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ORIGINAL PAPER

Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles

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Abstract Soybean production in South and North America has recently been threatened by the widespread dissemination of soybean rust (SBR) caused by the fungus *Phakopsora pachyrhizi*. Currently, chemical spray containing fungicides is the only effective method to control the disease. This strategy increases production costs and exposes the environment to higher levels of fungicides. As a first step towards the development of SBR resistant cultivars, we studied the genetic basis of SBR resistance in five F_2 populations derived from crossing the Brazilian-adapted susceptible cultivar CD 208 to each of five different plant introductions (PI 200487, PI 200526, PI 230970, PI 459025, PI 471904) carrying SBR-resistant genes (*Rpp*). Molecular mapping of SBR-resistance genes was performed in three of these PIs (PI 459025, PI 200526, PI 471904), and also in two other PIs (PI 200456 and 224270). The strategy mapped two genes present in PI 230970 and PI 459025, the original sources of *Rpp2* and *Rpp4,* to linkage groups (LG) J and G, respectively. A new SBR resistance locus, *rpp*5 was mapped in the LG-N. Together, the genetic and molecular analysis suggested multiple alleles or closely linked genes that govern SBR resistance in soybean.

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Introduction

Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. & P. Syd. is considered to be the most destructive foliar disease in soybeans (*Glycine max* (L.) Merr.) (Miles et al. 2003). The disease is disseminated through urediniospores carried by the wind and can develop rapidly, causing loss of foliar area and a severe reduction in grain yield.

The SBR disease was reported for the first time in 1902 in Japan, and was then described in other parts of Asia and Australia in 1934 (Kochman 1977), India in 1951 (Sharma and Mehta 1996), Hawaii in 1994 (Killgore and Heu 1994), and Africa in 1996 (Akinsanmi et al. 2001). In South America, the disease was first reported in 2001 in Paraguay (Paiva and Yorinori 2002) and Brazil (Yorinori et al. 2005), and in the following years, reached Argentina, Bolivia and Colombia (Rossi 2003). These authors estimated that the disease caused yield losses varying from 10 to 80%. In the United States, the disease appeared for the first time in November 2004 (Schneider et al. 2005), but environmental conditions have proven to be unfavorable to fungus spread and therefore, considerable crop damages have been avoided so far (Sconyers et al. 2006).

Phakopsora pachyrhizi development is favored by a temperature range of 15–29°C and high humidity. Under these conditions, disease symptoms can be detected

between 5 and 8 days after infection (Marchetti et al. 1976; Melching et al. 1989). Lesions initially show a polygonal shape measuring 2–5 mm and are tan colored. After 10–14 days, the reproductive structures appear on the abaxial face of the leaf. These are characterized by volcano-shaped growths, known as uredinia, where the urediniospores are produced (Marchetti et al. 1975). The disease progression culminates with premature defoliation.

Although fungicide applications are able to reduce losses in yield, the use of resistant or tolerant cultivars is seen as the best alternative for disease control due to factors such as reductions in cost, management facility and environmental concerns. Resistance to *P. pachyrhizi* occurs naturally in wild species of the genera *Glycine* (Burdon and Marshall 1981; Burdon 1988) and is conferred by a hypersensitive reaction (HR). This is a common type of response triggered when plant resistance genes (R-genes) are challenged by pathogen avirulence genes (Avr- genes) (McDowell and Simon 2006). Resistant genotypes show red–brown (RB) lesions and a spectrum ranging from no to high levels of sporulation depending on the genotype. Susceptible genotypes are characterized mostly by light brown (TAN) lesions with profuse sporulation.

Resistance genes have also been described in cultivated soybean. Presently, four different loci carrying dominant alleles have been reported: *Rpp1* identified in PI 200492 (McLean and Byth 1980), *Rpp2* from PI 230970 (Bromfield and Hartwig 1980), *Rpp3* (PI 462312) (Bromfield and Melching 1982) and *Rpp4* (PI 459025) (Hartwig 1986). An immune response has also been described for PI 200492 when it is inoculated with a particular type of *P. pachyrhizi* isolate (Bonde et al. 2006).

Although the use of resistance genes may offer an opportunity for disease control in soybean, their "race specific" nature may pose problems (Yamaoka et al. 2002; Bonde et al. 2006). For instance, in 2001 when SBR occurred for the first time in Brazil, all four of the resistance genes were effective against the disease, but in the following year, only *Rpp2* and *Rpp4* conferred resistance (Yorinori et al. 2005). Therefore, the discovery of new resistance genes is vital and stacking multiple resistance genes in a single cultivar (pyramiding) (Liu et al. 2000) could contribute to the sustainable development of SBR resistant cultivars.

