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Soybean Breeding Materials Useful for Resistance to Soybean Rust in Brazil

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Abstract

The development of resistant soybean varieties is an efficient way to manage soybean rust in Brazil. To date, resistant varieties effective in Brazil have been limited due to the presence of highly virulent rust populations. This study aimed to identify effective resistant varieties/resistance genes in selected soybean genotypes against a highly virulent Brazilian rust population, BRP-2. Sixty-six soybean genotypes, including 57 soybean rust-resistant/tolerant genotypes previously identified, were exposed to BRP-2 and their resistance was evaluated based on lesion characteristics. PI 587905 produced typical resistant and susceptible lesions and was excluded from the resistance classification. Hence, 47 (72.3%) of the 65 genotypes tested were classified as susceptible, 14 (21.5%) were classified as slightly resistant and 4 (6.2%) were classified as clearly resistant. Although 18 genotypes were placed into resistant categories, PI594767A was the only genotype on which neither uredinia nor urediniospores were produced in two weeks after inoculation. In addition, urediniospores were observed in all 16 varieties carrying one of the five known resistance genes (*Rpp1*-*Rpp5*) and in one genotype in which two resistance genes, *Rpp2* and *Rpp4*, were pyramided. Conversely, our screening of soybean genotypes by the rust population, BRP-2 inoculation revealed that two Chinese varieties, ‘Lu Pi Dou’ and ‘Hei Dou’, possessed a leaf-yellowing prevention characteristic. Leaf-yellowing, which might cause rapid defoliation leading to yield loss, was observed not only in susceptible varieties but also in a resistant variety ‘Shiranui’. The use of the resistance and/or tolerance identified in this study will assist in the development of resistant cultivars capable of enduring soybean rust in Brazil.

Discipline: Plant breeding
Additional key words: disease resistance, infection index, *Phakopsora pachyrhizi*, *Glycine max*, vertical resistance

Introduction

Soybean rust caused by the fungus *Phakopsora pachyrhizi* Sydow & Sydow has become one of the most serious threats to soybean [*Glycine max* (L.) Merr.] production in Brazil, due to its potential to reduce the soybean yield to less than 25% and the additional costs it entails to soybean production. From 2001 to 2007, this disease caused losses estimated at US$10 billion in Brazil[23, 42, 43]. The use of resistant cultivars is the most desirable solution, since it is economical and harmless to the environment[1, 29]. To develop suitable cultivars, soybean breeders must optimize the plant genotype by choosing the most promising resistant breeding materials and combining them to ensure the durability of resistance[27].

Five major genes resistant to soybean rust, *Rpp1*-*Rpp5*, have been identified and mapped in the soy-

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bean[6, 8, 12, 13, 14, 15, 21, 29, 32]. These major resistance genes are considered race-specific[5, 6, 12, 13]. Conversely, it is well-known that soybean rust presents high race variability[9, 25, 41], meaning soybean varieties carrying the resistance genes cannot be utilized for breeding in Brazil without considering the virulence of Brazilian rust populations. In our previous study, we found that a highly virulent rust population was present in Brazil, which limits the availability of useful resistance genotypes to employ in soybean breeding programs there[40]. In order to utilize known resistant varieties for the soybean breeding program in Brazil, we need to estimate the effectiveness of their resistance to the highly virulent rust population there.

Pyramiding resistance genes in a single cultivar can provide more durable resistance to certain plant diseases[7, 19, 33]. Here, the high pathogenic diversity of *P. pachyrhizi* in the field means this strategy may be crucial for improving soybean rust resistance. In our previous work[39], a resistant soybean line carrying two resistance genes, *Rpp2* and *Rpp4*, was developed. Conversely, utilizing field resistance or tolerance, usually revealed by minor genes, can also provide more durable resistance against soybean rust, particularly the one that presents high race variability. This kind of resistance has already been observed in the soybean[35]. However, its application in breeding programs has seldom been performed, since it is time-consuming and hard to evaluate[10].

Here, we investigated the resistance characteristics of rust lesions in known resistant/tolerant soybean genotypes inoculated with the highly virulent Brazilian rust population, BRP-2. The objective of this study was to identify useful genotypes resistant to the BRP-2 rust population. We also investigated two candidate characteristics with which to measure soybean rust-tolerance: the degree of leaf-yellowing and infection index. This paper describes the identification of soybean resources conferring tolerance against this disease.

Materials and methods

1. Preparation of inoculum

A population of soybean rust fungus previously sampled in Brazil, named Brazilian rust population-2 (BRP-2)[40], was used in this study. BRP-2 is a rust population putatively consisting of several races of soybean rust. Urediniospores of BRP-2, preserved in microtubes at under -80°C, were incubated at 39°C for 1 minute to break the dormancy, and suspended in distilled water with 0.04% (v/v) of polyoxyethylene sorbitan monolaurate (Tween 20, Sigma) to obtain a concentration of 50,000 spores/mL. This suspension was then used to inoculate samples to analyze resistance to BRP-2 based on the lesion features. Another spore suspension of 115,000 spores/mL was also used to evaluate the two candidate characteristics: the degree of leaf-yellowing and infection index. The conditions for multiplication and preservation of spores were previously described[40].

2. Plant materials

Sixty-six soybean genotypes (cultivars/lines), whose resistance to specific isolates/populations of soybean rust had been previously identified (Table 1), were evaluated for resistance to BRP-2. They comprised 16 resistant varieties (entries 2-17 in Table 1), identified as carrying one of the five known resistance genes (*Rpp1* - *Rpp5*); 40 soybean genotypes (entries 18-57 in Table 1), previously identified as resistant/tolerant to soybean rust; the line ‘An76-1’ (entry 1 in Table 1), which we developed before[39]; as well as nine susceptible varieties (entries 58-66 in Table 1). ‘An76-1’ carries a double-homozygous combination of the resistance alleles *Rpp2* and *Rpp4*, respectively derived from PI230970 and PI 459025 (Fig. 1). This line is derived from ‘An76’, a single plant selected from an F_2 population segregating these two resistance alleles, through marker-assisted selection (MAS)[39], by applying two flanking markers for each of the two resistance loci[32]. An76-1 was also previously crossed with cv. ‘Kinoshita’ and the resistance genes *Rpp2*, *Rpp4* and *Rpp5* were characterized in a previous study[18]. Of the 66 genotypes, 53 were submitted beforehand to Japanese rust population (JRP)[40].

The plant materials were divided into six experimental sets, consisting of up to 13 genotypes. All sets of experiments were held in growth chambers under the conditions of our previous work[40].

