Genetic Analysis of Soybean Plant Introductions with Resistance to *Phytophthora sojae*

S. G. Gordon, S. A. Berry, S. K. St. Martin, and A. E. Dorrance

First, second, and fourth authors: Department of Plant Pathology, The Ohio State University, Wooster, OH 44691; and third author: Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210.

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ABSTRACT

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Phytophthora sojae, which causes Phytophthora root and stem rot of soybean, is a serious disease worldwide and is managed primarily by deploying cultivars with resistance. Thirty-two soybean plant introductions (PIs), all but three of which were from South Korea, were proposed as new sources of single-gene resistance to *P. sojae*. The objective of this study was to characterize the inheritance of resistance to *P. sojae* in these PIs. Twenty-two soybean populations from crosses of these PIs and the susceptible cv. Williams were inoculated with *P. sojae* OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7), and OH25 (vir 1a, 1b, 1c, 1k, 7). These isolates were selected because they are virulent on soybeans with all known *Rps*

genes and many *Rps* gene combinations. Thirteen of the twenty-two populations had consistent segregation responses following inoculations between the two generations. In two PIs, resistance was conferred by two genes to OH17 and three genes to OH25. Resistance to both isolates was conferred by a single gene in PI 398440 although the individual families were not resistant to the same isolates. The data suggest that six of the populations have three-*Rps* gene combinations as previously proposed, while another four may have either a novel *Rps* gene or a four-*Rps* gene combination. Based on this phenotypic analysis, novel and uncharacterized *Rps* genes may be present in this material. More importantly, these PIs may serve as sources of novel *Rps* genes that can be used to more effectively manage Phytophthora root and stem rot.

Additional keywords: *Glycine max*, host resistance.

Phytophthora sojae Kaufmann & Gerdemann (*Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D.C. Erwin) causes root and stem rot of soybean (*Glycine max* L. Merr.), a serious and widespread disease (13). Phytophthora root and stem rot is predominantly managed by deploying single genes that confer resistance to this pathogen. In much of the north central soybean production region that extends from Ohio to Missouri and from North Dakota to Nebraska, *Rps1a*, *Rps1c*, and *Rps1k* are the most common *Rps* genes in commercial cultivars (3,4). Single-gene resistance has provided effective management of Phytophthora root and stem rot, albeit a single deployed *Rps* gene may only be effective for 8 to 15 years depending on the environment (22). Pathotypes of *P. sojae* exist with virulence to those soybeans that have many of the resistance genes deployed in commercial soybean cultivars; these pathotypes have been identified in fields throughout the north central region (1,10,14,15,20,26). *Rps1k* was widely deployed during the early 1980s but only recently has the occurrence of Phytophthora root and stem rot on soybeans with *Rps1k* been identified in commercial fields (1,2,10,14,15,20,26). This indicates that *Rps1k* conferred resistance to *P. sojae* for at least 20 years. As the complexity of *P. sojae* virulence pathotypes increases, new *Rps* genes must be identified, existing *Rps* genes encompassing different loci pyramided together, or *Rps* genes must be deployed in genetic backgrounds with high levels of partial resistance in order to effectively manage loss of soybean yield.

Plant germ plasm collections are sources of both described and uncharacterized resistance genes for a diversity of pathosystems. New sources of resistance genes have been identified through

