New genes conferring resistance to Asian soybean rust: allelic testing for the *Rpp2* and *Rpp4* loci

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Abstract – The objective of this study was to conduct allelic tests including crosses between a group of rust resistant genotypes from Embrapa's soybean germplasm collection and PI 230970 and PI 459025, which carry the *Rpp2* and *Rpp4* genes, respectively. Asian Soybean rust (ASR) caused by the fungus *Phakopsora pachyrhizi* has resulted in significant yield losses and concern among Brazilian farmers. Until recently, there were four resistance genes (*Rpp1* through *Rpp4*) described in the literature, but only *Rpp2* and *Rpp4* are still effective in Brazil. Twenty-six sources of resistance to *P. pachyrhizi* were crossed with PI 230970 and PI 459025 (*Rpp2* and *Rpp4 g*ene sources, respectively) and plants of their F_2 generations were infected with a suspension containing $2.5x10^4$ spores per milliliter and assessed in a greenhouse after 20 days, for the presence of resistant (RB) or susceptible (TAN) lesions. Chi-square tests were applied to investigate the hypotheses of independent or allelic resistance gene segregations. ASR resistant genes derived from PI 197182, PI 230971 and PI 417125 did not segregate in crosses with PI 230970, which indicates that these genotypes have a single resistance gene in the *Rpp2* locus. Crosses with the other 23 genotypes resulted in segregating populations, suggesting that their resistance genes do not belong to *Rpp2* or *Rpp4* loci.

Index terms: *Glycine max*, *Phakopsora pachyrhizi*, inheritance, vertical resistance.

Novos genes de resistência à ferrugem-asiática-da-soja: teste de alelismo para os locos *Rpp2* e *Rpp4*

Resumo – O objetivo deste estudo foi desenvolver testes de alelismo, inclusive cruzamentos entre um grupo de genótipos resistentes à ferrugem, do banco de germoplasma de soja da Embrapa, e as duas fontes de resistência com os genes *Rpp2* e *Rpp4*. A ferrugem-asiática-da-soja, causada pelo fungo *Phakopsora pachyrhizi*, tem gerado perdas significativas na produtividade da cultura e preocupado os agricultores brasileiros. Até recentemente, existiam quatro genes de resistência descritos na literatura, mas somente *Rpp2* e *Rpp4* continuam efetivos no Brasil. Vinte e seis fontes com resistência a *P. pachyrhizi* foram cruzadas com PI 230970 e PI 459025 (fontes dos genes *Rpp2* e *Rpp4*, respectivamente), e as plantas da geração F_2 foram infectadas com uma suspensão com $2,5x10^4$ esporos por mililitro e analisadas em casa de vegetação após 20 dias, para verificação da presença de lesões de resistência (RB) ou de suscetibilidade (TAN). Foram aplicados testes de qui-quadrado para investigar as hipóteses de segregação independente ou de alelos de resistência nos locos *Rpp2* e *Rpp4*. Genes de resistência provenientes de PI 197182, PI 230971 e PI 417125 não segregaram em cruzamentos com a PI 230970, o que indica que esses genótipos apresentam um único gene de resistência presente no loco *Rpp2*. Cruzamentos com os outros 23 genótipos resultaram em populações segregantes, o que indica que estas possuem genes que não pertencem aos locos *Rpp2* e *Rpp4*.

Termos para indexação: *Glycine max*, *Phakopsora pachyrhizi*, herança, resistência vertical.

Introduction

The development of cultivars resistant to Asian soybean rust (ASR) – caused by the *Phakopsora pachyrhizi* fungus – became the first priority of Brazilian soybean breeding programs since 2001, when the disease was first detected in Southern Brazil. Four dominant genes were described as sources of resistance

– *Rpp1*, *Rpp2*, *Rpp3* and *Rpp4* – identified among plant introductions (PIs) and cultivars (Bromfield & Hartwig, 1980; McLean & Byth, 1980; Bromfield & Melching, 1982; Hartwig, 1986). McLean & Byth (1980) studied the inheritance of rust resistance in PI 200492, Tainung 3 and Tainung 4, using the Australian Q-1 isolate, and concluded that resistance is dominant over susceptibility. Working with another Australian isolate (Q-2), the same authors observed that PI 200492 and Tainung 3 were susceptible, while Tainung 4 remained resistant and, therefore, they should carry different resistance genes. The authors named *Rpp1* the dominant resistance gene from PI 200492 (McLean & Byth, 1976).