The use of molecular markers is an effective tool for gene identification and transfer (Tanskley 1983; Tanskley and McCouch 1997), and can speed up the development of soybean cultivars carrying single or multiple resistance genes. Soybean has a reasonably dense molecular-marker linkage map (Song et al. 2004), and the association of markers to known genes has been pursued by many groups. Molecular mapping of SBR-resistance genes in soybean has previously been reported. Brogin et al. (2004) identified Single Sequence Repeat (SSR) markers linked to rust resistance present on the cultivar FT-2 in the linkage group (LG)-C2 of the previous soybean consensus map reported by Cregan et al. (1999). However, the locus could not be identified in the study. An SBR resistance gene from the cultivar Hyuuga was mapped at ~3-cM interval on LG-C2 between Satt134 and Satt460 (Monteros et al. 2007). Hyten (2007) recently mapped the *Rpp3* locus at same interval that Monteros et al. (2007). The *Rpp1* locus has been mapped to a ~1-cM interval on LG-G between Sct_187 and Sat_064 LG-G (Hyten et al. 2007).

The current study analyzed the genetic basis of SBR resistance in five distinct soybean genotypes. Three genes behaved as dominant genes and one showed incomplete dominance. The fifth genotype showed segregation distortion and the SBR resistance could not be determined. Molecular mapping of the SBR resistance genes in these populations as well as in two other populations carrying recessive resistance genes allowed us to identify at least three distinct loci conferring rust resistance in soybean.

Materials and methods

Plant material and genetic analysis

Genetic analysis was performed in five populations obtained by crossing a rust resistant parent with a susceptible parent. The resistant genotypes were chosen based on information in the literature (Bromfield and Hartwig 1980; Hartwig 1986), as well as on our preliminary allelism tests. The genotypes selected were: PI 200487 (Kinoshita), PI 200526 (Shira Nuhi); PI 230970, PI 459025 (Bing nan) and PI 471904 (Orba). PI 200487, PI 200526 and PI 230970 were collected in Japan in 1952, 1952 and 1956, respectively (GRIN 2008). PI 459025 was collected in China in 1981 and PI 471904 was collected in Indonesia in 1982 (GRIN 2008). PI 230970 and PI 459025 were previously described as having dominant genes at the *Rpp2* and *Rpp4* loci, respectively (Bromfield and Hartwig 1980; Hartwig 1986). The susceptible parent in every cross was the cultivar CD 208 and it was always used as the female parent. In spite of the large morphological differences of the exotic genotypes when compared with the Brazilian-adapted cultivar, the hybrid nature of the F_1 was confirmed by molecular marker analysis.

A portion of the F_1 seeds were planted and allowed to set seeds. Two hundred and four F_2 seeds from each cross were sowed (four plants per pot) in the greenhouse along with the parents and the remaining F_1 seeds. Experiments were performed during September 2005 (F_2 populations) and March 2006 ($F_{2:3}$ progeny test). F_2 plants were inoculated with *P. pachyrhizi,* as described below. After scoring the plants for the SBR disease, the plants were sprayed with

fungicide (Flutriafol 125 g/L, IMPACT®, Cheminova) and allowed to set seeds. A progeny ($F_{2:3}$) test was performed in order to confirm the phenotype and assign the genotype of the F_2 plants. Since a minimum of 11 plants (calculated according to the equation $n = \log(1 - P)/\log(1 - p)$) were needed to perform the progeny test with an acceptable confidence level ($P = 0.95$), final data were obtained for approximately 180 F_2 plants (varying with different populations). A Chi-square (χ^2) test was performed to verify whether the genetic segregation fit any expected model. Molecular analysis was conducted only for tested progeny of F_2 plants. Two other F_2 populations created by Calvo et al. (2008) were included in the molecular study. These populations carry recessive resistance genes and were developed by the crosses between resistant parents (PI 200456 and PI 224270) and the susceptible parent CD 208 (Calvo et al. 2008).

An allelism test was conducted only on the cross between PI 224270 and PI 230970. F1 plants were selfed and 430 F_2 seeds were planted and evaluated for SBR resistance as described below.