3. Evaluation of resistance based on lesion characteristics

We evaluated the lesion characteristics under the same conditions for cultivating plants and inoculating rust spores as in our previous study[40]. Five resistance characteristics, lesion color (LC), frequency of lesions with uredinia (%LU), number of uredinia per lesion (NoU), frequency of open uredinia (%OU), and sporulation level (SL), were scored under a stereomicroscope in the 66 soybean genotypes, two weeks after inoculation. The phenotypic values in three of the five resistance characteristics: %LU, NoU and SL, were individually classified as resistant or susceptible types and the standards that we primarily established for LC and SL[40] were applied in this evaluation. Phenotypic data of these five characteristics were obtained from 30 lesions in

Table 1. Soybean genotypes used in this study

Entry	Variety / Line	Common name	Characteristic regarding soybean rust (SBR)	Source[5)]	Reference
1	An76-1[1)]	−	Resistant line having *Rpp2* and *Rpp4*[2)]	JIRCAS	39
2	PI200492	Komata	Resistant variety having *Rpp1*	EMBRAPA	14
3	PI368039	Tainung 4	Resistant variety having *Rpp1*	EMBRAPA	41
4	PI587880A	Huang Dou	Resistant variety having *Rpp1*	EMBRAPA	30
5	PI587886	Bai Dou	Resistant variety having *Rpp1*	EMBRAPA	30
6	PI230970	No.3	Resistant variety having *Rpp2*	EMBRAPA	32
7	PI417125	Kyushu 31	Resistant variety having *Rpp2*	EMBRAPA	24
8	Iyodaizu B[1)]	−	Resistant variety having *Rpp2*[3)]	NICS	4
9	PI462312	Ankur	Resistant variety having *Rpp3*	EMBRAPA	15
10	FT2	−	Resistant variety having *Rpp3*	EMBRAPA	38
11	Hyuuga[1)]	−	Resistant variety having *Rpp3*	NICS	22
12	Hougyoku	−	Resistant variety having *Rpp3*?[4)]	NICS	4
13	BRSMS-Bacuri	−	Resistant variety having *Rpp3*?[4)]	EMBRAPA	16
14	PI459025	Bing Nan	Resistant variety having *Rpp4*	EMBRAPA	32
15	PI459025A	Bing Nan	Resistant variety having *Rpp4*	EMBRAPA	29
16	PI200526	Shiranui	Resistant variety having *Rpp5*	EMBRAPA	8
17	PI200487	Kinoshita	Resistant variety having *Rpp5*	EMBRAPA	8
18	PI416764	Akasaya	Resistant variety	EMBRAPA	1
19	PI587905	Xiao Huang Dou	Resistant variety	EMBRAPA	20
20	PI594767A	Zhao Ping Hei Dou	Resistant variety	EMBRAPA	20
21	GC00002-100	−	Resistant variety	AVRDC	2
22	GC00138-29	−	Resistant variety	AVRDC	26
23	GC60020-8-7-7-18	−	Resistant variety	AVRDC	26
24	GC84040-7-1[1)]	−	Resistant variety derived from entry 2	AVRDC	−
25	GC84040-16-1	−	Resistant variety derived from entry 2	AVRDC	3
26	GC84051-9-1	−	Resistant variety derived from entry 3	AVRDC	1
27	GC84058-18-4[1)]	−	Resistant variety	AVRDC	28
28	GC84058-21-2[1)]	−	Resistant variety	AVRDC	−
29	GC84058-21-4	−	Resistant variety	AVRDC	1
30	GC84051-32-1[1)]	−	Resistant variety derived from entry 3	AVRDC	26
31	GC85037-2-3-5-1	−	Resistant variety derived from entries 6 and 9	AVRDC	26
32	GC85039-1-2-1-1	−	Resistant variety derived from entry 2	AVRDC	40
33	GC86004-9	−	Resistant variety	AVRDC	3
34	SS86045-23-2	−	Resistant variety	AVRDC	26
35	GC87012-10-B-5	−	Resistant variety	AVRDC	26
36	GC87016-11-B-2	−	Resistant variety	AVRDC	26
37	GC87021-13-B-2[1)]	−	Resistant variety	AVRDC	26
38	GC87021-26-B-1[1)]	−	Resistant variety	AVRDC	26
39	GC95040-26-1-1-1[1)]	−	Resistant variety derived from entries 2 and 6	AVRDC	−
40	SRE-B-15C	−	Resistant variety	AVRDC	2
41	SRE-D-11C	−	Resistant variety	AVRDC	26
42	SRE-D-14A[1)]	−	Resistant variety	AVRDC	26
43	SRE-D-14B[1)]	−	Resistant variety	AVRDC	26
44	Xiao Jing Huang	−	Resistant variety	JAAS	37, 39
45	Niu Mao Huang	−	Resistant variety	JAAS	37, 39
46	Qin Dou	−	Resistant variety	JAAS	37, 39
47	Da Bai Qi	−	Resistant variety	JAAS	37, 39
48	6611	−	Resistant variety	JAAS	37, 39
49	Da Li Zi	−	Resistant variety	JAAS	37, 39
50	Himedaizu	−	Resistant variety	JIRCAS	37, 39
51	Sachiyutaka	−	Resistant variety	NICS	4
52	Lu Pi Dou	−	Tolerant variety: Less leaf-yellowing by SBR	JAAS	37, 39
53	Hei Dou	−	Tolerant variety: Less leaf-yellowing by SBR	JAAS	37, 39
54	BRS231	−	Tolerant variety: Low yield-loss by SBR	EMBRAPA	39
55	Abura	−	Resistant variety having major gene	EMBRAPA	17
56	BR01-17996	−	Breeding line resistant to SBR	EMBRAPA	40
57	BR01-18437	−	Breeding line resistant to SBR	EMBRAPA	29
58	TK5	−	Susceptible variety	EMBRAPA	41
59	PI548628	Wayne	Susceptible variety	EMBRAPA	41
60	RI75	−	Susceptible variety	EMBRAPA	40
61	Davis	−	Susceptible variety	EMBRAPA	38
62	EMBRAPA48	−	Susceptible variety	EMBRAPA	31
63	Misuzudaizu	−	Susceptible variety	Chiba Univ.	36
64	Moshidou Gong 503	−	Susceptible variety	Chiba Univ.	36
65	BRS184	−	Susceptible variety	EMBRAPA	39
66	Enrei[1)]	−	Susceptible variety	NICS	4

1) All genotypes except for these 13 were subject to the Japanese rust population (JRP) in our previous study[40]

2) An76-1 is a resistant line obtained by marker-assisted selection (MAS) from a segregant population (See Fig. 1)

3) The resistance gene of Iyodaizu B has been mapped at the same region where *Rpp2* is located (Yamanaka et al., unpublished)

4) Hougyoku and BRSMS-Bacuri putatively have the same resistance gene, *Rpp3*, as FT2 and Hyuuga[1, 4]

5) EMBRAPA: Brazilian Agricultural Research Corporation, AVRDC: Asian Vegetable Research and Development Center, JAAS: Jilin Academy of Agricultural Sciences, JIRCAS: Japan International Research Center for Agricultural Sciences, NICS: National Institute of Crop Science.