Bromfield & Hartwig (1980) found a single dominant gene determining the resistance of PI 230970 to India-73-1, Philippines-77-1 and Taiwan-72-1 isolates. Hartwig & Bromfield (1983) studied the relationship among the resistance of PI 200492, PI 230970 and PI 462312, using India-73-1 and Taiwan-72-1 isolates. They named *Rpp2* and *Rpp3* the resistance genes derived from PI 230970 and PI 462312, respectively. Another isolate of the fungus, Taiwan 80-2, broke the resistance of PI 200492, PI 230970 and PI 462312. The genotype PI 459025 was identified as resistant to Taiwan-80-2 and also to India-73-1 and Taiwan-72-1 isolates. Hartwig (1986) crossed the four PIs and concluded that PI 459025 carries a single resistance gene in a different locus (gene *Rpp4*, in the *Rpp4* locus). Still according to Hartwig (1986), Taiwan-72-1 isolate defeated the resistance of PI 200492 and PI 462312, but not those of PI 230970 and PI 459025. Other plant introductions as PI 417125, PI 203398 (Abura), PI 416764, PI 417115, PI 416819, PI 340050 and PI 417503 were studied and presented resistance reaction to *P. pachyrhizi* (Miles et al., 2006). More recently, other ASR major resistance genes, though recessive, were identified in the advanced Brazilian soybean line BR01-18437 (Pierozzi et al., 2008), and in PI 200456 and PI 224270 (Calvo et al., 2008). Finally, the fifth ASR resistance locus, *Rpp5*, and a recessive allele from PI 200456 and dominant alleles from PI 200526, PI 200487 and PI 471904 were identified (Garcia et al., 2008).

Historically, the ASR fungus has shown great capacity to develop new races and overcome each resistant gene along time. In Brazil, some genotypes, initially identified as resistant, had their resistance defeated by an isolate from Mato Grosso State (MT) (Yorinori et al., 2004), which also defeated the resistance provided by *Rpp1* and *Rpp3* genes. Recent tests involving soybean accessions from Brazil and USA germplasm banks indicated that there are several plant introductions expressing resistance to *P. pachyrhizi*. There is, however, little information regarding whether this resistance is conferred by genes different from those *Rpp1-Rpp4* already described. Knowledge about the genetic control of ASR resistance in these new available sources can increase the development efficiency of resistant soybean varieties and contribute to the disease management worldwide.

The objective of this work was to conduct allelic tests by crossing a group of 26 ASR resistant sources with the two remaining effective resistance *Rpp2* (PI 230970) and *Rpp4* (PI 459025) genes.

Materials and Methods

The genetic material selected for allelic testing included 26 PIs or cultivars that were identified as bearers of genes resistant to ASR, under greenhouse conditions at Embrapa Soja, Londrina, PR, Brazil: PI 197182, PI 230971, PI 417125, GC 84058-21-4, PI 408251, PI 379618 TC1, Nova Santa Rosa, PI 203398 (Abura), PI 423966, PI 416764, PI 417115, GC 84051-9-1, PI 398526, PI 416819, PI 339866, PI 340050, PI 417503, PI 417421, PI 203406, FT 87-17893, PI 417074, PI 408205, GC 84058-18-4, PI 416810, PI 200487 (Kinoshita) and PI 423962 (Hyuuga). A single plant harvested from each of resistant materials was used in crosses with the two testers PI 230970 and PI 459025 bearing the resistant genes *Rpp2* and *Rpp4*, respectively. Parents, their F_1 generation and the experimental materials, which included parents and their respective biparental cross derived F_2 generations in a total of approximately 4,730 individuals, were grown in a greenhouse at Embrapa Soybean, Londrina, PR, Brazil. Morphologic traits such as flower, pubescence and hilum color were used to eliminate selfed parent individuals from the F_1 and F_2 generations.