Phakopsora pachyrhizi inoculation and phenotyping

The isolate used in this study was obtained by collecting spores from naturally infected greenhouse plants of the susceptible cultivar BRSMS Bacuri, during the summer of 2004 in Cambé, PR, Brazil. This cultivar carries a rust resistance gene from FT-2 (Brogin et al. 2004) that had its resistance broken during the summer of 2003, shortly after the first appearance of the disease in Brazil (winter of 2001). Samples of the original isolate have been maintained in liquid N_2 (available upon request) and were used to infect greenhouse plants ('BRSMS Bacuri'). Since the isolate did not originate from a single lesion, it is possible that it represents a mixture of more than one race. Therefore, all multiplications of the isolate, as well as screening procedures, were monitored for the appearance of mixed (TAN and RB) types of lesion. Currently, no mixed lesion types have been observed in our greenhouse inoculations.

Phenotypic data from F_1, F_2 and $F_{2:3}$ plants were obtained by spraying the plants in the V3 developmental stage (Fehr and Caviness 1977) with an aqueous solution containing 50×10^3 *P. pachyrhizi* urediniospores per mL and Tween 20 (0.01% v/v). Urediniospores were collected from the abaxial leaf face of the susceptible cultivar BRSMS Bacuri by simply washing the leaf surface with water. Plants were inoculated with the spore solution at the end of the day, between 5:00 and 7:00 pm, and were kept in the greenhouse under 25°C/20°C ± 3°C night/day temperature and natural light. During the first 3 days after inoculation, the leaf surface was kept wet by regular spraying (mist) with an automated irrigation system. Two weeks after inoculation, the plants were scored for the presence of disease or resistance symptoms. Plants were considered resistant when they showed a red–brown (RB) lesion type and susceptible if they showed a TAN lesion. After scoring, plants were sprayed with fungicide (Flutriafol 125 g/L, IMPACT®, Cheminova) to control the disease and were grown to maturity.

DNA isolation

Healthy leaf tissue was collected from the parents and F_2 plants. Tissue was frozen in liquid nitrogen, freeze-dried, and ground to a fine powder with an A-10 mill (Janke & Kunkel IKA). DNA was extracted from the resulting powder using the protocol described by Shagai-Maroof et al. (1984).

Marker analysis

SSR markers were used for mapping studies. In order to ensure complete genome coverage, markers were chosen based on their distribution throughout the integrated molecular linkage map of soybean (Song et al. 2004). The sequence for each specific primer chosen for the study was retrieved from SoyBase and Toolbox (2007). Each SSR marker was tested for polymorphism between the parental lines.

PCRs were performed in a PTC-200 (Bio-Rad) thermocycler in a 20 µL reaction containing approximately 30 ng of template DNA, 0.25 µM of each primer, 0.15 µM dNTPs, 1.5 U of *Taq* DNA polymerase, 2.5 mM $MgCl_2$ and 1 × PCR buffer (20 mM Tris–HCl pH 8.3; 50 mM KCl). Amplification cycling conditions were: an initial cycle of 95°C for 3 min followed by 35 cycles of 94°C for 30 s for DNA denaturing; 55–60°C (depending on the annealing temperature of each primer pair) for 30 s for primer annealing; 72°C for 45 s for primer extension and a final cycle at 72°C for 10 min. PCR products were fractionated in a 3–4% MetaPhor (Lonza Bioscience) agarose gel and stained by adding 1 µL of ethidium bromide solution (10 mg/mL) to every 10 mL of gel solution. Gel images were obtained with a Typhoon (GE Healthcare) scanner.

Linkage analysis

Linkage of a SSR marker to the resistance trait in each F_2 population was initially obtained through bulk segregant analysis (BSA) according to the methods of Michelmore et al. (1991). Two different bulk groups were formed for each of the seven populations studied. The bulk groups were obtained by pooling an equal amount of DNA from 10 different plants, which were homozygous either for the resistance (Bulk R), or susceptibity (Bulk S) to SBR. SSR

markers that were polymorphic between the parents were testes. Those markers that showed a polymorphic pattern between the R and S bulks were considered to be potentially linked to the resistance gene and were further evaluated within individual F_2 plants from the corresponding bulk groups. Once a resistance gene was placed on a linkage group, the segregation of a new set of SSR loci in the same genomic region was evaluated in the entire F_2 population in order to map the resistance gene more precisely on the linkage group.

The SBR reactions were converted to data suitable for mapping according to the resistance gene action in each PI (dominant or recessive). The resistance in the incomplete dominance case was considered to be dominant. The data were based on the genotyping (homozygous dominant, recessive or heterozygous for the resistance) of the F_2 individuals with the $F_{2:3}$ test.

Before mapping, a Chi-Square (χ^2) test was performed on the dataset to determine if the markers were segregating according to expectations (1:2:1 ratio). All the SSR markers segregated as expected for co-dominat markers (data not shown). MAPMAKER/EXP 3.0 (Lincoln and Lander 1993) was used for linkage analyzes and map construction. A LOD score of 3.0 and Kosambi's mapping function with a maximum of 50 cM distance threshold were used for linkage confirmation. The maps obtained were compared to the soybean consensus map (Song et al. 2004).