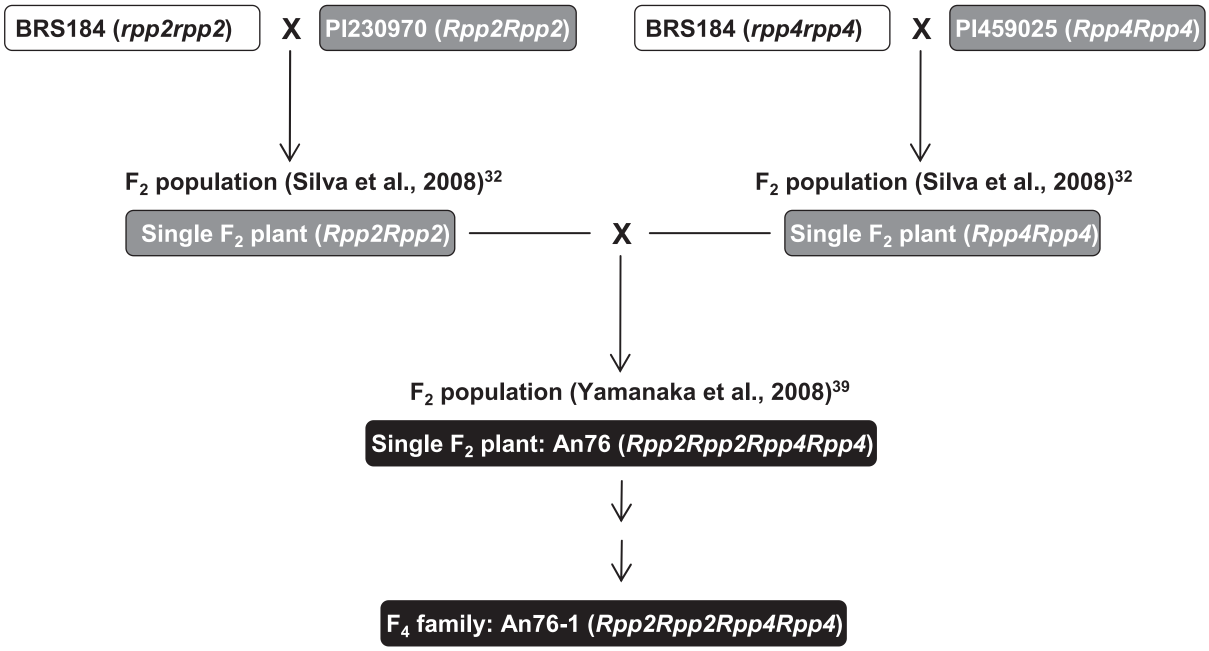


Fig. 1. Pedigree of the resistant genotype 'An76-1' with two genes resistant to soybean rust, *Rpp2* and *Rpp4*

Each single homozygous resistant genotype respectively for *Rpp2* and *Rpp4* was screened from the F_2 population by using DNA markers identified in the previous study[32]. Resistant alleles of two major genes are represented as *Rpp2* and *Rpp4* in this Figure. Arrow and 'X' mean selfing and cross, respectively.

□:Genotypes without major resistance gene, ■:Genotypes with single major resistance gene, ■:Genotypes with two major resistance genes.

three plants per genotype. Based on our prior work[40], we classified phenotypes of lesions into resistant/susceptible as follows: lesions with NoU and SL values $0.0 \leq x < 2.0$ and $2.0 \leq x$ were respectively classified as resistant and susceptible; and lesions with %LU values $0.0 \leq x < 70.0$ and $70.0 \leq x \leq 100.0$ were respectively classified as resistant and susceptible. Finally, soybean genotypes were classified into five types of resistance categories according to the phenotypes of %LU, NoU and SL as follows: "Susceptible" = having lesions with susceptible phenotypes in all three resistance characteristics (%LU, NoU and SL); "Slightly resistant" = having lesions that showed resistant phenotypes in any of three characteristics; "Resistant" = having lesions that showed resistant phenotypes in all three characteristics and possessing uredinia; "Highly resistant" = having lesions that showed resistant phenotypes in all three characteristics and with no uredinia; and "Immune" = having no lesions.

4. Evaluation of tolerance

In order to evaluate the reactions of tolerant varieties to BRP-2, the degree of leaf-yellowing and the infection index were also analyzed. Two Chinese varieties, 'Lu Pi Dou' (ID No. in China: ZDD00658) and 'Hei Dou' (ZDD00681), featured by their low leaf-yellowing[39], were used in this experiment. The names 'Lu Pi Dou' and 'Hei Dou' mean 'Green seed coat bean' and 'Black bean', respectively. Their basic agricultural characteristics[34] and the genetic relationship with Brazilian and Japanese soybean varieties[35] have already been reported. The Brazilian variety 'BRS231', that presents low yield-loss by soybean rust infection[39], and two soybean varieties, 'Shiranui' (*Rpp5*) and 'BRS 184', applied as resistant and susceptible controls, respectively, were also included in this experiment. The conditions of growth and inoculation by BRP-2 were the same as those for the evaluation based on lesion characteristics, except for the inoculum concentration and cultivation place. The urediniospore concentration in the inoculum was changed from 50,000 to 115,000 spores/mL, as described previously in this paper, and soybean plants were cultivated in a greenhouse rather than a growth chamber to promote leaf-yellowing. The degree of leaf-yellowing in each variety was determined based on a scale (Fig. 2). For the infection index, the number of lesions in a single leaflet (NoL), the single leaflet area obtained by a scanner (LA: cm^2), and the

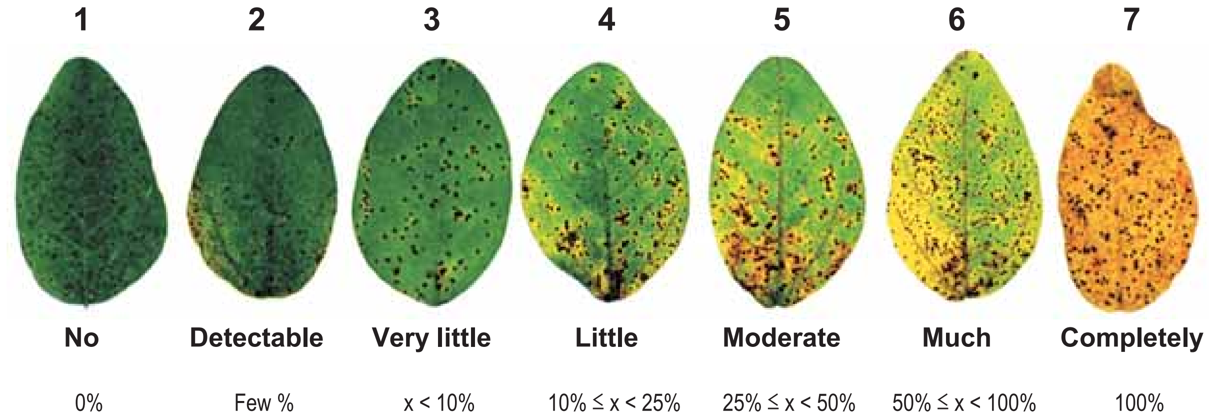


Fig. 2. Scale used to classify the character 'degree of leaf-yellowing' caused by soybean rust infection
The degree of yellowing is visually categorized into seven classes from 1: Not yellowed, to 7: Completely yellowed. The estimated yellowing area ratio (%) is also given in this Figure.

number of germinated spores in 1 mL of inoculated spore suspension (NGS), were applied to the following formula:

$$\text{Infection index} = \frac{10^4 \cdot \text{NoL}}{\text{LA} \cdot \text{NGS}}$$

In this formula, the infection index is 10^4 times for its numerically better representation. The infection index can be considered the number of rust lesions formed in 1 cm^2 of LA by inoculating a spore suspension with the concentration of 10^4 NGS. Thus, this index is based on the supposition that a uniform amount of inoculums was inoculated in one cm^2 leaflet.

The data for these two characteristics were obtained by using the first trifoliolate leaf from three independent plants. The leaf samples for evaluation were obtained two weeks after inoculation and no lesions derived from secondary infection were observed in this experiment. In addition, the same soybean genotypes were treated with 0.04% (v/v) of Tween 20 solution and maintained under the same condition as the inoculated plants. Uninoculated leaves from these plants were used as a negative control.

