Molecular Marker Analysis of Soybean Plant Introductions with Resistance to *Phytophthora sojae*

S. G. Gordon, K. Kowitwanich, W. Pipatpongpinoy, S. K. St. Martin, and A. E. Dorrance

First, second, third and fifth authors: Department of Plant Pathology, The Ohio State University, Wooster, OH 44691; and fourth author: Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210.
Accepted for publication 11 August 2006.

ABSTRACT

Gordon, S. G., Kowitwanich, K., Pipatpongpinoy, W., St. Martin, S. K., and Dorrance, A. E. 2007. Molecular marker analysis of soybean plant introductions with resistance to *Phytophthora sojae*. *Phytopathology* 97:113-118.

Molecular analysis of sources of resistance to plant pathogens should expedite and confirm novel gene discovery and consequently the development of disease resistant cultivars. Recently, soybean plant introductions (PIs) were identified that contain putative novel *Rps* genes for resistance to *Phytophthora sojae*. The number of resistance genes that confer resistance to *P. sojae* isolates OH17 (1b,1d,2,3a,3b,3c,4,5,6,7) and OH25 (1a,1b,1c,1k,7) was then determined in several of the PIs. The objective of this study was to determine if the *Rps* genes present in these PIs were associated with eight described *Rps* loci that have been mapped on soy-

bean molecular linkage groups F, G, J, and N. Nine F_{2:3} soybean populations were genotyped with simple sequence repeat (SSR) markers linked to previously mapped *Rps* loci. The nine PI populations all had SSR markers associated ($P < 0.01$) with resistance to *P. sojae* isolate OH17 in the *Rps1* region. *Rps1c* is a likely candidate in eight PIs but novel genes may also be possible, while novel genes may confer resistance in one PI to *P. sojae* isolate OH17. Two or more *Rps* genes, including some that are potentially novel, confer resistance to *P. sojae* isolate OH25 in eight of the populations. However, based on the response to these two isolates, virulence already exists for at least some of the novel genes identified in this study.

Additional keywords: *Glycine max*, host resistance.

Single, dominant R-genes to plant pathogens often occur in multigene clusters in many of the plant genomes that have been studied (23), including soybean (*Glycine max* L. Merr.) (1,2,4,13,14,19,20,32). In rice (*Oryza sativa* L.) and *Arabidopsis thaliana* (L.) Heynh., whose entire genomes have been sequenced, these resistance gene clusters are made up of active R-genes as well as numerous R-gene-like sequences (23). In soybean, these gene clusters consist of multiple R-genes that confer resistance to different pathogens, such as the *Rps2* (resistance to *Phytophthora sojae* Kauf. and Gerd.) and *Rmd* (resistance to powdery mildew) loci on molecular linkage group (MLG) J (25); or to different isolates or strains of the same pathogen, such as the tightly linked loci that confer resistance to *Soybean mosaic virus* on MLG F (17).

Numerous resources now exist for soybean genetic analysis, including both classical and molecular markers as well as genetic linkage maps (16,29). The most recent integrated genetic map of soybean is derived from five mapping populations and contains 20 linkage groups spanning more than 2,500 cM, defined by simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), random amplified DNA (RAPD), amplified fragment length polymorphism (AFLP), and classical markers (24,29). These markers are an important resource for both basic and applied research, including investigations to characterize the resistance to soybean pathogens (18).

P. sojae causes root and stem rot of soybean, and is a well-described pathosystem (10). Fourteen resistance genes to *P. sojae* (*Rps*) have been described and mapped to eight loci in the soybean genome (*Rps1* to *Rps8*) (7,8,12,17) (Fig. 1). *Rps1* and *Rps7*

are located on MLG N (22,31); *Rps2* on MLG J (25); *Rps3* and *Rps8* on MLG F (7,8,12); and *Rps4* and *Rps6* on MLG G (7,27). Current evidence suggests that *Rps5* is located on MLG G and is linked to *Rps4* (7,8). Both *Rps1* and *Rps3* have multiple alleles (17).

Incorporation of *Rps* genes into soybean cultivars has been the primary strategy for managing *Phytophthora* root and stem rot (3,17,28). *Rps1a* was the first resistance gene to be widely deployed. It was first deployed during the 1960s and remained effective for approximately 8 years (28). Subsequently, *Rps1c*, *Rps1k*, *Rps3a*, and *Rps6* were deployed in the north central region of the United States, with *Rps1k* remaining effective for almost 20 years (9). Thus, single gene-mediated resistance is an effective means for managing this soilborne disease; however, new genes that are effective against the current *P. sojae* populations are needed.