Two experiments were carried out to evaluate the segregation pattern of the F_2 generations. The first one was performed with included six individuals of each of the 21 parents and 60 to 140 individuals of the F_2 generations, and was sown on October 15, 2004. Plants were grown in greenhouse in randomly

distributed pots each one containing five to seven plants from the same cross. Plants were individually scored. The second experiment was installed to confirm the segregation pattern obtained for some of the resistance sources: PI 230971, PI 416819, PI 200487 (Kinoshita), GC 84058-18-4 and GC 84051-9-1, on Feb 01, 2005. Three plants of each parent and 70 to 100 F_2 plants from each cross were grown in pots randomly distributed in the greenhouse. Twenty-four plants of the susceptible cultivar BRSMS Bacuri were also sown in each experiment, to check the inoculation efficiency. Each pot contained three plants that were individually scored.

The *P. pachyrhizi* isolate derived from MT isolate (originated in Mato Grosso State), and which defeated the resistance expressed by the *Rpp1* and *Rpp3* genes, was used for the inoculations. Based on ITS sequence, this isolate is probably related to the MUT Zimbabwe isolate (GenBank accession no. AF333499), showing an identity of 99.8% (Silva et al., 2008). The MT isolate was constantly inoculated, at greenhouse conditions, on BRSMS Bacuri soybean cultivar, which served as filter against the Southern Brazilian isolate to which it is resistant. Spores were collected from leaves showing profuse sporulation, by using a soft brush, and then were placed in distilled water. Since the spores were not collected from a single lesion, they may represent a mixture of multiple races. However, the presence of RB and TAN reactions on the same leave was not observed in our assessments. Inoculum preparation involved a Neubauer chamber to obtain $2.5x10^4$ spores per milliliter solution to which Tween 20 was added. Thirteen days after the emergence, the inoculum was sprayed only once on plants, with an atomizer, after 18h, to ensure longer leaf moisture permanence and to avoid deleterious sun effects on the germinating spores. During the night following spraying, the plants were submitted to a fine water spray during 15 s every 3 hours. Evaluation was carried out between 20 to 27 days after inoculation (between V6 and V9 soybean growth stage), the observation of all leaves of the plants and classification of their reaction types as reddish-brown (RB – indicating resistance) and light-brown or "tanish" (TAN – indicating susceptibility). The RB lesions are dark with little sporulation, while TAN lesions are lighter with abundant sporulation.

Chi-square tests (χ^2) to check the expected 3:1, 15:1, 13:3, 63:1 theoretical ratios for one, two or three independent genes segregating were applied to the F_2 generation. Only those crosses showing at least 60 F_2 individuals and clear reaction type were considered in the analyses. The parental and BRSMT Bacuri cultivar reactions were used as checks to assess the presence of false resistant scores.

Results and Discussions

The experimental conditions allowed good expression of the genotype reactions to ASR with lesions on leaves becoming visible between the seventh and eighth day after inoculation. The spore mass was visible between the 12^{th} and the 15^{th} days after inoculation. The mixed reaction commonly observed on ASR evaluations (Miles et al., 2006), where both RB and TAN lesions appear on the same plant, was not observed in the experiments, including the check plants of susceptible cultivar BRSMS Bacuri. The use of a single isolate as inoculum and the greenhouse environment free of other isolates were the most likely cause of plant homogeneous reactions.

The susceptibility reaction (TAN lesions) of cultivar BRSMS Bacuri and PI 200492 (*Rpp1* gene) and PI 462312 (*Rpp3* gene) and the resistance reaction (RB lesions) of PI 230970 (*Rpp2*) and PI 459025 (*Rpp4*) were consistent with the predominance of the MT isolate in both experiments. According to Hartwig (1986), Taiwan-72-1 *P. pachyrhizi* isolate broke the resistance of PI 200492 and PI 462312, but not the resistance of PI 230970 and PI 459025. This reaction pattern is similar to that obtained in this study with the Brazilian isolate, suggesting that the Taiwan-72-1 and MT isolates may belong to the same race. Until present studies on ASR races being carried out in Brazil, no results concerning these two isolates are conclusive. Reference should be also made to the study of Silva et al. (2008), based on ITS sequence, which found 99.8% identity between the MT and MUT Zimbabwe isolates (GenBank accession no. AF333499).