Results

1. Genes and varieties resistant to BRP-2

Table 2 shows the phenotypes of 66 soybean genotypes, regarding five resistance characteristics evaluated in this study. Only one variety, PI594767A, showed a highly resistant phenotype, in which no uredinia were formed within two weeks of inoculation (Fig. 3, Table 2). Three varieties, SRE-D-11C, GC84058-18-4, and GC84058-21-4, were classified as resistant. The resistance genes of these four varieties are unknown. Fourteen genotypes, seven of which carried at least one of the three resistant alleles *Rpp3*, *Rpp4*, and *Rpp5*, showed resistant phenotypes in one of the characteristics %LU (frequency of lesions with uredinia), NoU (number of uredinia per lesion), or SL (sporulation level), being classified as the slightly resistant category. Ten varieties, carrying one of the following resistant alleles, *Rpp1*, *Rpp2*, and *Rpp3*, were classified as susceptible together with another 37 varieties. One variety, PI587905, was excluded from the classification once it presented two visibly different types (one of which was whitish-colored with sporulation; another was reddish brown without sporulation) of lesions on the inoculated leaves.

Consequently, more than 70% of the tested genotypes were classified as susceptible, and only 1.5, 4.6 and 21.5% were highly resistant, resistant, and slightly resistant, respectively (Fig. 4). The proportion of resistant categories obtained from BRP-2 inoculation differed dramatically from that previously obtained by the inoculation of the Japanese rust population (Fig. 4), in which the various types of resistance totaled 50.9%.

2. Comparison of characteristics between An76-1 and its ancestors

The resistance characteristics LC, NoU, and SL were used to compare An76-1 with PI230970 and PI 459025, the source varieties of the resistance alleles, *Rpp2* and *Rpp4*, respectively. The average values of NoU and SL in PI230970 based on 30 lesions were significantly higher than those of An76-1 and PI459025 (Tables 2, 3), whilst between the latter two, those of NoU and SL (t-test following the F-test, p = 0.7406 and Mann-Whitney U-test, p = 0.2226, respectively) did not differ. The average values of LC increased considerably

Table 2. Phenotypes of five resistance characteristics in 66 soybean genotypes subject to Brazilian rust population 2 (BRP-2)

Entry	Genotype (Genes)	LC		%OU	%LU	NoU		SL		Resistance classification
		Average	SD			Average	SD	Average	SD	
20	PI594767A	1.3	0.5	0.0%	0.0%	0.0	0.0	0.0	0.0	Highly resistant
41	SRE-D-11C	1.9	0.6	100.0%	6.7%	0.1	0.3	0.0	0.2	Resistant
27	GC84058-18-4	3.6	1.5	44.4%	63.3%	0.9	0.8	0.5	0.8	Resistant
29	GC84058-21-4	3.9	2.1	37.5%	36.7%	0.8	1.0	0.6	0.9	Resistant
15	PI459025A (*Rpp4*)	3.3	0.8	61.2%	83.3%	1.6	1.1	0.9	0.8	Slightly resistant
44	Xiao Jing Huang	5.1	0.8	88.2%	96.7%	3.4	1.7	1.2	0.5	Slightly resistant
26	GC84051-9-1	4.7	0.7	55.6%	70.4%	2.3	0.8	1.2	1.0	Slightly resistant
1	An76-1 (*Rpp2*+*Rpp4*)	2.2	0.6	68.2%	93.3%	1.5	0.9	1.2	1.2	Slightly resistant
14	PI459025 (*Rpp4*)	5.0	0.0	100.0%	100.0%	1.5	0.6	1.5	0.7	Slightly resistant
16	Shiranui (*Rpp5*)	4.1	0.3	87.5%	100.0%	1.6	0.7	1.5	0.7	Slightly resistant
11	Hyuuga (*Rpp3*)	3.8	0.7	93.1%	93.3%	2.4	0.9	1.6	0.7	Slightly resistant
18	PI416764	4.0	0.0	91.1%	100.0%	1.5	0.6	1.6	0.6	Slightly resistant
17	Kinoshita (*Rpp5*)	3.8	0.6	86.3%	96.7%	1.7	0.7	1.6	0.6	Slightly resistant
33	GC86004-9	5.7	0.4	87.1%	100.0%	3.4	1.2	1.8	0.5	Slightly resistant
25	GC84040-16-1	4.5	0.5	91.0%	83.3%	4.5	1.7	1.9	1.1	Slightly resistant
36	GC87016-11-B-2	5.1	1.0	84.2%	96.7%	3.2	1.4	1.9	0.7	Slightly resistant
9	PI462312 (*Rpp3*)	5.0	0.0	87.5%	100.0%	1.8	1.0	2.0	0.8	Slightly resistant
12	Hougyoku (*Rpp3?*)	2.3	0.5	58.6%	96.7%	3.9	1.8	2.0	1.2	Susceptible
63	Misuzudaizu	5.3	0.5	79.1%	96.7%	4.6	2.1	2.2	0.7	Susceptible
49	Da Li Zi	5.5	0.5	84.8%	100.0%	2.6	1.3	2.3	0.7	Susceptible
32	GC85039-1-2-1-1	5.5	0.6	82.5%	100.0%	4.0	1.6	2.3	0.7	Susceptible
8	Iyodaizu B (*Rpp2*)	2.9	0.5	87.1%	100.0%	2.3	1.1	2.4	0.8	Susceptible
4	PI587880A (*Rpp1*)	5.2	0.4	84.7%	100.0%	2.8	1.2	2.4	0.7	Susceptible
34	SS86045-23-2	5.6	0.5	83.3%	100.0%	5.8	1.8	2.4	0.5	Susceptible
60	RI75	6.0	0.2	80.7%	100.0%	4.8	2.5	2.6	0.7	Susceptible
55	Abura	4.4	0.6	94.4%	100.0%	1.8	1.0	2.7	0.5	Slightly resistant
31	GC85037-2-3-5-1	5.4	0.5	85.0%	100.0%	3.3	1.4	2.7	0.5	Susceptible
57	BR01-18437	4.5	0.5	83.8%	100.0%	3.5	1.5	2.7	0.7	Susceptible
30	GC84051-32-1	5.9	0.3	93.5%	100.0%	4.1	1.8	2.7	0.7	Susceptible
22	GC00138-29	4.9	0.3	85.4%	100.0%	3.2	1.1	2.8	0.4	Susceptible
24	GC84040-7-1	4.7	0.5	95.3%	100.0%	2.8	0.8	2.8	0.4	Susceptible
50	Himedaizu	5.5	0.6	100.0%	100.0%	2.7	1.3	2.8	0.4	Susceptible
7	PI417125 (*Rpp2*)	4.8	0.4	85.2%	100.0%	2.9	1.3	2.8	0.4	Susceptible
23	GC60020-8-7-7-18	5.5	0.5	73.5%	96.7%	6.0	2.9	2.8	0.6	Susceptible
54	BRS231	5.5	0.5	88.0%	100.0%	4.2	1.2	2.9	0.3	Susceptible
58	TK5	4.1	0.3	94.3%	100.0%	3.5	1.3	2.9	0.3	Susceptible
28	GC84058-21-2	6.0	0.2	95.5%	100.0%	3.7	1.1	2.9	0.5	Susceptible
5	PI587886 (*Rpp1*)	5.1	0.3	88.0%	100.0%	3.6	1.4	2.9	0.3	Susceptible
53	Hei Dou	5.9	0.3	95.4%	100.0%	3.6	1.1	2.9	0.3	Susceptible
38	GC87021-26-B-1	5.8	0.4	90.4%	100.0%	4.6	1.5	2.9	0.2	Susceptible
65	BRS184	5.9	0.3	94.7%	100.0%	4.4	1.7	3.0	0.2	Susceptible
59	Wayne	5.8	0.4	80.0%	100.0%	4.7	1.6	3.0	0.2	Susceptible
56	BR01-17996	5.7	0.5	90.3%	100.0%	2.4	0.7	3.0	0.0	Susceptible
13	BRSMS-Bacuri (*Rpp3?*)	5.6	0.5	95.7%	100.0%	3.1	1.2	3.0	0.0	Susceptible
66	Enrei	5.1	0.5	83.3%	100.0%	3.2	2.2	3.0	0.0	Susceptible
42	SRE-D-14A	6.0	0.0	93.9%	100.0%	3.3	1.0	3.0	0.0	Susceptible
43	SRE-D-14B	5.9	0.3	96.2%	100.0%	3.5	1.4	3.0	0.0	Susceptible
6	PI230970 (*Rpp2*)	3.0	0.0	94.6%	100.0%	3.7	1.8	3.0	0.0	Susceptible
39	GC95040-26-1-1-1	5.6	0.5	98.2%	100.0%	3.7	1.2	3.0	0.0	Susceptible
61	Davis	5.9	0.3	86.6%	100.0%	4.2	1.5	3.0	0.0	Susceptible
64	Moshidou Gong 503	5.9	0.3	96.1%	100.0%	4.3	1.4	3.0	0.0	Susceptible
62	EMBRAPA48	5.8	0.4	96.2%	100.0%	4.4	1.7	3.0	0.0	Susceptible
46	Qin Dou	6.0	0.0	91.2%	100.0%	4.5	1.9	3.0	0.0	Susceptible
37	GC87021-13-B-2	5.8	0.4	92.0%	100.0%	4.6	1.4	3.0	0.0	Susceptible
3	PI368039 (*Rpp1*)	5.0	0.2	92.9%	100.0%	4.8	1.7	3.0	0.0	Susceptible
2	PI200492 (*Rpp1*)	4.9	0.4	93.8%	100.0%	4.9	1.5	3.0	0.0	Susceptible
10	FT2 (*Rpp3)*	5.3	0.4	88.4%	100.0%	4.9	1.6	3.0	0.0	Susceptible
21	GC00002-100	4.9	0.6	38.1%	100.0%	4.9	1.5	3.0	0.0	Susceptible
51	Sachiyutaka	5.3	0.7	93.3%	100.0%	5.0	1.7	3.0	0.0	Susceptible
40	SRE-B-15C	5.6	0.5	89.4%	100.0%	5.0	1.7	3.0	0.0	Susceptible
35	GC87012-10-B-5	5.5	0.5	75.4%	100.0%	5.6	2.2	3.0	0.0	Susceptible
52	Lu Pi Dou	6.0	0.0	93.2%	100.0%	5.9	2.6	3.0	0.0	Susceptible
47	Da Bai Qi	5.4	0.5	98.4%	100.0%	6.1	2.0	3.0	0.0	Susceptible
48	6611	4.5	0.5	95.1%	100.0%	6.1	2.8	3.0	0.0	Susceptible
45	Niu Mao Huang	5.7	0.4	74.6%	100.0%	6.3	1.8	3.0	0.0	Susceptible
19	PI587905[1)]	–	–	–	–	–	–	–	–	(Mixed types of lesions)

