioned on the center for comparison. The genetic distances (in cM) are shown on the left side.

Table 4. Soybean rust (SBR) response in the F_1 generation resulted from the cross between different soybean resistant accessions and the two breeding lines TMG_0012 and TMG_001.

Resistant genotype	*Rpp* gene	Cross and SBR response	
		× TMG06_0012	× TMG06_0011
PI 594760B	*Rpp1*[?]	RB†	TAN‡
PI 561356	*Rpp1*[?]	RB	TAN
PI 587905	*Rpp1*[?]	RB	TAN
PI 224270	*rpp2*[?] (Recessive)	TAN	TAN
CG 84058-18	*Rpp4*[?]	RB	RB
CG 84058-21	*Rpp4*[?]	RB	RB
PI 200456	*rpp5*(Recessive)	TAN	TAN
PI 200526	*Rpp5*[?]	RB	RB
PI 200487	*Rpp5*[?]	RB	RB
PI 471904	*Rpp5*[?]	RB	RB

†RB, reddish-brown (resistant lesion type).

‡TAN, tan (susceptible lesion type).

same F_2 resistant plants with the commercial cultivar CD205 (Coodetec), which is susceptible to SBR. The F_2 resistant plants from the cross of PI 561356 × TMG06_0011 will be referred from here as "356_011-F_2R." From the backcross "356_011-F_2R" × TMG06_0011, all the F_1–derived plants were susceptible. On the F_2 generation, 188 plants had the TAN lesion type and 74 were immune, confirming the segregation of a single recessive resistance gene (3:1 S:R, $\chi^2 = 1.66$, $p = 0.20$ [nonsignificant]; 13:3 S:R, $\chi^2 = 15.50$, $p < 0.01$). For the cross "356_011-F_2R" × CD205, all the F_1–derived plants were resistant and showed the RB lesion type. The segregation ratio of the F_2 plants was 136:272:144 TAN:RB:immune (expected ratio for a single gene 1:2:1, $\chi^2 = 0.35$, $p = 0.84$ [nonsignificant]), suggesting that the single resistant gene has an incompletely dominant action in this cross and that the heterozygous plants develop the RB phenotype. If we combine the RB lesion and immune resistant phenotypes the segregation also fits the expected 3:1 R:S single dominant gene ratio (416:136 R:S, $\chi^2 = 0.04$, $p = 0.84$ [nonsignificant]).

DISCUSSION

In this paper we report the alternation of gene action type whereby resistance to SBR conferred by a single resistant allele can be inherited as dominant or recessive, depending on the susceptible allele involved in the cross. To our knowledge, this is the first time that this phenomenon has been reported for a soybean pathosystem. The presence of a second independent gene in the susceptible parent TMG06_0011, acting as a suppressor of SBR resistance, was our first hypothesis to explain the phenomenon. However, the clear single-gene segregation observed in all the crosses and the molecular marker data completely ruled out this possibility. A second possible explanation could be seed contamination in our PI stock such that plants were of different genotypes. However, the fact that we always performed the crosses using the same individual plant as the donor resistant parent excludes this possibility.

PI 594760B was described as highly resistant to SBR as both seedlings and adult plants and in greenhouse and field evaluations, where low severities (less than 0.5%) and RB lesions with low sporulation levels were observed (Miles et al., 2006, 2008). The genetic basis of the resistance was not determined in these previous studies; however, here we report that a single gene controls the SBR resistance in PI 594760B derived populations. Interestingly, the resistance is dominant in the cross PI × TMG06_0012 and recessive in the cross PI × TMG06_0011.

The fact that we conducted the SBR screenings using a greenhouse population of *P. pachyrhizi* that cannot be verified as a pure culture could be viewed as a limitation to our study. We do not believe that this was a problem, however, since it has been proven that consistent genetic and molecular analyses can be done in carefully monitored screenings with field (Ray et al., 2009) and greenhouse populations (Silva et al., 2008; Calvo et al., 2008; Garcia et al., 2008). We also performed a validation test using spores obtained from a single lesion. Because of the limited amount of spores, this validation was limited to the parents (PI 594760B, TMG06_0012, and TMG06_0011), F_1, and a portion of $F_{2:3}$ families, but the SBR responses obtained from these tests (data not shown) were in perfect agreement with the ones obtained using our greenhouse- collected spores.

PI 561356 was described as having a mixed (RB and TAN lesions in the same leaf) reaction type in seedling screenings where the plants were inoculated with a mixture of isolates from Thailand (TH01-1), Brazil (BZ01-1), Paraguay (PG01-2), and Zimbabwe (ZM01-1) (Miles et al., 2006). In our screenings PI 561356 showed an immune reaction (no obvious lesion symptoms) when challenged with our isolated. Also, this PI was scored as resistant in other recent study conducted in Brazil (Camargo et al., 2009). The discrepancies between these disease studies are most likely a consequence of the different *P. pachyrhizi* isolates used, since SBR resistance genes respond differently when challenged with different isolates (Bonde et al., 2006; Pham et al., 2009).