Dorrance and Schmitthenner (10) identified 32 plant introductions (PIs) that were proposed to contain novel *Rps* genes, combinations of novel and known *Rps* gene(s), or combinations of three known *Rps* loci. In a subsequent study (11), 12 of these PIs were crossed with the susceptible cv. Williams and were evaluated for segregation of resistance to two *P. sojae* isolates, OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and OH25 (vir 1a, 1b, 1c, 1k, 7), in the resulting populations. The two isolates used in this previous study were virulent to soybeans with all characterized *Rps* genes except *Rps8* (10,12). Based on the segregation data in the previous study, many of the PIs have two or more *Rps* loci, some of which appear to be novel. More specifically, resistance to OH17 was conferred by one locus in 12 populations and two loci in two populations. Resistance to OH25 was conferred by two loci in seven populations, by three loci in four populations, and by a single locus in one population (11).

These putatively novel *Rps* genes may be *Rps8* or novel alleles at known loci or new genes at loci where no *Rps* genes have been

Corresponding author: A. E. Dorrance; E-mail address: dorrance.1@osu.edu

DOI: 10.1094/PHYTO-97-0113

© 2007 The American Phytopathological Society

previously reported. There are other regions in the soybean genome where resistance genes exist to other pathogens, but to which no *Rps* genes have been mapped (19). Therefore, the objectives of this study were to determine if the *Rps* genes in these PIs were associated with previously described *Rps* loci by genotyping with SSR markers linked to known *Rps* loci. For this study a subset of the PIs with specific phenotypic resistance responses to *P. sojae* isolates OH17 and OH25 were chosen (11). This strategy of phenotypic and genotypic analysis to identify associations to known *Rps* loci will provide insight as to which *Rps* genes may be present in these PIs. In addition, any lack of association with SSRs and *Rps* loci would then indicate that these PIs contain *Rps* genes at novel loci.

MATERIALS AND METHODS

Soybean populations and phenotypic data. Nine populations were selected for genotyping with SSRs based on population size and consistent segregation ratios for both *P. sojae* isolates between the F₂ and F₃ generations (Table 1). The resistance response to both isolates from the prior study was used in the molecular analyses.

The population development and phenotypic screen were described previously (11). Briefly, each PI was crossed with the susceptible cv. Williams and the F₁ and F₂ plants from each of these crosses were allowed to self-pollinate to produce populations of F_{2:3} families. For phenotypic analysis, 10 to 20 soybean seedlings

Fig. 1. Locations of *Rps* loci on major linkage group (MLG) in the soybean genome and their genetic distance from simple sequence repeat loci used in this study. Distances are based on a merger of genetic maps from several reference soybean populations (6,12,15,30).

TABLE 1. *Rps* locus-linked simple sequence repeat markers mapped to major linkage groups (F, G, J, and N) used to genotype F_{2:3} populations of resistant soybean plant introductions (PIs) crossed to the susceptible cv. Williams, and the number of families in each population

Resistant parent	F _{2:3} families	F (<i>Rps</i> 3, 8)	G (<i>Rps</i> 4, 6)	J (<i>Rps</i> 2)	N (<i>Rps</i> 1, 7)
PI273483D	58	Satt510 Satt663	Satt191 Satt199	Satt244 Satt287 Satt431	Satt152
PI398440	36	Satt510 Satt114	Satt199 Satt472	Sat_144 Sat_393	Satt152 Satt159
PI398694	75	Satt510 Satt663	Satt191 Satt199	Sat_144 Sat_393	Satt152
PI399036	92	Satt335 Satt510 Satt663	Sat_064 Satt191	Sat_144 Satt440	Satt631 Satt683
PI399079	90	Satt334 Satt114	Satt191 Sat_372	Sat_395 Satt440	Sat_084 Satt152
PI407974B	94	Satt510 Satt516	Satt191 Satt199	Satt244	Satt159
PI408097	93	Satt335 Satt510	Satt472 Satt199	Satt431	Satt152 Satt159
PI408211B	93	Satt510	Satt191	Satt215	Satt152 Satt159 Satt584
PI424354	92	Satt335 Satt510 Satt114	Satt191	Satt440 Sat_144	Satt159 Satt631 Satt683