All values in this table were rounded off to the first decimal place, whereupon the list arrangement was determined by the values of the five resistance characteristics in the following order: first - sporulation level (SL); second - number of uredinia (NoU). The values for the lesion color (LC), NoU, and SL are obtained from 30 lesions. Classification in terms of resistant (shading) or susceptible phenotypes in each characteristic was determined according to our previous study[40], however the frequency of open uredinia (%OU) was excluded from the classification. Phenotypic data for 13 varieties (Entries 2-7, 9, 14, 16, 18, 19, 58 and 59) shown in this table were obtained in our previous study[40]. [1)] Two types of lesions clearly different were obtained in PI587905 hence phenotypic data of this genotype was excluded from the analysis.

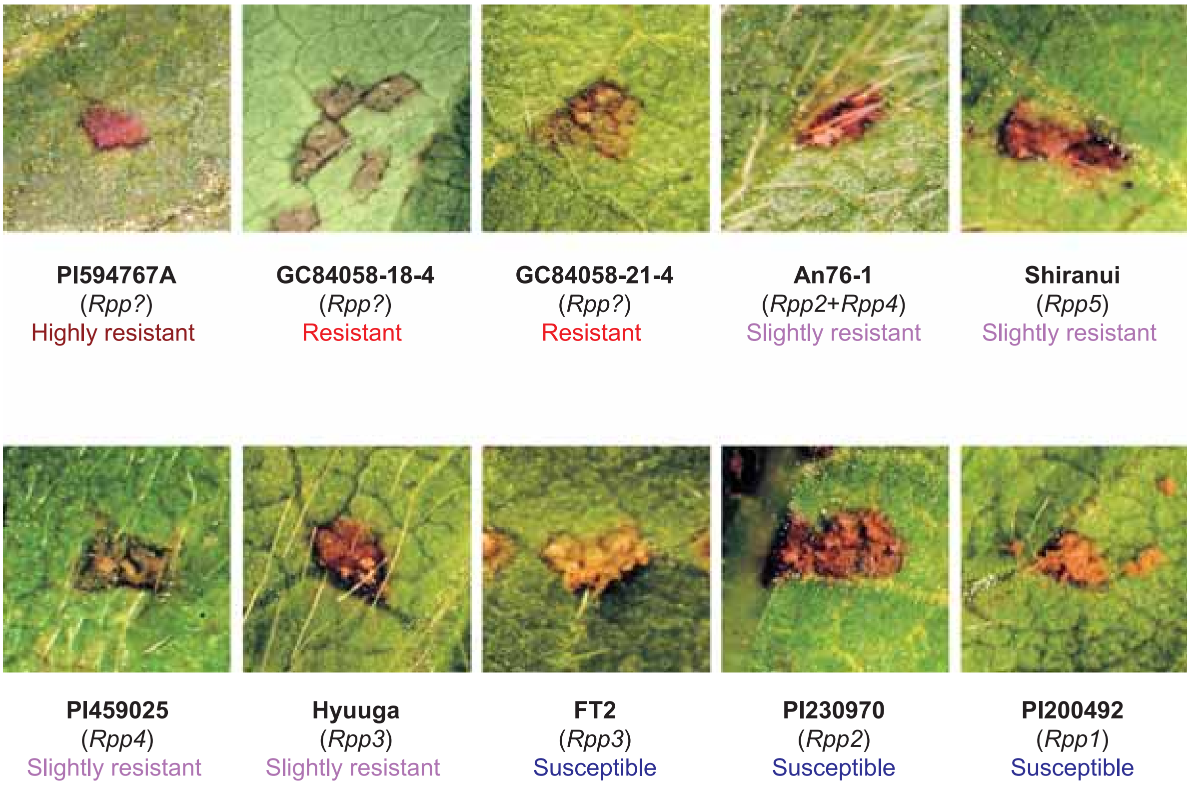


Fig. 3. Example lesions obtained from one 'Highly resistant' and two 'Resistant' genotypes and from seven genotypes having one or two kinds of major resistance genes

The resistance genes and categories are shown with the name of the soybean genotypes.