The analyses of our data allowed screening of three genotypes with resistant genes belonging to the *Rpp2* locus and 23 genotypes with new resistant genes, which showed independent segregation from both the *Rpp2* and *Rpp4* loci. These two loci remain effective against the MT soybean rust isolate. No new source bearing resistance genes in the *Rpp4* locus was identified.

Table 1 shows the segregation pattern obtained in the F_2 populations derived from crosses between each investigated resistance source with PI 230970 and PI 459025. The total number of plants evaluated in each cross depended on the number of F_2 seeds produced and on the difficulties to classify plants with few lesions or without lesions. Therefore, only those individual F_2 plants expressing typical TAN or RB reaction were considered for analyses.

The investigated resistant sources were divided in five groups: I, F_2 generations that did not show segregation for the *Rpp2* locus and segregated in a

Table 1. Segregation pattern obtained from the F_2 generations derived from crosses between each resistance source with PI 230970 and PI 459025 testers, including the theoretical ratio not rejected by a chi-square test (χ^2), the number of evaluated plants (N), lesion type (Lesion), observed (O) and expected (E) number of plants, degrees of freedom (df) and the probability level (p>0.05).

Resistance source	*Rpp2* - PI 230970								*Rpp4* - PI 459025							
	Ratio	N	Lesion	O	E	df	χ^2	p	Ratio	N	Lesion	O	E	df	χ^2	p
PI 197182	1:0	60	RB	60	60.00	-	-	-	15:1	101	RB	96	94.69	1	0.29^{ns}	0.58
			TAN	0	0.00						TAN	5	6.31			
PI 230971	1:0	92	RB	92	92.00	-	-	-	13:3	95	RB	76	77.19	1	0.10^{ns}	0.75
			TAN	0	00.00						TAN	19	17.81			
PI 417125	1:0	99	RB	99	99.00	-	-	-	15:1	65	RB	59	60.94	1	0.98^{ns}	0.32
			TAN	0	00.00						TAN	6	4.06			
GC 84058-21-4	15:1	89	RB	81	83.44	1	1.14^{ns}	0.28	15:1	69	RB	64	64.69	1	0.11^{ns}	0.73
			TAN	8	5.56						TAN	5	4.31			
PI 408251	15:1	83	RB	81	77.81	1	2.09^{ns}	0.15	15:1	88	RB	82	82.50	1	0.05^{ns}	0.82
			TAN	2	5.19						TAN	6	5.50			
PI 379618 TC1	15:1	140	RB	128	131.25	1	1.28^{ns}	0.25	15:1	76	RB	71	71.25	1	0.01^{ns}	0.90
			TAN	12	8.75						TAN	5	4.75			
Nova Santa Rosa	15:1	117	RB	112	109.69	1	0.78^{ns}	0.37	15:1	79	RB	75	74.06	1	0.19^{ns}	0.66
			TAN	5	7.31						TAN	4	4.94			
PI 203398 (Abura)	15:1	100	RB	95	93.75	1	0.26^{ns}	0.60	15:1	100	RB	93	93.75	1	0.09^{ns}	0.75
			TAN	5	6.25						TAN	7	6.25			
PI 423966	15:1	87	RB	82	81.56	1	0.03^{ns}	0.84	15:1	79	RB	77	74.06	1	1.86^{ns}	0.17