The cross PI 561356 × TMG06_0011 and the backcross "356_011-F_2R" × TMG06_0011 ("356_011-F_2R" = F_2 resistant plants from PI 561356 × TMG06_0011) resulted in the inheritance of resistance as a single recessive gene and the progenies only showed TAN and immune reactions in the F_2 populations. On the other hand, the cross "356_011-F_2R" × CD205 resulted in a single locus with 1:2:1 immune:RB:TAN F_2 segregation. Because we used F_2 recessive resistant plants (356_011-F_2R) in the backcross with TMG06_0011 and in the cross with CD205, the contrasting genetic inheritances observed for the resistance (dominant and recessive) could only have occurred if a single locus was involved in the inheritance, with the allele from PI 561356 conferring the SBR resistant phenotype, the susceptible allele

from TMG06_0011 being dominant to the resistance, and the susceptible allele from CD205 being recessive.

The resistance pattern with three phenotypic responses to SBR (immune, RB, and TAN) in the F_2 generation also occurred for PI 587866 and PI 587880 in response to a field inoculation in Paraguay during the 2007/2008 growing season (Ray et al., 2009). In that case, the authors identified a single locus—a putative *Rpp1* allele—acting with incomplete dominance. A dosage effect where the *Rpp1/Rpp1* genotype produces the immune reaction, the *Rpp1/rpp1* leads to the RB lesion, and the *rpp1/rpp1* conferred the TAN lesion type was proposed (Ray et al., 2009). A single locus with incomplete dominance also appears to be acting in our population. Circumstantial evidence of immune reactions was reported for the *Rpp4* (Hartwig, 1986) and *Rpp3* (Bonde et al., 2006) locus, but it has only been consistently observed for the *Rpp1* locus in response to some of *P. pachyriyzi* isolates (McLean and Byth, 1980; Bonde et al., 2006; Hyten et al., 2007; Pham et al., 2009; Ray et al., 2009). Moreover, up to now the incomplete dominance resulting in the RB phenotype of the heterozygous genotype has only been clearly reported for the possible *Rpp1* gene described by Ray et al. (2009).

The genetic linkage map location of the dominant (POP-1, cross PI 594760B × TMG06_0012) and the recessive (POP-2, cross PI 594760B × TMG06_0011) genes reported here is in the same region as *Rpp1* (Hyten et al., 2007), *Rpp1b* (Chakraborty et al., 2009), and the likely *Rpp1* allele discovered in PI 587886 (Ray et al., 2009). A single dominant gene for PI 561356 was also mapped in the *Rpp1* vicinity (Camargo et al., 2009). The map distances are not the same in our two mapping populations and also vary from those in previous studies. Nonetheless, these differences are common in small genetic intervals, since the linkage maps are a measure of recombinant events. They may result from the different types of mapping populations, different numbers of individuals and/or markers, deviations in the segregation, or from small genotyping and/or phenotyping errors. The most relevant point is that the order of the markers in the map is in agreement with the order of the soybean linkage consensus map (Song et al., 2004).

Taken together, the genetic and molecular data from PI 594760B-derived populations and the genetic data from PI 561356-derived populations indicate that the same single locus is involved in the dominant, incomplete dominant, and recessive SBR resistant phenotypes. Although it is possible that the gene reported here is an independent resistance locus linked to *Rpp1*, it is more likely that the gene is a different allele at the *Rpp1* locus. However, allelism tests between the PIs reported here and the sources of other *Rpp1* alleles, and the SBR reactions of the progenies to different *P. pachyrhizi* isolates, will be necessary to clearly answer this question.

The dominant, incomplete dominant, and recessive nature of the SBR resistance genes (*Rpps*) are well documented. For instance, at least two dominant alleles are reported for the *Rpp1* locus (McLean and Byth, 1980; Chakraborty et al., 2009). Dominant alleles are also known for *Rpp2*, *Rpp3*, *Rpp4*, *Rpp5*, and [*Rpp?*]_PI506764 (Bromfield and Hartwig, 1980; Bromfield and Melching, 1982; Hartwig, 1986; Garcia et al., 2008; Monteros et al., 2007). Incomplete dominance has been observed for the possible *Rpp5* allele from PI 471905 (Garcia et al., 2008) and for the possible *Rpp1* allele from PI 587886 and PI 587880A (Ray et al., 2009). The first single recessive genes controlling SBR resistance were reported in PI 224270 and in PI 200456 (Calvo et al., 2008). In subsequent studies it was elucidated that PI 224270 posses a possible recessive allele of *Rpp2* and that PI 200456 has a recessive allele at *Rpp5* (Garcia et al., 2008). However, a single resistant allele in the same plant acting as recessive and dominant (or incomplete dominant) was never reported.