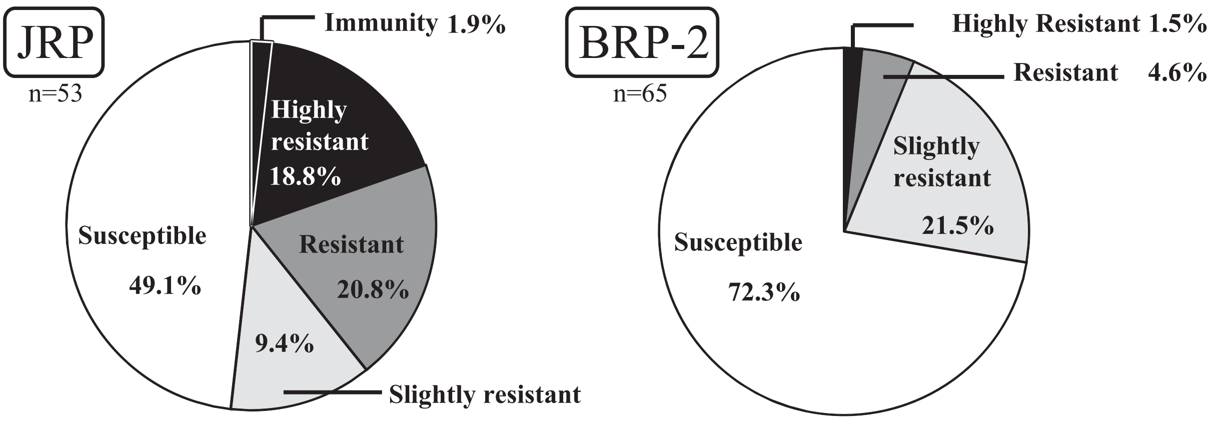


Fig. 4. The proportion of the five resistance categories: 'Immunity', 'Highly resistant', 'Resistant', 'Slightly resistant' and 'Susceptible', in 53 and 65 soybean genotypes respectively inoculated with Japanese rust population (JRP) and Brazilian rust population 2 (BRP-2)

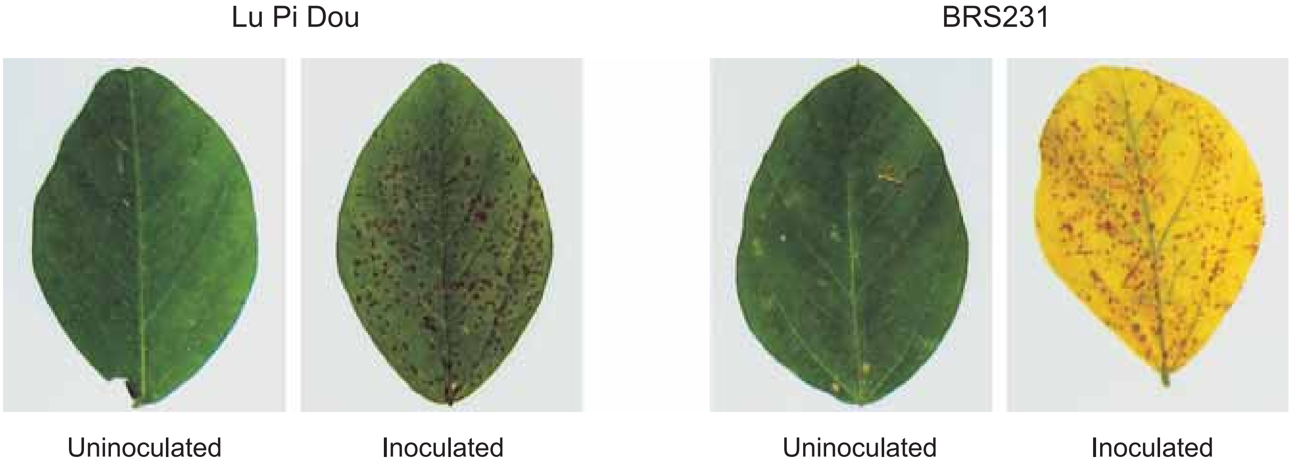
No immunity phenotype was observed, regarding BRP-2 inoculation. The frequency data of the 53 genotypes inoculated by JRP was obtained from our previous study[40]. PI587905 was excluded from the analysis of BRP-2 inoculation since it has shown mixed types of lesions.

in PI230970 compared to An76-1, and in PI459025 compared to PI230970 (Tables 2, 3). These indicate that An76-1 produced significantly darker lesions than PI 230970 and PI459025, and showed a similar level of NoU and SL with PI459025 (Table 3).

3. Leaf-yellowing prevention in two Chinese varieties

We identified that two Chinese varieties, 'Lu Pi

A



B

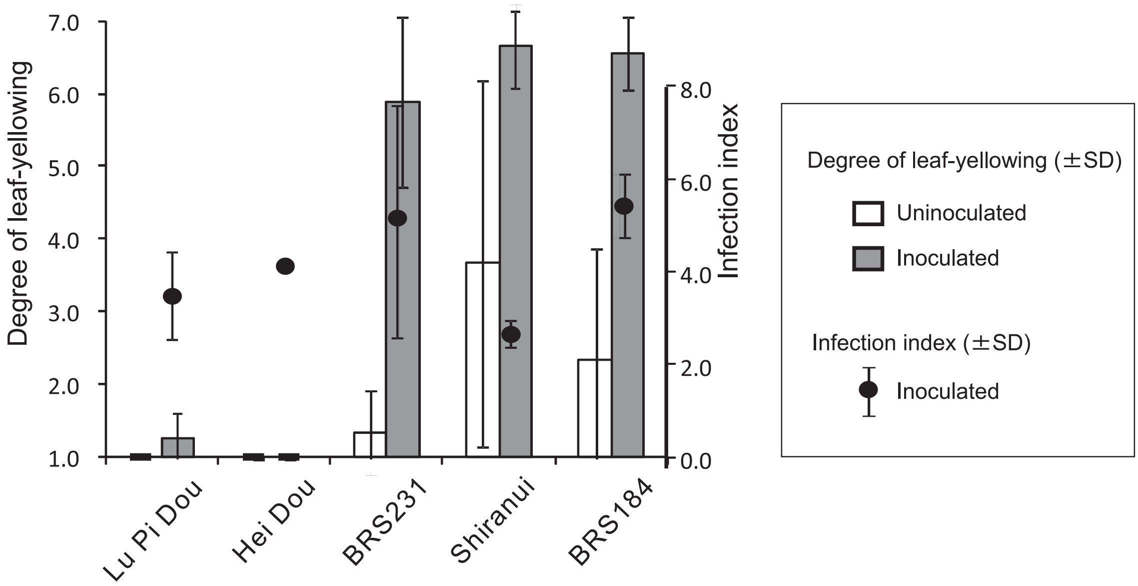


Fig. 5. Evaluation of two characteristics, the degree of leaf-yellowing and the infection index, in five soybean varieties

A: Example of leaves inoculated and uninoculated with BRP-2 in the 'Lu Pi Dou' and 'BRS231' varieties.

B: Degree of leaf-yellowing and infection index of five soybean varieties.
Uninoculated leaves were obtained from healthy plants grown under the same condition as uninoculated plants.