Results

Inheritance of rust resistance

The results of rust resistance segregation for the different F_2 and $F_{2:3}$ populations are shown in Table 1. The segregation ratios for the PI 459025 population are suggestive of the presence of a single dominant gene. The segregation ratios in F_2 and $F_{2:3}$ also indicate that a single dominant gene is responsible for resistance in the PI 200487 and PI 200526 populations.

Close inspection of the phenotypic segregation for PI 459025 and PI 200526 shows that the data also fits the 13 resistant to 3 susceptible ratio ($\chi^2 = 0.22$; $P > 0.05$ and $\chi^2 = 2.583$; $P > 0.05$, respectively). These results would support the hypothesis for epistatic action of two independent resistance genes. However, the BSA data (see below) for these populations, as well as for all other populations showed that only one genomic region was involved in resistance. In addition, a previous allelism test between PI 200526 and PI 200487 (data not shown) did not show any evidence of a second gene. For these reasons, we discarded the possibility that two independent genes are involved in disease resistance in these PIs. Genetic segregation for the PI 471904 population also suggested the presence of a single gene based on the $F_{2:3}$ progeny test (Table 1) although this gene has an incomplete dominance.

The data for PI 230970, which has been previously described to carry a dominant allele at the *Rpp2* locus (Bromfield and Hartwig 1980) showed a distorted segregation pattern that did not fit a 3:1 (Table 1), a 9:7 ($\chi^2 = 6.895$; $P < 0.05$) or any other clear ratio. Therefore, we could not conclusively determine the genetic basis of SBR resistance inheritance in this population.

Genetic mapping

The number of markers evaluated for each population and the polymorphism frequencies are shown in Table 2. In the PI 459025 population that carries a dominant resistance gene and is the original source of *Rpp4* locus (Hartwig

Table 1 Phenotypic and genotypic segregation from populations derived by crossing different resistant parents to the susceptible parent CD 208

Resistant parent	F_2 test					$F_{2:3}$ test					
	No of plants					No of lines					
	R	*S*	Total	Expected ratio	χ^2	*R*	*H*	*S*	Total	Expected ratio	χ^2
PI 200526	156	47	203	3:1	0.369 NS	48	85	44	177	1:2:1	0.458 NS
PI 200487	150	49	199	3:1	0.015 NS	45	69	36	150	1:2:1	2.040 NS
PI 459025	158	43	201	3:1	1.395 NS	53	88	34	175	1:2:1	4.131 NS
PI 471904	103	91	194	1:1	0.742 NS	39	101	34	174	1:2:1	4.793 NS
PI 230970	123	64	187	3:1	8.487*	19	108	73	200	1:2:1	30.44*
PI 200456[a]	53	148	201	1:3	0.201 NS	41	80	52	173	1:2:1	2.376 NS
PI 224270[a]	43	152	195	1:3	0.904 NS	34	87	53	174	1:2:1	4.149 NS

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

* Statistical significance of the Chi-square value ($P = 0.05$)

[a] Population developed in the study made by Calvo et al. (2008), TMG Tropical Melhoramento e Genética Ltda., Brazil

Table 2 For each cross, the corresponding allele, the number of SSR loci tested, percentage that were parentally polymorphic, number of SSR used for map construction and number of individuals genotyped during mapping are shown

Resistant parent × 'CD 208'	*Rpp* allele	No of SSRs tested	% Polymorphic SSRs	No of markers used for mapping	No of individuals used for mapping
PI 224270	*rpp2[?](PI 224270)*	177	38	10	174
PI 230970 *(Rpp2)*	*Rpp2[?]*	175	36	–	–
PI 459025 *(Rpp4)*	*Rpp4[?]*	169	41	06	175
PI 200456	*rpp5(PI 200456)*	182	40	08	173
PI 200526	*Rpp5(PI 200526*	192	43	04	177
PI 200487	*Rpp5(PI 200487)*	190	42	–	–
PI 471904	*Rpp5 (PI471904)*	177	40	06	174

1986), the BSA analysis only showed linkage with molecular markers from linkage group G. Since a *P. pachyrhizi* isolate different from the one used by Hartwig (1986) was used in the present study, we cannot be certain that the locus identified here is indeed *Rpp4*. Therefore, we are temporarily designating this locus as *Rpp4[?]* according to the suggestions of the Soybean Genetics Committee (SGC, personal communication).