in each F_{2:3} and F_{2:4} family were inoculated with *P. sojae* isolates OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and OH25 (vir 1a, 1b, 1c, 1k, 7) by the hypocotyl method (12). Seedlings were scored as susceptible (expanding brown lesion) or resistant (necrotic lesion restricted to the inoculation site) 7 days following inoculation. Each family was then scored based on the percentage of susceptible responses as homozygous resistant (RR), 0 to 20%; heterozygous (Rr), 21 to 79%; or susceptible (rr), 80 to 100%. This scoring was used to represent the F₂ plant genotype. These data were then used to determine the number of *Rps* loci that conferred resistance to each *P. sojae* isolate (11).

SSR marker analysis. Tissue from 10 to 15 seedlings from each of the F_{2:3} families was bulked, placed in liquid nitrogen, lyophilized, and ground for DNA extraction. DNA was extracted by a cetyltrimethylammoniumbromide method (26), yielding 500 µl of sample with DNA concentrations ranging from 100 to 125 ng/µl.

SSR markers were chosen from the integrated soybean genetic map (6,30) based on their linkage (3 to 20 cM) to previously mapped *Rps* loci on MLGs F, G, J, and N (7,27,31) (Fig. 1). The SSR markers were screened for polymorphisms between the PIs and Williams, and those SSRs used to genotype each population are listed in Table 1. SSRs were amplified using standard polymerase chain reaction (PCR) protocols (16) with the exception that a touchdown PCR program was used to drop the annealing temperature 1 degree per cycle for eight cycles, from 58° to 50°C, and then 30 cycles were run with an annealing temperature of 50°C.

Single factor analyses using the *P. sojae* resistance response for each isolate as the dependent variable were completed for each marker in each population using SAS PROC GLM (SAS Institute, Cary, NC). The model was $y_{ij} = \mu + \alpha_i + e_{ij}$ where y_{ij} is the trait value of the j th genotype of the i th marker class, μ is the population mean, α_i is the effect of the i th marker class, and e_{ij} is the experimental error.

Power analysis. For an R-gene phenotype analysis, the expression of one R-gene will partially mask the presence of the second when they are both effective against a specific isolate. In order to determine the statistical power to detect associations between SSRs and phenotypic response to *P. sojae* governed by multiple *Rps* loci, and therefore minimize both type I (false negative) and type II (false positive) error, a power analysis was performed (21). Power is the probability of detecting linkage (by obtaining a significant result that rejects the null hypothesis of independent assortment), given that linkage of a given intensity exists and a population of a given size is sampled.

Power to detect association between SSRs and *Rps* loci was estimated using a 1-tailed z test at alpha = 0.05 for a binomial frequency, based on the relative frequencies of the phenotypic classes most sensitive to linkage, i.e., the combined frequency of resistant (MM) and heterogeneous (Mm) classes versus that of the resistant (MM) and heterogeneous (Mm) classes (21). If MM, Mm, and mm are the three genotypic classes of the SSR marker and resistant (R), heterozygous (H), and susceptible (S) are the three phenotypic classes of the resistance assay, then the classes (MM+R) and (Mm+H) represent combinations of one recombinant and one parental (nonrecombinant) gamete, and (MM+H) and (Mm+R) represent combinations of recombinant gametes. The relative proportions of (MM+R) + (Mm+H) and of (MM+H) + (Mm+R) are very sensitive to linkage. Let $p = [(MM+R) + (Mm+H)] / [(MM+R) + (Mm+H) + (MM+H) + (Mm+R)]$. Then, for example, when there is no linkage, $p = 0.511$. (This is shown by making the Punnett square and performing the necessary algebra.)

If complete linkage between M/m and R/r is present, then $p = 0.833$. If the two loci are 10 cm apart, then $p = 0.738$, and so on. The binomial test of linkage is a test of the null hypothesis that $p = 0.511$ (no linkage) against the alternative hypothesis that

$p > 0.511$ (linkage). This test included only those individuals from the assay that fell into the four classes (MM+R, Mm+H, MM+H, and Mm+R) that collectively make up approximately 70% of the population. The null hypothesis was rejected when the observed ratio (p^*) falls in the rejection region. The rejection region consists of the right tail (5%) of the distribution and lies above 0.511. Given the population size and the true value of p , 0.738, for example, the power of the binomial test equals the probability that p^* falls in the rejection region.