Dou' and 'Hei Dou', presented leaf-yellowing prevention upon BRP-2 inoculation (Fig. 5). The degree of leaf-yellowing in these varieties was significantly lower than those in the susceptible variety 'BRS184', in the *Rpp5*-harboring resistant variety 'Shiranui', and in the tolerant variety 'BRS231', despite no significant difference in the infection index by BRP-2 inoculation in these five varieties (Fig. 5B).

Table 3. Statistic comparison of three resistance characteristics: lesion color (LC), number of uredinia (NoU), and sporulation level (SL) among the genotypes: PI230970, PI459025, and An76-1[1)]

Genotype combination \ Resistance characteristic	LC[2)]	NoU[3)]	SL[2)]
PI230970 (*Rpp2*) − PI459025 (*Rpp4*)	$u = 900$, $z = 6.653^*$	5.885*	$u = 855$, $z = 5.988^*$
PI230970 (*Rpp2*) − An76-1 (*Rpp2* + *Rpp4*)	$u = 765$, $z = 4.657^*$	5.937*	$u = 795$, $z = 5.101^*$
PI459025 (*Rpp4*) − An76-1 (*Rpp2* + *Rpp4*)	$u = 900$, $z = 6.653^*$	0.333	$u = 532$, $z = 1.220$

1) Phenotypic values from 30 lesions in three genotypes were used for analysis.
2) The *u*- and *z*-values were calculated from the Mann-Whitney *U-test*.
3) The *t*- value was calculated from the Student's *t*- test.
* The difference between genotypes is significant at a 1% level ($p < 0.01$, two-tailed test).

Discussion

1. Limited number of resistant genotypes to BRP-2

In this study, a very limited number of resistant soybean genotypes were identified as useful for breeding to confer satisfactory resistance to the BRP-2 rust population, corroborating the estimations previously performed[40]. All five genotypes carrying *Rpp4* or *Rpp5* showed a slightly resistant phenotype against the BRP-2, whereas all seven genotypes carrying *Rpp1* or *Rpp2* showed susceptible phenotype against the BRP-2 (Fig. 3, Table 2). Interestingly, we found a variation of resistance in five varieties which putatively carry the *Rpp3* resistance gene (Fig. 3, Table 2). A similar finding was observed in soybean varieties carrying the *Rpp1* gene under inoculation of the Japanese rust population[40]. These varieties may have different alleles on the *Rpp3* locus and/or additional quantitative trait loci (QTLs) for resistance. *Rpp4* and *Rpp5* resistance genes, and also *Rpp3* in some varieties, contributed to the resistance to BRP-2 by reducing the number of uredinia and the urediniospore production, hence these sources can be used for breeding, even though the sporulation by the BRP-2 population on the same is not completely averted. A resistance survey of 66 genotypes revealed that four varieties, PI594767A, SRE-D-11C, GC84058-18-4 and GC84058-21-4, were more resistant to BRP-2 than those with one of the known *Rpp* resistance genes (Fig. 3, Table 2). The resistance genes inherited in these varieties are still unknown, but the varieties may be more useful than the genotypes carrying known *Rpp* resistance genes.

In this study, we evaluated the resistance of the soybean genotype 'An76-1' carrying resistant alleles of *Rpp2* and *Rpp4* as homozygous. Regarding the NoU (number of uredinia per lesion) and SL (sporulation level), the resistance of An76-1 was significantly higher than PI230970 (*Rpp2*), but not PI459025 (*Rpp4*). A possible explanation is that the resistant allele of *Rpp2*, derived from PI230970, was ineffective against the BRP-2 rust population. Consequently, no high resistance based on NoU and SL to BRP-2 was observed in An76-1. Conversely, LC in An76-1 was significantly darker than both PI230970 and PI459025 (Fig. 3, Table 3). The difference of LC between PI230970 and PI459025 was also significant. Therefore, LC and the other two characteristics, NoU and SL, were assumed to be independently controlled in soybean plants under our experimental conditions.

2. Classification criteria for resistance to BRP-2

In this study, we modified the resistance classification of varieties by excluding the character %OU (frequency of open uredinia), as used in our previous study[40], since we observed that this character can produce a misclassification of resistant genotypes into susceptible ones. The %OU in SRE-D-11C was 100% representing susceptible phenotype, however, this value was obtained from only 2 uredinia produced in 2 of 30 lesions (Table 2). Though all two uredinia were opened, it is reasonable to consider this variety as resistant to the BRP-2, since neither uredinia nor urediniospores were observed in the other 28 lesions. An index of open uredinia, i.e. by multiplying %OU by NoU, may be more suitable to judge resistance in the genotypes than solely %OU.

3. Varieties showing "Leaf-yellowing Prevention" as a soybean rust resistance source

Severe infection with soybean rust promotes early leaf-yellowing and defoliation[11]. However, we observed a clear difference in the degree of leaf-yellowing among two Chinese varieties and three control varieties, despite having similar infection indexes (Fig. 5). In addition, these two Chinese varieties showed clear susceptible lesions by BRP-2 infection (Table 2), meaning the candidate tolerance achieved by the leaf-yellowing prevention observed in ‘Lu Pi Dou’ and ‘Hei Dou’ subject to BRP-2 could not be caused by less infection and the resistance related to lesions’ characteristics usually conferred by the major resistance genes. ‘Lu Pi Dou’ and ‘Hei Dou’ were previously identified as showing leaf-yellowing prevention by soybean rust infection in the 267 soybean varieties[39]. Moreover, these two varieties also possess a unique characteristic, namely a green cotyledon color. The leaf-yellowing prevention might be a peculiar characteristic for soybean varieties having green cotyledon, since this comprises only four of 267 varieties. Therefore, a highly resistant variety might be developed by introducing the characteristic leaf-yellowing prevention along with major resistance genes if leaf-yellowing prevention is independent from the green cotyledon color. In addition, the relationship between leaf-yellowing and defoliation must be analyzed to estimate the usefulness of the leaf-yellowing prevention characteristic.

In conclusion, the Brazilian rust population 2 (BRP-2) employed in this study is virulent to all 16 varieties that have one of the *Rpp1-5* resistance genes as well as most previously identified resistant varieties. This result suggested that the high virulence of the Brazilian rust population limits the number of useful resistant varieties in Brazil. Therefore, a resistant cultivar effective against soybean rust in Brazil should be developed, not only by pyramiding some major resistance genes but also by introducing tolerance such as leaf-yellowing prevention.