The populations derived from the crosses with PI 230970 (original source of *Rpp2*; Bromfield and Hartwig 1980) and PI 224270 showed evidence of linkage to the same group of markers located in a region of linkage group J. Therefore, we decided to perform an allelism test between these two PIs. No segregation occurred in the F_2 progeny of the cross PI 224270 × PI 230970, indicating that the SBR resistance in these two cultivars is conferred by alleles of the same locus (possibly *Rpp2*; Table 3). We designated the resistance allele in PI 224270 as *rpp2[?](PI 224270)* since this PI carries a recessive SBR resistance gene. Due to the segregation distortion observed in the cross CD-208 × PI 230970 (Table 1), this population was not used for mapping purposes. Instead, we chose to use the population derived from the cross CD-208 × PI 224270 (Calvo et al. 2008) for mapping the SBR gene.

Resistance in all the remaining PIs evaluated (PI 200456; Calvo et al. 2008, PI 200487, PI 200526 and PI 471904) was associated with primers that mapped to a specific genomic region from LG-N. Preliminary allelism tests indicated that PI 200487 and PI 200526 carry the same allele (data not shown). Since the population derived from PI 200526 had a larger number of F_2 plants, this population was chosen for mapping. PI 200456 and PI 471904 were also selected for mapping due to the distinct (recessive and incomplete dominance, respectively) nature of the resistance genes and because no allelism tests were available for these two parents.

Molecular mapping was performed after genotyping individual F_2 plants from each population. The number of SSR markers and individuals used for mapping, as well as the corresponding *Rpp* locus (if any) is shown on Table 2. The resistant *Rpp4[?]* locus from PI 459025 (original source of *Rpp4*) was mapped at 2.8 cm from Satt288 and 31.3 cM from Satt191 on LG-G (Fig. 1). Based on the consensus map (Song et al. 2004), other SSR markers exist in this interval but unfortunately they were not polymorphic in this population. The agreement with the consensus map was verified further by mapping three other SSR markers (Sat_199, Satt517 and Sat_143) in the region. Based on PI 459025 × CD 208 map the order of the markers is in good agreement with the consensus map (Fig. 1). The recessive gene from PI 224270 (*rpp2[?]*), which is a possible recessive allele of *Rpp2,* was mapped to LG-J between the SSR loci Satt215 (4.3 cM) and Sat_361 (4.7 cM) (Fig. 2).

The recessive gene from PI 200456, the dominant gene from PI 200526 and the incomplete dominant gene from PI 471904 all mapped between the same SSR loci in LG-N of the soybean consensus map, although the distances between the SSR loci varied among the three populations (Fig. 3). The gene from PI 200456 was mapped at 1.6 cM from Sat_275 and 7.2 cM from Sat_280. In the PI 471904 population, these distances were 0.6 and 3.6 cM and for the PI 200526 population the distances were 4.3 and 6.5 cM, respectively. Since no allelism tests are available for these populations, we cannot conclusively determine if they are alleles of the same locus or closely linked genes. This is a potential new locus and will be referred as *Rpp5*.

Table 3 Results of the allelism test performed between PI 230970 (contains *Rpp2*; Bromfield and Hartwig 1980) and PI 224270 (*rpp2[?]*)

Cross	No of plants				
	R	S	Total	Expected ratio	χ^2
PI 230970 × PI 224270	430	0	430	1:0	0.000 NS

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

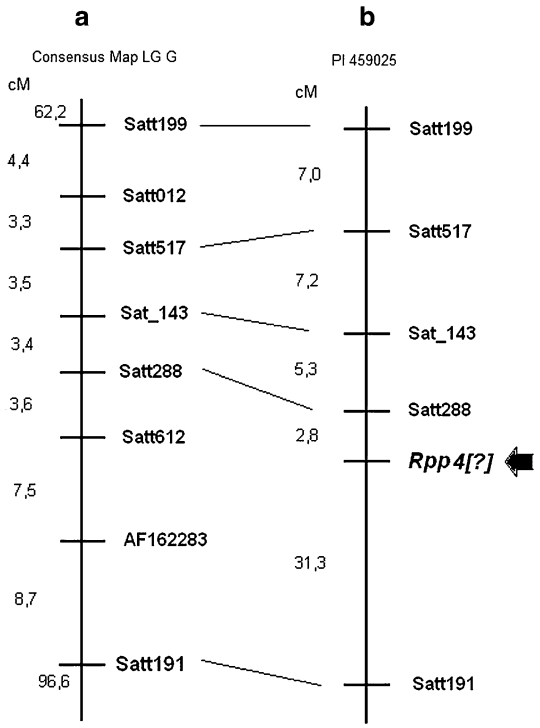

Fig. 1 Genetic linkage map constructed with SSR markers. **a** Represents a fragment of the consensus soybean linkage group G (Song et al. 2004); **b** illustrates the map derived from the F_2 population CD 208 × PI 459025. The *lines* indicate the corresponding position of the markers between the two maps. The *arrow* shows the position of the soybean rust resistance gene *Rpp4[?]*. The genetic distances (cM) are shown on the left side. Only the markers closest to the gene are shown in the figure