Example of calculations. For 100 F₂ lines, the number falling in the four pertinent classes is expected to be 70. The rejection region can be calculated as any p^* exceeding 0.609. If linkage exists at 10 cM, the expected value of p is 0.738, as mentioned above. The probability is .99 that a sample of 70 individuals will give a p^* greater than 0.609 if the true value of p is 0.738, i.e., that is the power of the test in that situation. Calculation of the rejection region and the power is done by using a normal approximation to the binomial distribution.

RESULTS AND DISCUSSION

The statistical power analysis was completed for one-, two-, and three-locus models and a theoretical population size of 94 F₂ families, and for a single-locus model in a population size of 50 families (Table 2). For the larger population of 94 F₂ families, the power analysis indicates that for genetic distances of 15 cM or less between *Rps* and SSR loci, there was greater than 90% probability of detecting a statistical association for a particular SSR when either one or two segregating *Rps* loci were present (Table 2). For a distance of 20 cM, there was greater than 80% probability of detecting a statistical association between an SSR and an *Rps* locus. For the three-*Rps* locus model there was greater than 90% probability of detecting an association at a distance of 10 cM, greater than 79% probability of detecting an association at a distance of 15 cM, and greater than 62% probability of detecting an association at 20 cM (Table 2). These calculations, since they are based on a relatively crude test for linkage, probably underestimate the power of the statistical analysis that was used in this study. In addition, the test is based on a single SSR marker. For most of the populations, there was more than one marker per *Rps* locus (Table 1), and in these situations the power to detect associations with at least one marker is greater than the results of the power analysis.

In the first study (10) these PIs were identified based on their resistance to a number of *P. sojae* isolates whose combined virulence specificities encompassed soybeans with all known *Rps*

TABLE 2. Statistical power^a to detect association of simple sequence repeat (SSR) markers with *Rps* loci responsible for resistance to *Phytophthora sojae* isolates OH17 and OH25

Recombination frequency	1 gene ^b	2 genes ^b	3 genes ^b	1 gene ^c
0	1.00	1.00	0.99	1.00
0.05	1.00	0.99	0.98	0.98
0.10	1.00	0.99	0.92	0.90
0.15	0.99	0.95	0.79	0.75
0.20	0.93	0.81	0.62	0.57
0.25	0.73	0.60	0.45	...
0.30	0.46	0.39	0.30	...
0.35	0.25	0.24	0.20	...
0.40	0.13	0.14	0.13	...
0.45	0.07	0.08	0.09	...

^a Power was calculated for one-, two-, and three-*Rps* locus models in a population of 94 F_{2:3} families and for a one-*Rps* locus model in a population of 50 F_{2:3} families. Probability is the likelihood of detecting linkage at a given recombination frequency between *Rps* and SSR loci at alpha = 0.05.

^b Power to detect associations in a population of 94 families.

^c Power to detect associations in a population of 50 families.

TABLE 3. Statistical association^a of simple sequence repeat markers linked to *Rps* loci from soybean major linkage groups F, G, J, and N in F_{2:3} populations^b that have resistance conferred by a single gene to *Phytophthora sojae* isolate OH17 (1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and two genes to *P. sojae* isolate OH25 (1a, 1b, 1c, 1k, 7)

Resistant parent	Number of families	<i>P. sojae</i> isolate	F (<i>Rps</i> 3, 8)	G (<i>Rps</i> 4, 6)	J (<i>Rps</i> 2)	N (<i>Rps</i> 1, 7)
PI273483D	58	OH17	ns	ns	*	***
		OH25	ns	ns	ns	ns
PI398694	75	OH17	ns	ns	ns	***
		OH25	ns	**	ns	ns
PI407974B	94	OH17	ns	ns	ns	***
		OH25	ns	***	ns	ns
PI408097	93	OH17	ns	ns	ns	***
		OH25	*	***	ns	ns

^a ns, *, **, and *** indicate that the statistical association was not significant, significant at $P < 0.05$, significant at $P < 0.01$, and significant at $P < 0.001$, respectively.

^b Populations of segregating F_{2:3} families were developed from crosses of resistant PI with susceptible cv. Williams.