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References

1. Arias, C. A. A. et al. (2008) Asian rust in Brazil: Varietal resistance. *In* JIRCAS Working Report No. 58, eds. Kudo, H. et al., JIRCAS, Tsukuba, Japan, 29−30.
2. Asian Vegetable Research and Development Center (1989) Progress Report 1989. AVRDC Publication, Shanhua, Taiwan.
3. Asian Vegetable Research and Development Center (1992) Progress Report 1992. AVRDC Publication, Shanhua, Taiwan.
4. Benitez, E. R. et al. (2008) Evaluation of selected Japanese varieties for resistance to *Phakopsora pachyrhizi*. *Breed. Res*., **10**, Suppl. 1, 70.
5. Bromfield, K. R. (1984) Soybean Rust - Monograph No. 11, The American Phytopathological Society, St. Paul, MN, USA.
6. Bromfield, K. R. & Hartwig, E. E. (1980) Resistance to soybean rust and mode of inheritance. *Crop Sci*., **20**, 254−255.
7. Castro, A. J. et al. (2003) Pyramiding quantitative trait locus (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. *Crop Sci*., **43**, 651−659.
8. Garcia, A. et al. (2008) Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: discovery of a novel locus and alleles. *Theor. Appl. Genet*., **117**, 545−553.
9. Harakava, R. (2005) Distinção molecular das ferrugens asiática e americana da soja. *Summa Phytopathologica*, **31**, Suppl., 119.
10. Hartman, G. L. Miles, M. R. & Frederick, R. D. (2005) Breeding for resistance to soybean rust. *Plant Dis*., **89**, 664−666.
11. Hartman, G. L. & Hill, C. B. (2010) Chapter 13: Diseases of soybean and their management. *In* The soybean: Botany, production and uses, ed. Singh, G., CABI, Wallingford, UK, 276−299.
12. Hartwig, E. E. (1986) Identification of a 4th major gene conferring resistance to soybean rust. *Crop Sci*., **26**, 1135−113.
13. Hartwig, E. E. & Bromfield, K. R. (1983) Relationships among 3 genes conferring specific resistance to rust in soybeans. *Crop Sci*., **23**, 237−239.
14. Hyten, D. L. et al. (2007) Map location of the Rpp1 locus that confers resistance to soybean rust in soybean. *Crop Sci*., **47**, 835−838.
15. Hyten, D. L. et al. (2009) Bulked segregant analysis using the GoldenGate assay to locate the *Rpp3* locus that confers resistance to soybean rust in soybean. *Crop Sci*., **49**, 265−271.
16. Kato, M. & Yorinori, J. T. (2008) A study on a race composition of *Phakopsora pachyrhizi* in Brazil: a difficulty of race identification. In JIRCAS Working Report No. 58, eds. Kudo, H. et al. JIRCAS, Tsukuba, Japan, 94−98.
17. Laperuta, L. D. C. et al. (2008) New genes conferring resistance to Asian soybean rust: allelic testing for the *Rpp2* and *Rpp4* loci. *Pesquisa Agropecuária Brasileira*, **43**, 1741−1747.
18. Lemos, N. G. et al. (2011) Characterization of genes

Rpp2, *Rpp4*, and *Rpp5* for resistance to soybean rust. *Euphytica*, doi: 10.1007/s10681-011-0465-3.
19. Liu, J. et al. (2000) Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. *Plant Breed*. **119**, 21−24.
20. Miles, M. R., Frederick, R. D. & Hartman, G. L. (2006) Evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. *Plant Health Progress*, doi 10.1094/PHP-2006-0104-01-RS.
21. McLean, R. J. & Byth, D. E. (1980) Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. *Aust. J. Agric. Res*., **31**, 951−956.
22. Monteros, M. J. et al. (2007) Mapping and confirmation of the 'Hyuuga' red-brown lesion resistance gene for Asian soybean rust. *Crop Sci*., **47**, 829−834.
23. Navarro, J. C. et al. (2004) First report of "Asian" soybean rust in Bolivia. *In* Abstracts of contributed papers and posters / VII World Soybean Research Conference, IV International Soybean Processing and Utilization Conference, III Congresso Brasileiro de Soja. Londrina Brazil. Embrapa-Soja, Londrina, Brazil, 85−86.
24. Nogueira, L. M. et al. (2008) Os genes de resistência á ferrugem asiática da soja, *Rpp2* e *Rpp4*, apresentam efeitos nao-aditivos quando acumulados em uma variedade. *Tropical Plant Pathol*., **33**, Suplemento, p. 204.
25. Oliveira, A. C. B., Godoy, C. V. & Martins, M. C. (2005) Avaliação da tolerância de cultivares de soja à ferrugem asiática no Oeste da Bahia. *Fitopatologia Brasileira*, **30**, 658−662.
26. Oloka, H. K. et al. (2008) Reaction of exotic soybean germplasm to *Phakopsora pachyrhizi* in Uganda. *Plant Dis*., **92**, 1493−1496.
27. Palloix, A., Ayme, V. & Moury, B. (2009) Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytol*., doi: 10.1111/j.1469−8137.2009.02827.x.
28. Pham, T. A. et al. (2009) Differential responses of resistant soybean entries to isolates of *Phakopsora pachyrhizi*. *Plant Dis*., **93**, 224−228.
29. Pierozzi, P. H. B. et al. (2008) New soybean (*Glycine max* Fabales, Fabaceae) sources of qualitative genetic resistance to Asian soybean rust caused by *Phakopsora pachyrhizi* (Uredinales, Phakopsoraceae). *Genet. Mol. Biol*., **31**, 505−511.
30. Ray, J. D. et al. (2009) Genetics and mapping of adult plant rust resistance in soybean PI 587886 and PI 587880A. *Theor. Appl. Genet*., **119**, 271−280.
31. Ribeiro, A. S. et al. (2008) Genetic control of soybean (*Glycine max*) yield in the absence and presence of the Asian rust fungus (*Phakopsora pachyrhizi*). *Genet. Mol. Biol*., **31**, 98−105.
32. Silva, D. C. G. et al. (2008) Molecular mapping of two loci that confer resistance to Asian rust in soybean. *Theor. Appl. Genet*., **117**, 57−63.
33. Singh, S. et al. (2001) Pyramiding three bacterial blight resistance genes (*xa5*, *xa13*, *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet*., **102**, 1011−1015.
34. The database of soybean genetic resources in Northeast China. http://www.jircas.affrc.go.jp/DB/guide-eng.html.
35. Wang, T. C. & Hartman, G. L. (1992) Epidemiology of soybean rust and breeding for host resistance. *Plant Prot. Bull*., **34**, 109−124.
36. Yamanaka, N. et al. (2001) An informative linkage map of soybean reveals QTLs for flowering time, leaflet morphology and regions of segregation distortion. *DNA Res*., **8**, 61−72.
37. Yamanaka, N. et al. (2007a) Genetic relationship of Japanese, Chinese and Brazilian soybean gene pools revealed by SSR markers. *Genet. Mol. Biol*., **30**, 85−88.
38. Yamanaka, N. et al. (2007b) Application of DNA markers for identifying genes for resistance to soybean diseases in South America and for evaluating genetic relationships among soybean gene pools. *In* JIRCAS Working Report No. 51, eds. Suenaga, K. et al. JIRCAS, Tsukuba, Japan, 47−54.
39. Yamanaka, N. et al. (2008) Identification of DNA markers and characterization of the genes for resistance against Asian soybean rust. *In* JIRCAS Working Report No. 58, eds. Kudo, H. et al. JIRCAS, Tsukuba, Japan, 99−107.
40. Yamanaka, N. et al. (2010) Development of classification criteria for resistance to soybean rust and differences in virulence among Japanese and Brazilian rust populations. *Tropical Plant Pathol*., **35**, 153−162.
41. Yamaoka, Y. et al. (2002) Pathogenic races of *Phakopsora pachyrhizi* on soybean and wild host plants collected in Japan. *J. Gen. Plant Pathol*., **68**, 52−56.
42. Yorinori, J. T. (2008) Soybean germplasms with resistance and tolerance to Asian rust and screening method. *In* JIRCAS Working Report No. 58, eds. Kudo, H. et al. JIRCAS, Tsukuba, Japan, 70−87.
43. Yorinori, J. T. et al. (2005) Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. *Plant Dis*. **89**, 675−677.