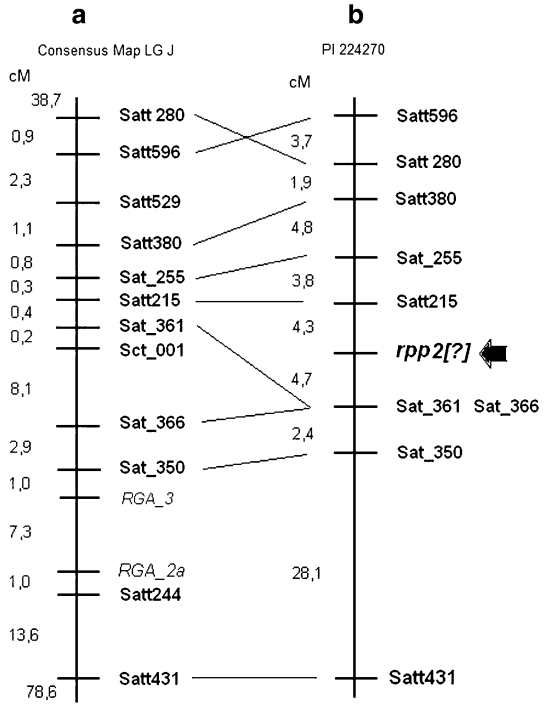

Fig. 2 Genetic linkage map constructed with SSR markers. **a** Represents a fragment of the consensus soybean linkage group J (Song et al. 2004); **b** illustrates the map derived from the F_2 population CD 208 × PI 224270. The *lines* indicate the corresponding position of the markers between the two maps. The *arrow* shows the position of the soybean rust resistance gene *rpp2[?](PI 224270)*. The genetic distances (cM) are shown on the left

Control maps were constructed to check the mapping data due to the map expansion observed on the five populations. These maps were made removing either the *Rpp* genes or each SSR marker used for the mapping on an individual basis, and the results remained almost the same (data not shown).

Discussion

SBR is currently the most damaging disease for soybean production in Brazil. As a first step towards the development of long lasting SBR rust resistant soybean cultivars, we are carrying out studies to increase our understanding of the genetic basis of this trait. Since *Rpp1* and *Rpp3* do not confer resistance to the *P. pachyrhizi* "race" used in our study, we were unable to conduct genetic and mapping studies for these loci.

PI 230970 is the source of the *Rpp2* reference allele initially described by Bromfield and Hartwig (1980). We were not able to confirm the presence of a single resistance gene because the segregation of the PI 230970 derived population did not fit any clear ratio. Difficulties in the genetic analysis of rust resistance have been previously reported (Burdon 1988). We currently do not have a clear explanation for this discrepancy. One possibility may be that we worked with a different isolate from the ones (India-73-1; Philippines-77-1 and Taiwan-72-1) used by Bromfield and Hartwig (1980). Moreover, distinct genetic behavior of rust resistance genes has been reported in wheat when challenged with different rust isolates and different conditions, including temperature and genetic background (Kolmer 1996).

The allelism test between PI 230970 and PI 224270 indicated that the SBR resistance genes are located in the same locus and the *rpp2[?]* allele from PI 224270 was mapped to LG-J. Interestingly, this genomic region also hosts several other disease resistance genes including *Rps2*, *Rmd* and *Rjs2* (Polzin et al. 1994), *Rbs1, Rbs3, Rcs3* and QTLs for cyst nematode resistance (SoyBase and Toolbox 2007).