TABLE 4. Statistical association^a of simple sequence repeat markers from soybean major linkage groups F, G, J, and N linked to *Rps* loci in F_{2:3} populations^b that have resistance conferred by a single gene to *Phytophthora sojae* isolate OH17 (1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and three genes to *P. sojae* isolate OH25 (1a, 1b, 1c, 1k, 7)

Resistant parent	Number of families	<i>P. sojae</i> isolate	F (<i>Rps</i> 3, 8)	G (<i>Rps</i> 4, 6)	J (<i>Rps</i> 2)	N (<i>Rps</i> 1, 7)
PI399036	92	OH17	ns	*	ns	***
		OH25	ns	***	ns	ns
PI408211B	93	OH17	ns	ns	ns	***
		OH25	ns	ns	ns	*

^a ns, *, **, and *** indicate that the statistical association was not significant, significant at $P < 0.05$, significant at $P < 0.01$, and significant at $P < 0.001$, respectively.

^b Populations of segregating F_{2:3} families were developed from crosses of resistant plant introductions (PIs) with susceptible cv. Williams.

genes and two-gene combinations as well as most three-gene combinations with the exception of the gene combination(s) of *Rps*1c, 2, 3 (a or b), and 4. Therefore, the primary hypothesis was that these PIs contain a combination of *Rps*1c, 2, 3 (a or b), 4, or novel gene(s) plus known *Rps* genes. Based on phenotypic analysis, resistance to OH17 and OH25 was conferred by one and two loci, respectively, in PI273483D, PI398694, PI407974B, and PI408097. SSRs linked to *Rps*1 and *Rps*7 on MLG N were associated ($P < 0.001$) with resistance to OH17 for all four PIs (Table 3). Of these four PIs, only one was identified with markers significantly associated with resistance at two loci for OH25. None of the *Rps* loci were associated with resistance to OH25 in PI273483D with a moderate population size of 50 families. Resistance to OH25 in PI398694, PI407974B, and PI408097 was associated with SSRs linked to *Rps*4 or *Rps*6 ($P < 0.001$) (Table 3). *P. sojae* isolate OH25 has avirulence to soybeans with both *Rps*4 and *Rps*6; therefore one of these two linked loci is likely the resistance locus. SSRs were associated ($P < 0.05$) with resistance to OH25 at the *Rps*3 and *Rps*8 loci in PI408097 (Table 3). Even though resistance to OH17 was conferred by one locus, one PI had SSRs at additional loci associated with resistance to this isolate ($P = 0.05$): PI273483D with SSRs linked to *Rps*2. For PI273483D and PI399036, more resistance loci were detected than predicted by the segregation ratio to OH17, which was 3:1. The P values for the additional SSR loci associated with resistance were marginally significant ($P = 0.01$ to 0.05); these results could also be false positives. All of the associations between SSR markers and *P. sojae* resistance, while convincing, are statistical functions and must be further scrutinized. Further experiments are in progress, including genetic mapping in advanced generations, to confirm the proposed novel *Rps* loci, and to develop populations for allelism tests. Only PI408097 fit the proposed model of *Rps* gene combinations (*Rps*1c, 2, 3 [a or b], and 4) based on the genotypic evaluations. Resistance to OH17 was associated with MLG N where *Rps*1 mapped, thus indicating that *Rps*1c may be involved and that resistance to OH25 was linked to MLGs F (*Rps*3) and G (*Rps*4, PI408097).

In two PI populations (PI399036 and PI408211B) where a single locus conferred resistance to OH17 and three loci conferred resistance to OH25, SSRs linked to *Rps*1 and *Rps*7 on MLG N were associated with resistance to OH17 ($P < 0.001$) (Table 4). Resistance to OH25 in PI399036 was associated with SSRs linked to *Rps*4 or *Rps*6 on MLG G (Table 4) but no significant associations were detected for the remaining two loci. Resistance to OH25 in PI408211B was linked to *Rps*1 on MLG N (Table 4) which suggests the presence of a novel gene on MLG N in PI408211B. This is very strong evidence for a novel gene as none of the previously described *Rps* genes in the *Rps*1 locus confer resistance to both OH17 and OH25.

Resistance to *P. sojae* isolates OH17 and OH25 was conferred by a single locus in PI398440. However, in the previous study the F_{2:3} families did not respond consistently to both isolates; some families that were resistant to OH17 were susceptible to OH25 or vice versa (11). For PI398440, only SSRs linked to *Rps*1 and *Rps*7 on MLG N were associated with resistance to OH17 ($P < 0.001$), while no SSRs were associated with resistance to OH25 (Table 5). These results suggest that resistance to OH25 in PI398440 may reside at a novel locus. This genotypic evaluation confirms that different regions of the genome confer resistance to each isolate. This is not only strong evidence for novel genes in these populations but for novel virulence in the *P. sojae* isolates as well. The virulence that exists in OH17 and OH25 to soybeans with these novel *Rps* genes may prevent them from being useful for management of *Phytophthora* root and stem rot.