			TAN	5	5.44						TAN	2	4.94			
PI 416764	15:1	67	RB	60	62.81	1	2.01^{ns}	0.15	15:1	95	RB	88	89.06	1	0.20^{ns}	0.65
			TAN	7	4.19						TAN	7	5.94			
PI 417115	15:1	94	RB	87	88.13	1	0.23^{ns}	0.63	15:1	75	RB	73	70.31	1	1.64^{ns}	0.20
			TAN	7	5.88						TAN	2	4.69			
PI 416819	15:1	96	RB	87	90.00	1	1.60^{ns}	0.20	15:1	97	RB	91	90.94	1	0.00^{ns}	0.98
			TAN	9	6.00						TAN	6	6.06			
GC 84058-18-4	15:1	68	RB	64	63.75	1	0.01^{ns}	0.90	15:1	81	RB	76	75.94	1	0.00^{ns}	0.98
			TAN	4	4.25						TAN	5	5.06			
PI 398526	13:3	97	RB	76	78.81	1	0.53^{ns}	0.46	13:3	79	RB	69	64.19	1	1.92^{ns}	0.16
			TAN	21	18.19						TAN	10	14.81			
PI 339866	13:3	117	RB	96	95.06	1	0.05^{ns}	0.82	15:1	87	RB	85	81.56	1	2.32^{ns}	0.12
			TAN	21	21.94						TAN	2	5.44			
PI 340050	13:3	88	RB	67	71.50	1	1.51^{ns}	0.22	15:1	69	RB	67	64.69	1	1.32^{ns}	0.25
			TAN	21	16.50						TAN	2	4.31			
PI 417503	13:3	103	RB	90	83.69	1	2.54^{ns}	0.11	15:1	76	RB	72	71.25	1	0.12^{ns}	0.72
			TAN	13	19.31						TAN	4	4.75			
PI 417421	13:3	82	RB	66	66.63	1	0.03^{ns}	0.86	15:1	103	RB	94	96.56	1	1.08^{ns}	0.29
			TAN	16	15.38						TAN	9	6.44			
PI 203406	13:3	101	RB	88	82.06	1	2.29^{ns}	0.13	15:1	102	RB	95	95.63	1	0.06^{ns}	0.79
			TAN	13	18.94						TAN	7	6.38			
FT 87-17893	13:3	80	RB	67	65.00	1	0.33^{ns}	0.56	15:1	105	RB	98	98.44	1	0.03^{ns}	0.86
			TAN	13	15.00						TAN	7	6.56			
PI 417074	13:3	117	RB	90	95.06	1	1.43^{ns}	0.23	15:1	81	RB	74	75.94	1	0.79^{ns}	0.37
			TAN	27	21.94						TAN	7	5.06			
PI 408205	13:3	71	RB	57	57.69	1	0.04^{ns}	0.83	15:1	91	RB	89	85.31	1	2.55^{ns}	0.11
			TAN	14	13.31						TAN	2	5.69			
GC 84051-9-1	15:1	82	RB	80	76.88	1	2.03^{ns}	0.15	13:3	96	RB	82	78.00	1	1.09^{ns}	0.29
			TAN	2	5.13						TAN	14	18.00			
PI 416810	15:1	73	RB	68	68.44	1	0.04^{ns}	0.83	13:3	98	RB	82	79.63	1	0.37^{ns}	0.53
			TAN	5	4.56						TAN	16	18.38			
PI 200487 (Kinoshita)	15:1	72	RB	70	67.50	1	1.48^{ns}	0.22	13:3	86	RB	74	69.88	1	1.29^{ns}	0.25
			TAN	2	4.50						TAN	12	16.13			
PI 423962 (Hyuuga)	15:1	67	RB	62	62.81	1	0.16^{ns}	0.68	13:3	76	RB	62	61.75	1	0.00^{ns}	0.94
			TAN	5	4.19						TAN	14	14.25			

15:1 or 13:3 ratio (resistant to susceptible) for the *Rpp4* locus; II, F_2 generations that segregated in a 15:1 ratio for both *Rpp2* and *Rpp4* loci; III, F_2 generations that segregated in a 13:3 ratio for both *Rpp2* and *Rpp4* loci; IV, F_2 generations that segregated in a 13:3 ratio for the *Rpp2* locus and in a 15:1 ratio for the *Rpp4* locus; V, F_2 generations that segregated in a 15:1 ratio for the *Rpp2* locus and in a 13:3 ratio for the *Rpp4* locus.