We detected a single genomic region in LG-G associated with resistance in PI 459025. This PI contains *Rpp4,* as previously reported (Hartwig 1986) and it is possible that the gene present in this population is indeed *Rpp4*. However, because the *P. pachyrhizi* isolate used for our resistance screening was different from those isolates used by Hartwig (1986), the SBR resistance gene found in this PI may

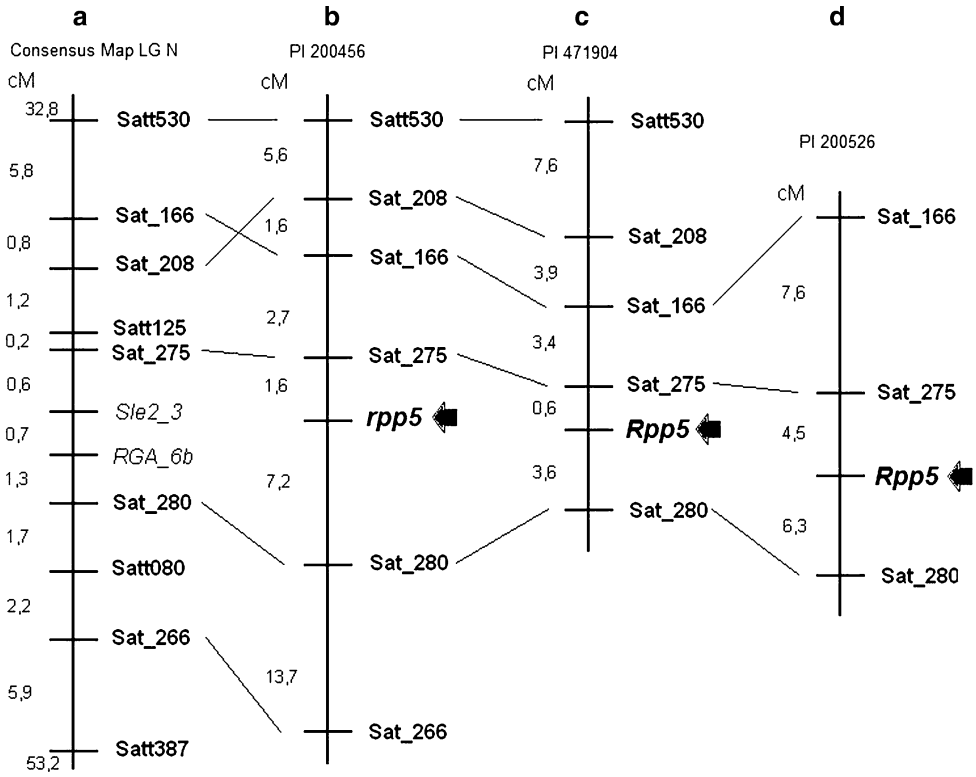

Fig. 3 Genetic linkage map constructed with SSR markers. **a** Represents a fragment of the consensus soybean linkage group N (Song et al. 2004); **b** illustrates the map derived from the F_2 population CD 208 × PI 200456; **c** shows the map derived from the F_2 population CD 208 × PI 471904; **d** illustrates the map derived from the F_2 population CD 208 × PI 200526. The *lines* indicate the corresponding position of the markers between the four maps. The *arrows* show the position of the soybean rust resistance genes. The genetic distances (cM) are shown on the left

represent a locus unrelated to *Rpp4*. Although the Chi-square test also fits the ratio for two independent loci controlling SBR resistance in PI 459025, we were unable to detect two genomic regions associated with the trait using our BSA approach. Taking the genetic and mapping data together, our data indicate that a single dominant locus controls SBR resistance in PI 459025, as proposed by Hartwig (1986). Recently, the *Rpp1* locus was also mapped to this same linkage group, although in another genomic region (Hyten et al. 2007).

Since we have used a different isolate from the one used by Bromfield and Hartwig (1980) and Hartwig (1986), we cannot rule out the possibility that the SBR resistance genes mapped in PI 224270 and PI 459025 are allelic to *Rpp2* and *Rpp4*, respectively. However, for the time being, we have designated the loci present in these PIs as *rpp2[?]* and *Rpp4[?]*, respectively.

Kato and Yorinori (2006) have studied the virulence of eight single lesion rust isolates collected from different parts of Brazil. Although they were able to show pathogenic differences among the isolates upon their inoculation into 14 different genotypes (including PI 230970, PI 459025, PI 200492 and PI 200526), all the isolates behaved identically (RB lesion) in PI 230970 (*Rpp2*), PI 459025 (*Rpp4*), and PI 200526(*Rpp5*). Therefore, the virulence diversity of the isolates was more related to the *Rpp1* (PI 200492) and *Rpp3* (PI 462312) loci, that had their resistance broken in Brazil during the summer of 2003.

The PI 471904 derived population showed a phenotypic segregation in the F_2 plants of 103 R:91 S that fits a 1:1 ratio which can be explained by the presence of incomplete dominance at this locus. The presence of a single gene controlling the resistance in this PI was further confirmed by the progeny test.