Two loci conferred resistance to OH17 in PI399079 and PI424354 (11). In both of these populations, SSRs linked to *Rps*1 and *Rps*7 were associated with resistance ($P < 0.001$) (Table 6). No additional SSRs were associated with resistance to OH17 in PI399079, while SSRs linked to *Rps*3 or *Rps*8 were identified in PI424354. Resistance to OH25 was associated with SSRs linked to *Rps*4/*Rps*6 on MLG G in the PI424354 population ($P < 0.001$); no SSRs were associated with resistance for PI399079. These results indicate that these PIs may contain a novel locus. OH17 has virulence for genes at all of the described *Rps* loci except for

TABLE 5. Statistical association^a of simple sequence repeat markers from soybean major linkage groups F, G, J, and N linked to *Rps* loci in F_{2:3} population^b of Williams by plant introduction (PI) 398440 that has resistance conferred by a single gene to both *Phytophthora sojae* isolates OH17 (1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and OH25 (1a, 1b, 1c, 1k, 7)

Resistant parent	Number of families	<i>P. sojae</i> isolate	F (<i>Rps</i> 3, 8)	G (<i>Rps</i> 4, 6)	J (<i>Rps</i> 2)	N (<i>Rps</i> 1, 7)
PI398440	36	OH17	ns	ns	ns	***
		OH25	ns	ns	ns	ns

^a ns, *, **, and *** indicate that the statistical association was not significant, significant at $P < 0.05$, significant at $P < 0.01$, and significant at $P < 0.001$, respectively.

^b Populations of segregating F_{2:3} families were developed from crosses of resistant PI with susceptible cv. Williams.

TABLE 6. Statistical association^a of simple sequence repeat markers from soybean major linkage groups F, G, J, and N linked to *Rps* loci in F_{2:3} populations^b that have resistance conferred by two genes to *Phytophthora sojae* isolate OH17 (1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and either two (424234B) or three genes (424354) to *P. sojae* isolate OH25 (1a, 1b, 1c, 1k, 7)

Resistant parent	Number of families	<i>P. sojae</i> isolate	F (<i>Rps</i> 3, 8)	G (<i>Rps</i> 4, 6)	J (<i>Rps</i> 2)	N (<i>Rps</i> 1, 7)
PI399079	90	OH17	ns	ns	ns	***
		OH25	ns	ns	ns	ns
PI424354	92	OH17	*	ns	ns	***
		OH25	ns	***	ns	ns

^a ns, *, **, and *** indicate that the statistical association was not significant, significant at $P < 0.05$, significant at $P < 0.01$, and significant at $P < 0.001$, respectively.

^b Populations of segregating F_{2:3} families were developed from crosses of resistant plant introductions (PIs) with susceptible cv. Williams.

*Rps*1 alleles 1a, 1c, and 1k. Thus, the second gene conferring resistance is indeed novel. Of most interest is the locus in PI399079, in which only SSRs linked to a single known *Rps* locus were associated with resistance to OH17. In addition, the lack of association for the second locus and for OH25 also indicate that this population has the higher probability of identifying another region in the soybean genome where the second *Rps* locus resides.

The PIs used as the resistant parents in this study originated primarily from South Korea. Burnham et al. (5) evaluated the genetic diversity of these PIs and concluded that they are genetically distant from U.S. and Chinese genotypes based on SSR analysis. In addition to being sources of *P. sojae* resistance, these PIs have the potential to broaden the genetic diversity of U.S. soybean germ plasm. Using chi-square analysis of SSR allele frequencies, Burnham et al. (5) also found that MLG A2 SSRs were significantly associated with resistant PIs but not with susceptible PIs. In the current study three populations were genotyped with MLG A2 SSRs and no association with *P. sojae* resistance was found (data not shown).

In summary, this study confirms the existence of multiple *Rps* loci in these soybean PIs, including some that are potentially novel. These potentially novel loci fall into two categories: novel alleles of known loci and putatively new genes at new loci. While all these PIs are resistant to *P. sojae* isolates OH17 and OH25, it is clear from the results that the resistance is quite diverse in its genetic basis. In addition to novel resistance loci, these data demonstrate the existence of undescribed virulence genes in *P. sojae* which also require further characterization. The usefulness of these PIs for management of *Phytophthora* root and stem rot will ultimately depend on the proportion of the *P. sojae* population to which these genes confer resistance.