screening with different races (pathotypes) of a specific pathogen. Buchwaldt et al. (6) identified 12 lentil (*Lens culinaris* [Medik.]) accessions with resistance to *Colletotrichum truncatum* (Schwein.) out of 1,771 evaluated. In an evaluation of 13 spring barley (*Hordeum vulgare* L.) accessions, Brooks et al. (5) identified both known and novel genes for resistance to *Puccinia hordei* G. Oth., the causal agent of leaf rust. The U.S. Department of Agriculture (USDA) Soybean Germplasm Collection is a source of potential new *Rps* genes and sources of high partial resistance to *P. sojae* (11,16,17,19,21). Lohnes et al. (19) evaluated 726 accessions from central China (plant introduction [PI] numbers 567290 to 567781). All 726 of the PIs were susceptible to one or more of the following *P. sojae* races: 1, 7, 17, and 25. Kyle et al. (16) evaluated 628 accessions from southern China and found 36 accessions that were resistant to all 10 races of *P. sojae* tested. Kyle et al. (16) also identified three provinces from which a high percentage of resistant accessions originated and concluded that *P. sojae* resistance in China is extremely diverse. In another study, four Japanese PIs were found to harbor multiple known *Rps* genes including *Rps1a*, 1b, 1c, and 3 as well as a unique *Rps* allele, designated *Rps?* based on responses of segregating populations to 19 different races of *P. sojae* (21).

Thirty-two accessions, all but three of which are from South Korea, were identified in a previous study as possible new sources of *Rps* genes (11). In that study, *P. sojae* isolates with specific virulence pathotypes were chosen for inoculations to assay for all described *Rps* genes and most two- and three-gene combinations of *Rps* genes. The 32 PIs identified were resistant to three *P. sojae* isolates with the following virulence pathotypes: OH1 (vir 7), OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7), and OH25 (vir 1a, 1b, 1c, 1k, 7). Collectively, these isolates cause susceptible interactions with all previously identified *Rps* genes except *Rps8*. In addition, the 32 PIs were resistant to five *P. sojae* isolates with

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

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virulence pathotypes chosen to elicit a susceptible response from soybeans with specific two- and three-*Rps* gene combinations. However, following inoculation with these five isolates, soybeans with the following three-gene combinations—*Rps1c*, *Rps3a*, *Rps4*; *Rps1c*, *Rps3b*, *Rps4*; *Rps1c*, *Rps2*, *Rps3a*; and *Rps1c*, *Rps2*, *Rps3b*—still exhibited a resistant response (11). Therefore, these 32 PIs either have one of the above three-*Rps* gene combinations, a novel *Rps* gene, or a novel *Rps* gene in combination with a described *Rps* gene. One novel gene, *Rps8*, has already been described from among these 32 PIs (7).

Specific races or pathotypes have been used in other host-pathogen systems to identify the number and expression of resistance genes in a given host. Different pathotypes of *Pyrenophora tritici-repentis* (Died.) Drechs., were used to characterize resistance genes in wheat (*Triticum aestivum* L.) (24). Based on a phenotypic analysis, the same recessive gene that controlled resistance to necrosis was identified in all seven cultivars (24). Therefore, it should also be possible to determine how many *Rps* genes are segregating in soybean populations, and which may have novel genes or combination of genes, based on a phenotypic analysis of F_{2:3} and F_{2:4} families with well characterized pathotypes (races) of *P. sojae*.

The hypothesis of this study is that selecting a combination of isolates with specific virulence pathotypes to soybeans with all known *Rps* genes should expedite the process of identifying novel genes in PIs with multiple *Rps* genes. *Rps8* is considered a novel gene in this study, primarily due to its recent discovery (7). Much of this research was completed prior to and during the time the gene was characterized.

Table 1 briefly outlines expected outcomes of F_{2:3} and F_{2:4} populations following inoculation with three specific pathotypes of *P. sojae*. For example, if a population is segregating for a novel single gene, then following inoculation with race 1 (vir *Rps7*), an F_{2:3} population should segregate 3 resistant:1 susceptible. If there's one novel gene in this PI parent that confers resistance to all *P. sojae* races used, then all of the F_{2:3} families would have the same response to all *P. sojae* isolates. If the F_{2:3} families did not have the same response, then there exists the possibility of uncharacterized virulence pathotypes within the isolates to the *Rps* genes in this PI.