SBR resistance in three different PIs (PI 200526, PI 471904, and PI 200456) showing three distinct types of gene action (dominance, incomplete dominance and recessive) mapped to the same genomic region of LG-N. These data, along with our discovery of the *rpp2[?]* allele in PI 224270 and PI 230970 (source of *Rpp2*) have led us to speculate that there are multiple alleles or closely linked genes at the LG-N and LG-J SBR resistance loci. We are currently conducting allelism tests in very large F_2 populations to confirm this hypothesis.

Indirect evidence that different alleles of the same (or closely linked) locus may confer distinct resistance properties has been provided by recent publications by Monteros et al. (2007) and Hyten (2007). The first authors mapped the SBR resistance locus present in the cultivar Hyuuga to a ~3-cM interval on LG-C2 between Satt134 and Satt460. This is the same genomic region that Hyten (2007) mapped de SBR resistance locus *Rpp3* in PI 462312. In our screenings Hyuuga confers a RB type lesion while PI 462312, the original source of *Rpp3,* is completely susceptible to the SBR race prevalent in Brazil.

It will be interesting to see whether the different alleles behave differently when challenged with distinct rust isolates or in different genetic backgrounds. The diversity in *Rpp* genes revealed by the gene action types also explains some of the variation that we see in the performance of the sources of resistance. Although all of the SBR resistant germplasm carrying *Rpp* genes triggers a HR response, the intensity of the reaction and the behavior through the developmental stage of the plant vary considerably among the different genotypes (Bonde et al. 2006).

The maps created from the five populations (PI 200456, PI 200526, PI 224270, PI 459025 and PI 471904) were in good agreement with the consensus map created by Song et al. (2004) regarding markers order but differ with regard to the distances between each marker in each population and from the consensus map created by Song et al. (2004). A phenotypic error during the SBR resistance screening or a genotypic error for the SSR screening could account for these maps expansions. Due to the nature of the phenotype analyzed (lesion color) it is more likely that phenotype screening is causing this discrepancy with the consensus map.

Since *Rpp1* (Hyten et al. 2007), *Rpp3* (Hyten 2007*), rpp2[?](PI 224270)* (this study), present on the original source of *Rpp2*, and *Rpp4[?]*(this study), found on the original source of *Rpp4*, have been mapped to different genomic regions than LG-N, the locus mapped here in LG-N is potentially a new locus. Therefore, following the approval of the SGB, we are designating the locus identified in PI 200456 as *rpp5*.

In spite of being closely linked genes or alleles of the same gene, the existence of the new alleles reported here provide novel genetic variability for breeding programs in order to develop rust resistant soybean cultivars. From a plant breeding perspective, one important issue regarding SBR resistance is its duration in the field. Although some rust resistance genes have lasted over 20 years in barley, this is not always the case. For instance, wheat leaf rust resistance conditioned by single race-specific genes in Brazil does not last more than an average of 2 years (Barcellos et al. 1997). The fact that the resistance conferred by the *Rpp1* and *Rpp3* loci has been simultaneously broken only 2 years after the first appearance of SBR in Brazil illustrates the difficulties associated with breeding for SBR rust resistance.

However, unlike *Rpp1* and *Rpp3*, the *Rpp2* and *Rpp4* loci remain effective in Brazil 6 years after SBR appearance in the country and there are no signs the pathogen has overcome these resistance alleles so far. In Asia, the resistance conferred by *Rpp4* lasts approximately 20 years (Hartman et al. 2005), which is a good length of time from a breeding perspective. The combination of genetic approaches with other measures of controlling SBR may certainly help to extend the lifetime of SBR resistance genes in Brazil. For instance, adoption of a quarantine period (ninety days without any soybean crop in the field before a new soybean growing season starts) has considerably delayed the occurrence of the SBR epidemic in Mato Grosso state, consequently reducing the number of fungicide applications required for disease control in susceptible soybean cultivars.

Another alternative for extending the effectiveness of resistance genes would be to pyramid genes conferring resistance to different races of the pathogen within the same genotype. The availability of molecular markers linked to the different *Rpp* loci as shown here should make this task more feasible. In addition, it should provide soybean breeders with the ability to select resistant plants at an early stage of plant development, even in the absence of the pathogen.

The results of our study indicate that there is a complex arrangement (multiple alleles and/or very close genes with different modes of gene action) of *Rpp* loci in soybean. Our results also support the observation that the R genes in soybean also occur in clusters (Graham et al. 2002). The molecular mapping of the *Rpp* genes presented here may also help lay the foundations for map-based cloning of these genes in soybean.

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