Our findings point toward a complex interaction and it seems that the nature of the single *Rpp* resistant genes is a relative status. We propose that a dominant susceptible allele, present in TMG06_0011, is interfering in the SBR resistance leading to the inversion of dominance observed in each cross. Therefore, a multiallelic series is acting, where the TMG06_0011 susceptible allele is dominant over the PI 594760B and PI 561356 resistant alleles, which in turn are dominant to the TMG06_0012 and CD205 susceptible alleles.

Further molecular analysis is needed to explain the inversion of dominance reported here. By now, due to the fact that the same resistant allele (most likely a *Rpp1* allele) was present in the dominant and the recessive inheritance analysis, we are speculating that a gene silencing mechanism is taking part in this pathosystem. This could be happening at the transcriptional or posttranscriptional level (Mlotshwa et al., 2002; Rahman et al., 2008), triggered by an inverted repeat and/or a truncated gene at the resistance locus or in a neighboring gene, leading to formation of double-stranded RNA (dsRNA).

It is notable that very often two or more genes or parts of the genes are arranged as inverted repeats. Many of such inverted repeats are dominant silencing loci, repressing the expression of homologous genes (Muskens et al., 2000). If a transcript of one repeat extends into the other, the result would be production of antisense RNA of the second repeat, allowing for the formation of dsRNA that could trigger posttranscriptional gene silencing mechanisms. A similar phenomenon was reported for silencing soybean *CHS* (chalcone synthase) in seed coats (Clough et al., 2004). The i^i locus contains a 10.9 kb cluster of 3 *CHS* genes inversely duplicated, as well as a neighboring subtilisin gene that is truncated and transcribed toward the *CHS* cluster (Clough et al., 2004). The large inverted

duplication of the *CHS* gene cluster and the presence of the neighboring truncated subtilisin gene can lead to production of antisense or aberrant *CHS* RNAs that could potentially be involved in the silencing of *CHS* expression in seed coats (Clough et al., 2004; Tuteja et al., 2004, 2009).

Inverted repeats of endogenous sequences are clearly the result of duplications (Muskens et al., 2000). Resistance genes are found both as isolated genes (singletons) and as tightly linked arrays of related genes (gene clusters) and these clusters are mainly a result of tandem duplications (Leister 2004). Gene families rapidly evolving under stress, such as these complex disease-resistant gene clusters, are prime candidates for young small RNA-generating loci in plants (Voinnet, 2004). The soybean genome contains many duplicated regions with approximately 75% of the genes occurring as multiple copies (Schoemaker et al., 1996; Schmutz et al., 2010). Moreover, the recent molecular characterization of the SBR resistance gene *Rpp4* (Meyer et al., 2009) revealed that three resistance genes candidates from the same family are arranged in a tandem manner. Also, the *Rpp4* locus is duplicated elsewhere in the soybean genome (Meyer et al., 2009). If the *Rpp1* genomic region evolved in a similar manner as *Rpp4*, it is possible that an inverted sequence could have arisen at the TMG06_0011 locus related to SBR resistance. The molecular cloning of this genomic region in our plant material and/or the detailed annotation of the soybean genome sequence will be necessary to test this hypothesis.

We performed several crosses with other sources of known SBR resistant genes and our two breeding lines. The inversion of dominance did not occur for the *Rpp2*, *Rpp4*, and *Rpp5* populations, but it was observed in populations derived from the possible *Rpp1* accessions PI 594760B, PI 561356, and PI 587905. We could not test the *Rpp3* locus because there were no *Rpp3* alleles known to condition resistance to the *P. pachyrhizi* isolate used in our study. However, it appears that the phenomenon is exclusively related to the *Rpp1* genomic region. This genomic region has a great diversity for the SBR resistance manifested by the different alleles or loci (Hyten et al., 2007; Chakraborty et al., 2009; Ray et al., 2009). Also, it is the only genomic region with SBR resistance loci and/or alleles that consistently gives an immune reaction to some *P. pachyrhizi* isolates (McLean and Byth, 1980; Bonde et al., 2006; Hyten et al., 2007; Pham et al., 2009; Ray et al., 2009). Even a quantitative trait locus related to disease severity was mapped to the *Rpp1* region (Chakraborty et al., 2009).

This diversity is particularly interesting for a breeding perspective. The original *Rpp1* allele from PI 200492 (McLean and Byth, 1980) is no longer effective in Brazil (Yorinori et al., 2005). In spite of that, the SBR resistance sources reported here probably have *Rpp1* alleles or genes at a closely linked locus that are effective under greenhouse screenings in our country. This expands the potential sources of resistance genes that could be used in the development of SBR-resistant cultivars, providing breeders and researchers with more tools to stay ahead of *P. pachyrhizhi* in the host versus pathogen race. In addition, we described the presence of a dominant susceptible allele that can suppress the SBR resistance. This is crucial information for breeders make effective and durable genetic combinations, and it is possible that many other SBR resistance genes are hidden behind susceptible alleles.

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