ACKNOWLEDGMENTS

We thank R. Fioritto, S. McIntyre, G. Mills, and B. Sugerman for development of plant populations; S. A. Berry, A. Mills, and K. Burnham for plant inoculations; and B. Bishop for assistance with data analysis. This project was sponsored by Ohio's soybean producers' check-off dollars through the Ohio Soybean Council. Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.

LITERATURE CITED

- Anderson, T. R., and Buzzell, R. I. 1992. Inheritance and linkage of the *Rps*7 gene for resistance to *Phytophthora* rot of soybean. *Plant Dis.* 76:958-959.
- Ashfield, T., Danzer, J. R., Held, D., Clayton, K., Keim, P., Saghai-Maroo, M. A., Webb, D. M., and Innes, R. W. 1998. *Rpg*1, a soybean gene effective against races of bacterial blight, maps to a cluster of previously identified disease resistance genes. *Theor. Appl. Genet.* 96:1013-1021.
- Athow, K. L. 1985. *Phytophthora* root rot of soybean. Pages 575-581 in: *Proc. World Soybean Res. Con. III*. R. Shibles, ed. Westview Press, Boulder, CO.
- Bhattacharyya, M. K., Narayanan, N. N., Gao, H., Santra, D. K., Salimath, S. S., Kasuga, T., Liu, Y., Espinosa, B., Ellison, L., Marek, L., Shoemaker, R., Gijzen, M., and Buzzell, R. I. 2005. Identification of a large cluster of coiled coil-nucleotide binding site-leucine rich repeat-type genes from the *Rps*1 region containing *Phytophthora* resistance genes in soybean. *Theor. Appl. Genet.* 111:75-86.
- Burnham, K. D., Francis, D. M., Dorrance, A. E., Fioritto, R. J., and St. Martin, S. K. 2002. Genetic diversity patterns among *Phytophthora* resistant soybean plant introductions based on SSR markers. *Crop Sci.* 42:338-343.
- Cregan, P. B., Jarvik, T., Bush, A. L., Shoemaker, R. C., Lark, K. G., Kahler, A. L., Kaya, N., VanToai, T. T., Lohnes, D. G., Chung, J., and Specht, J. E. 1999. An integrated genetic linkage map of the soybean genome. *Crop Sci.* 39:1464-1490.
- Demirbas, A., Rector, B. G., Lohnes, D. G., Fioritto, R. J., Graef, G. L., Cregan, P. B., Shoemaker, R. C., and Specht, J. E. 2001. Simple sequence repeat markers linked to the soybean *Rps* genes for *Phytophthora* resistance. *Crop Sci.* 41:1220-1227.
- Diers, B. W., Mansur, L., Imsande, J., and Shoemaker, R. C. 1992. Mapping *Phytophthora* resistance loci in soybean with restriction fragment polymorphism markers. *Crop Sci.* 32:377-383.
- Dorrance, A. E., McClure, S. A., and de Silva, A. 2003. Pathogenic diversity of *Phytophthora sojae* in Ohio soybean fields. *Plant Dis.* 87:139-146.
- Dorrance, A. E., and Schmitthenner, A. F. 2000. New sources of resistance to *Phytophthora sojae* in the soybean plant introductions. *Plant Dis.* 84:1303-1308.
- Gordon, S. G., Kowitwanich, K., Pipatpongpinoy, W., St. Martin, S. K., and Dorrance, A. E. 2007. Genetic analysis of soybean plant introduction with resistance to *Phytophthora sojae*. *Phytopathology* 97:113-118.
- Gordon, S. G., St. Martin, S. K., and Dorrance, A. E. 2004. Mapping *Rps*8, a gene for resistance to *Phytophthora* root and stem rot in soybean. Page 320 in: *Crop Sci. Soc. America Annu. Mtg., ASA-CSA-SS*, Madison, WI.
- Graham, M. A., Marek, L. F., Lohnes, D., Cregan, P., and Shoemaker, R. C. 2000. Expression and genome organization of resistance gene analogs in soybean. *Genome* 43:86-93.