The scenario is different for PIs where multiple *Rps* genes are present. If two *Rps* genes are present in the PI, assuming one of

the genes is not *Rps7*, then after inoculation with race 1 a 15:1 segregation ratio in F₂ plants is expected. *P. sojae* isolate OH17 has virulence to soybeans with *Rps1b*, 1d, 2, 3a, 3b, 3c, 4, 5, 6, and 7, and OH25 has virulence to soybeans with *Rps1a*, 1b, 1c, 1k, and 7. In a population with a combination of two novel genes, a 15:1 segregation ratio is expected to both isolates. If there is a novel gene present in combination with one known *Rps* gene, then one expects a 15:1 ratio to the isolate that lacked virulence to the soybeans with the known *Rps* gene and a 3:1 ratio for reaction to the isolate which has virulence to soybeans with the known *Rps* gene.

Based on a previous study (11) there is also the possibility of four three-gene combinations in these PIs: *Rps1c*, *Rps3a*, *Rps4*; *Rps1c*, *Rps3b*, *Rps4*; *Rps1c*, *Rps2*, *Rps3a*; and *Rps1c*, *Rps2*, *Rps3b*. *P. sojae* isolate OH25 has virulence to soybeans with *Rps1c* and OH17 has virulence to soybeans with *Rps2*, *Rps3a*, *Rps3b*, and *Rps4*. In these cases, following inoculation with OH17, a 3:1 ratio is expected where *Rps1c* is segregating. Conversely, in the same population, following inoculations with OH25, a 15:1 ratio is expected for soybeans with the combinations of *Rps3a* and *Rps4*; *Rps3b* and 4; *Rps2* and 3a; and *Rps2* and 3b.

The objectives of this study were to determine the number of *Rps* genes in these PIs by following segregation ratios in soybean populations derived from crosses between these resistant PIs and the susceptible cv. Williams, and based on this phenotypic analysis, determine which of these PIs may have a novel gene.

MATERIALS AND METHODS

Plant material. Soybean PIs and the cv. Williams were obtained from the USDA Soybean Germplasm Collection in Urbana, IL. The PIs used in this study were first characterized by Dorrance and Schmitthenner (11) as resistant to eight *P. sojae* races, including 1, 17, and 25. Most of the PIs listed in Table 2 originated from South Korea. The crosses used to evaluate the PIs were Williams (*rps/rps*) × PI (*Rps* unknown). Williams was the seed parent for all crosses, except those with PIs 398440 and 424247A, in which it was the pollen parent. Plants derived from accidental self-fertilization were distinguished from F₁ hybrids based on flower color and plant type in the F₁. The F₁ plants from each of these crosses were allowed to self-pollinate to produce

TABLE 1. Expected ratios for F_{2:3} families segregating for resistance following inoculation with isolates of *Phytophthora sojae* with specific virulence pathotypes

Number and type of <i>Rps</i> gene in plant introduction (PI)	OH25 (1a,1b,1c,1k,7)	OH17 (1b,1d,2,3a,3b,3c,4,5,6,7)	Comments	Additional inoculations
No unidentified virulence				
One novel gene	3:1	3:1	All families should be the same.	None
Two novel genes	15:1	15:1	All families should be the same.	None
One novel plus known gene (S to OH25)	3:1	15:1	Families S to OH1 and OH17 should be the same.	Additional inoculations with isolates with virulence to 1a, 1b, or 1c can be used to identify which known <i>Rps</i> allele is present.
One novel plus known S to OH 17	15:1	3:1	Families S to OH1 and OH25 should be the same and those should also be S to OH17.	Additional inoculations with isolates can be used to identify known <i>Rps</i> gene.
1c, 3a, 4 1c, 3b, 4 1c, 2, 3a 1c, 2, 3b	15:1	3:1	Family that is S to OH1 should be S to all races. Families S to OH25 and OH17 may be different.	Inoculate with an isolate with 1c, 3a, or 1c, 3b virulence to confirm. If true, should be 3:1.
Undescribed virulence				
One novel gene	3:1	3:1	PI would not have been S to isolates originally.	
Two genes	3:1	3:1	Susceptible families are different among the isolates.	Additional isolates may give similar or different patterns.
1 is	15:1	3:1		
undescribed	3:1	15:1		

populations of approximately 100 to 300 F₂. The F₂ plants were selfed and each plant was threshed individually to yield F_{2:3} seed. These F_{2:3} families were used for inoculations with the *P. sojae* isolates. Approximately 20 to 70 remnant F_{2:3} seeds per family from all populations (Table 3) were planted and increased. F₄ seeds were bulk-harvested from each family, from 40 to 50 F₃ plants, and F_{2:4} seedlings were used for inoculations as described below.