Genotypes PI 197182, PI 230971 and PI 417125 were classified in group I, thus each one of these carry a single resistance gene located in the same linkage group of the *Rpp2* locus already described in the literature. The *Rpp2* locus was mapped on soybean linkage group J (Silva et al., 2008) and, with our data, it is not possible to know if the resistant genes derived from those PIs are alleles or if they are in different linked loci. Another resistant gene, though recessive, derived from PI 224270, was also mapped into this same linkage group (Garcia et al., 2008). The difference in dominance mode action from one source to another shows that they may be multiple alleles in the *Rpp2* locus. Therefore, breeders trying to pyramiding those genes may not be successful.

Genotypes GC 84058-21-4, PI 408251, PI 379618 TC1, Nova Santa Rosa, PI 203398 (Abura), PI 423966, PI 416764, PI 417115, PI 416819 and GC 84058-18-4 were all included in group II. Therefore, each of these genotypes carries resistance genes at a locus different from *Rpp2* and *Rpp4*. The obtained segregation pattern in the F_2 generations of each cross also supported the hypothesis that each new source carries a single dominant gene controlling resistance. The soybean breeding line BR01-18437, studied by Pierozzi et al. (2008), probably received its resistant gene from PI 203398 (Abura), which was used as high protein genetic source. However, the gene from BR01-18437 is recessive, while the gene from PI 203398 studied here is dominant. Complementary studies are being developed to clarify how dominance of this gene works.

Only one genotype, PI 398526, was classified into group III. The observed segregation pattern, 13 resistant to 3 susceptible, indicated that its resistant gene is recessive or does not express dominance when crossed with PI 230970 and PI 459025. Recessive ASR-resistance genes have been first reported in the soybean line BR01-18437 (Pierozzi et al., 2008), and then in PI 200456 and PI 224270 (Calvo et al., 2008). The existence of recessive ASR-resistance genes will increase the breeders attention to the possibility of obtaining F_1 plants with TAN reaction that should not be discarded.

The segregation pattern obtained in the F_2 generation for the resistant sources PI 339866, PI 340050, PI 417503, PI 417421, PI 203406, FT 87-17893, PI 417074, PI 408205, GC 84051-9-1, PI 416810, PI 200487 (Kinoshita) and PI 423962 (Hyuuga) indicated that each one of these genotypes has one gene for resistance to ASR in a locus distinct from *Rpp2* and *Rpp4*. Considering that *Rpp2* and *Rpp4* genes in the testers are dominant, a 15:1 segregation ratio would be expected, if the resistance genes in the tested genotypes were also dominant, and a 13:3 ratio would be expected if they were recessive.

The results obtained in groups IV and V, however, can only be explained considering a different reaction type of the heterozygous genotype for the resistance locus derived from the tested genotypes, in combination with the homozygous recessive genotype for the *Rpp2* and *Rpp4* loci. The probability of this genotype combination is 1/8 (or two in each 16 plants). Those genotypes in group IV would produce 13RB to 3TAN and 15RB to 1TAN for the *Rpp2* and *Rpp4* loci, respectively, while in group V, similar results would be obtained for the *Rpp4* and *Rpp2* loci, respectively. Therefore, epistatic gene action may have occurred, since the expression of the heterozygous genotype seems to have depended on the tester and, for groups IV or V, i.e. the gene dominance depended on the tester, displaying from complete dominance (15:1) to absence of dominance (13:3). Three types of dominance action were assigned to resistance to ASR, from partial to complete dominant genes (Garcia et al., 2008) and recessive genes (Pierozzi et al., 2008; Calvo et al., 2008).