14. Graham, M. A., Marek, L. F., and Shoemaker, R. C. 2002. PCR sampling of disease resistance-like sequences from a disease resistance gene cluster in soybean. *Theor. Appl. Genet.* 105:50-57.
15. Grant, D., Imsande, M. I., and Shoemaker, R. C. 2002. Soybase; The USDA-ARS Soybean Genome Database. Published online by USDA-ARS.
16. Grau, C. R., Dorrance, A. E., Bond, J., and Russin, J. 2004. Fungal diseases. Pages 679-763 in: *Soybeans: Improvement, Production, and Uses*. 3rd ed. H. R. Boerma and J. E. Specht, eds. Agron. Monogr. 16. ASA, CSSA, and SSSA, Madison, WI.
17. Hayes, A. J., Jeong, S. C., Gore, M. A., Yu, Y. G., Buss, G. R., Tolin, S. A., and Saghai-Marooof, M. A. 2004. Recombination within a nucleotide-binding-site/leucine-rich-repeat gene cluster produces new variants conditioning resistance to *Soybean mosaic virus* in soybeans. *Genetics* 166:493-503.
18. Hegstad, J. M., Nickell, C. D., and Vodkin, L. O. 1998. Identifying resistance to *Phytophthora sojae* in selected soybean accessions using RFLP techniques. *Crop Sci.* 38:50-55.
19. Kanazin, V., Marek, L. F., and Shoemaker, R. C. 1996. Resistance gene analogs are conserved and clustered in soybean. *Proc. Natl. Acad. Sci. USA* 93:11746-11750.
20. Lewers, K., Heinz, R., Beard, H., Marek, L., and Matthews, B. 2002. A physical map of a gene-dense region in soybean linkage group A2 near the black seed coat and *Rhg4* loci. *Theor. Appl. Genet.* 104:254-260.
21. Liu, B. 1998. *Statistical Genomics*. CRC Press, NY.
22. Lohnes, D. G., and Schmitthenner, A. F. 1997. Position of the *Phytophthora* resistance gene *Rps7* on the soybean molecular map. *Crop Sci.* 37:555-556.
23. Meyers, B. C., Kaushik, S., and Nandety, R. S. 2005. Evolving disease resistance genes. *Curr. Opin. Plant Biol.* 8:129-134.
24. Palmer, R. G., Pfeiffer, T. W., Buss, G. R., and Kilen, T. C. 2004. Qualitative genetics. Pages 679-763 in: *Soybeans: Improvement, Production, and Uses*. 3rd ed. H. R. Boerma and J. E. Specht, eds. Agron. Monogr. 16. ASA, CSSA, and SSSA, Madison, WI.
25. Polzin, K. M., Lohnes, D. G., Nickell, C. D., and Shoemaker, R. C. 1994. Integration of *Rps2*, *Rmd*, and *Rj2* in linkage group J of the soybean molecular map. *J. Hered.* 85:300-303.
26. Saghai-Marooof, M. A., Soliman, K. M., Jorgensen, R. A., and Allard, R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014-8018.
27. Sandhu, D., Gao, H., Ciano, S., and Battacharyya, M. K. 2004. Deletion of a disease resistance nucleotide-binding-site leucine-rich-repeat-like sequence is associated with the loss of *Phytophthora* resistance gene *Rps4* in soybean. *Genetics* 168:2157-2167.
28. Schmitthenner, A. F. 1985. Problems and progress in control of *Phytophthora* root rot of soybean. *Plant Dis.* 69:362-368.
29. Shoemaker, R. C., Cregan, P. B., and Vodkin, L. O. 2004. Soybean genomics. Pages 235-263 in: *Soybeans: Improvement, Production, and Uses*. 3rd ed. H. R. Boerma and J. E. Specht, eds. Agron. Monogr. 16. ASA, CSSA, and SSSA, Madison, WI.
30. Song, Q. J., Marek, L. F., Shoemaker, R. C., Lark, K. G., Concibido, V. C., Delannay, X., Specht, J. E., and Cregan, P. B. 2004. A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* 109:121-128.
31. Weng, C., Yu, K., Anderson, T. R., and Poysa, V. 2001. Mapping genes conferring resistance to *Phytophthora* root rot of soybean, *Rps1a* and *Rps7*. *J. Hered.* 92:442-446.
32. Yu, Y. G., Buss, G. R., and Saghai-Marooof, M. A. 1996. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. *Proc. Natl. Acad. Sci. USA* 93:11751-11756.