***P. sojae* inoculations.** To determine how many genes were present in these PIs, three isolates of *P. sojae* were used in this study; all were maintained at the Department of Plant Pathology, Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, and were collected in Ohio from soybeans with *Phytophthora* stem rot. These isolates were shared with all of the land grant universities in the north central region in September 2002 as well as being deposited in the National Soybean Pathogen Collection Center at the University of Illinois, Urbana, IL. Initially, *P. sojae* race 1 isolate OH1 (vir 7) was used to inoculate 100 7-day-old F₂ seedlings to determine the number of *Rps* genes in the PI. *P. sojae* isolates OH25 (vir 1a, 1b, 1c, 1k, 7) and OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) were used to inoculate 10 to 20 seedlings from each F_{2:3} family. For each population, between 35 and 277 families were evaluated, depending on the number of seeds available per family and the size of the population (Table 2). Fifteen to twenty-five F_{2:4} seedlings were inoculated (Table 3).

The seedlings were inoculated using a variation of the hypocotyl inoculation technique (7,23). Briefly, seeds were placed between germination paper wetted with water, rolled up, and stored in the dark in plastic containers on top of wire mesh. Seven days later, the germination papers were unrolled and the seedlings were inoculated. A 7-day-old culture of *P. sojae* grown on lima bean (*Phaseolus lunatus* L.) agar (50 g of lima beans, 12 g of agar per liter) was cut and placed in a hypodermic syringe and then the agar was forced through the syringe to create a slurry. The slurry was placed back into the syringe and seedlings were inoculated by scratching the hypocotyl with the needle of the hypodermic syringe and placing 0.2 to 0.4 ml of the agar/mycelium mixture onto the wound. Following inoculation,

the towels were re-rolled and placed back into plastic containers as before. Seven days after inoculation the seedlings were scored for disease reactions. Individual F₂ seedlings were scored as either resistant (R) or susceptible (S). Individuals with a brown expanding lesion were scored as S and those with a necrotic lesion restricted to the inoculation site were scored as R. The F_{2:3} and F_{2:4} families were scored based on the percentage of seedlings with susceptible reactions. Families in which 0 to 20%, 21 to 79%, or 80 to 100% of the seedlings developed lesions were designated as R, heterozygous (H), or S, respectively. The segregation ratios represented the F₂ plant genotype, and for purposes of calculating chi-square statistics and maximum likelihoods, the R and H classes were combined.

Differential checks were used in each test to ensure that the isolates were consistent in their reaction to corresponding pathotypes. The differentials used in this study were 'Williams' (universal susceptible); 'Harlon' (*Rps1a*), 'Harosoy 13XX' (*Rps1b*), 'Williams 79' (*Rps1c*); PI103091 (*Rps1d*); 'Williams 82' (*Rps1k*); L76-1988 (*Rps2*); L83-570 (*Rps3*); PRX 146-36 (*Rps3b*); PRX 145-48 (*Rps3c*); L85-2352 (*Rps4*); L85-3059 (*Rps5*); and 'Harosoy 62XX' (*Rps6*) and 'Harosoy' (*Rps7*).