The ASR-resistance gene from PI 200487 (Kinoshita) source from the previous discussed group was assigned to the locus *Rpp5*, which was mapped in the linkage group N of soybean (Garcia et al., 2008), confirming that it is independent of loci *Rpp2* and *Rpp4* (linkage groups J and G, respectively). Data from Garcia et al. (2008) showed that only one gene with complete dominance confers the resistance of PI 200487, which confirms the results obtained from the cross with the gene *Rpp2* (15RB:1TAN) but not with the gene *Rpp4* (13RB:3TAN).

Similarly to PI 200487 (Kinoshita), PI 423962 (Hyuuga) showed ratios 15RB:1TAN and 13RB:3TAN in crosses with *Rpp2* and *Rpp4*, respectively. The ASR-resistance locus of PI 423962 has been mapped on the linkage group C2 (Monteros et al., 2007), confirming its independence. Another resistance gene derived from the Brazilian cultivar FT-2 had been previously mapped into this same linkage group, but this gene has been already defeated by ASR fungus.

The results of the allelic tests allowed the identification of three investigated sources, which carry genes in the same linkage group where the *Rpp2* locus (linkage group J of soybean) is located, and 23 sources with resistance genes mapping out of *Rpp2* or *Rpp4* loci, which can be used by breeders to develop cultivars resistant to ASR. A summary of these results can be seen in Table 2, which shows the segregation ratios not rejected by chi-square test (χ^2 with $p>0.05$) for both testers.

At the beginning of this research the objective was to develop allelic tests for all *Rpp1* through *Rpp4* loci described in the literature. This objective was changed due to difficulties of maintaining and experimenting with two isolates of the soybean rust fungus and also to the loss of interest of Brazilian breeders in the *Rpp1* and *Rpp3* genes, which have been defeated by the MT isolate. As consequence, the genes classified in Table 2 as new genes can be different alleles in the *Rpp1* or *Rpp3* loci or, alternatively, genes derived of nondescribed *Rpp* loci.

Table 2. Summary of the results, showing the segregation ratio obtained for each test cross and the conclusions from allele testings.

Sources	Ratio with *Rpp2*	Ratio with *Rpp4*	Conclusion
PI 197182	1:0	15:1	Gene *Rpp2*
PI 230971	1:0	13:3	Gene *Rpp2*
PI 417125	1:0	15:1	Gene *Rpp2*
GC 84058-21-4	15:1	15:1	New gene
PI 408251	15:1	15:1	New gene
PI 379618 TC1	15:1	15:1	New gene
Nova Santa Rosa	15:1	15:1	New gene
PI 203398 (Abura)	15:1	15:1	New gene
PI 423966	15:1	15:1	New gene
PI 416764	15:1	15:1	New gene
PI 417115	15:1	15:1	New gene
PI 416819	15:1	15:1	New gene
GC 84058-18-4	15:1	15:1	New gene
PI 398526	13:3	13:3	New gene
PI 339866	13:3	15:1	New gene
PI 340050	13:3	15:1	New gene
PI 417503	13:3	15:1	New gene
PI 417421	13:3	15:1	New gene
PI 203406	13:3	15:1	New gene
FT 87-17893	13:3	15:1	New gene
PI 417074	13:3	15:1	New gene
PI 408205	13:3	15:1	New gene
GC 84051-9-1	15:1	13:3	New gene
PI 416810	15:1	13:3	New gene
PI 200487 (Kinoshita)	15:1	13:3	New gene
PI 423962 (Hyuuga)	15:1	13:3	New gene

Conclusions

1. A group of 23 soybean rust-resistant sources – GC 84058-21-4, PI 408251, PI 379618 TC1, Nova Santa Rosa, PI 203398 (Abura), PI 423966, PI 416764, PI 417115, PI 416819, GC 84058-18-4, PI 398526, PI 339866, PI 340050, PI 417503, PI 417421, PI 203406, FT 87-17893, PI 417074, PI 408205, GC 84051-9-1, PI 416810, PI 200487 (Kinoshita) and PI 423962 (Hyuuga) – carry resistance genes that are not in the *Rpp2* or in the *Rpp4* loci.

2. Three of the studied resistant sources – PI 197182, PI 230971 and PI 417125 – have at least one allelic or closely linked gene to the *Rpp2* locus.

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