Statistical analysis. To estimate the number of resistance genes in each of these PIs, segregation data for response to both isolates were used to calculate the likelihood (27) for models with one, two, three, or four resistance genes for each population. The model equation for a single-gene model (0.75 R and 0.25 S) was $(R + H) \times \log(0.75) + S \times \log(0.25)$, where R = the frequency of the resistant class, H = that of the heterozygous class, and S = that of the susceptible class. The two-, three-, and four-gene values were computed using the appropriate segregation proportions of 15/16 and 1/16, 63/64 and 1/64, and 255/256 and 1/256, respectively. Where the model incorporated more than one locus, the assumption was of independently segregating loci with duplicate (or triplicate or quadruplicate) gene action (epistasis). These expected proportions were used to obtain the common logarithm of the likelihood function for each model. The model with the maximum likelihood was selected and considered significantly ($P < 0.01$) better than the other models if its log likelihood had a smaller negative value than the others by two or

TABLE 2. Results of maximum likelihood and chi-square (χ^2) analyses of soybean F_{2:3} families to determine number of genes conferring resistance to *Phytophthora sojae* OH17 (1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) in plant introductions (PI) crossed to the susceptible cv. Williams

PI	F _{2:3} family reactions ^a for <i>P. sojae</i> OH17							χ^2	P
	R	H	S	Log L1 ^b	Log L2	Log L3	Ratio		
273483D	35	37	26	-47.9	-55.0	-69.8	3:1	0.12	0.73
274456	52	67	35	-72.5	-81.0	-101.8	3:1	0.42	0.51
398295	47	64	41	-72.2	-85.5	-110.3	3:1	0.32	0.57
398440	28	43	23	-43.6	-50.7	-65.0	3:1	0.01	0.91
398643	33	44	23	-47.0	-52.8	-66.6	3:1	0.21	0.64
398666	21	44	29	-43.3	-55.7	-74.6	3:1	1.72	0.19
398693	42	68	25	-60.8	-65.7	-81.8	3:1	3.03	0.08
398694	27	46	28	-47.0	-57.3	-75.0	3:1	0.40	0.53
398697	33	61	40	-62.3	-78.4	-104.0	3:1	1.68	0.19
399036	28	55	27	-49.7	-59.1	-76.9	3:1	0.01	0.91
399079	83	95	19	-90.0	-81.3	-91.2	15:1	3.87	0.05
404159	41	29	18	-44.3	-45.1	-53.6	3:1	0.97	0.32
407861C	58	57	24	-66.5	-66.9	-79.4	3:1	4.4	0.04
407974B	25	53	22	-44.3	-51.4	-66.4	3:1	0.48	0.49
408029	22	52	19	-40.3	-46.4	-59.9	3:1	1.04	0.31
408097	22	38	31	-43.3	-56.7	-76.1	3:1	3.99	0.05
408211B	36	32	17	-41.5	-43.0	-51.8	3:1	1.13	0.29
408319C	16	38	22	-34.3	-43.7	-58.4	3:1	0.63	0.43
416840	22	35	21	-36.4	-43.7	-56.9	3:1	0.15	0.69
417229	32	49	25	-49.1	-56.3	-71.9	3:1	0.11	0.74
424247A	74	132	71	-127.0	-151.8	-197.5	3:1	0.06	0.81
424354	51	66	12	-57.8	-52.6	-59.6	15:1	2.05	0.15

^a Families were classified as R (homozygous resistant) if 0 to 20% developed susceptible lesions, H (heterozygous or segregating) if 21 to 79% developed susceptible lesions, and S (homozygous susceptible) if 80 to 100% developed susceptible lesions.

^b Log Ln refers to the logarithm of the likelihood associated with a model positing n segregating *Rps* genes. L1 = one gene, L2 = two genes, and L3 = three genes.

more units, corresponding to a likelihood ratio of 100 or more (Tables 2 through 5).

Chi-square analyses were also performed using SAS (version 9.1, SAS Institute, Cary, NC) to test for goodness of fit to the gene model of one, two, or three genes that was most likely. For chi-square analysis, the $F_{2:3}$ and $F_{2:4}$ data were pooled to reconstitute an F_2 genotype for analysis.

RESULTS

F_2 populations. One hundred F_2 plants from 17 of the 22 PI $\times$ Williams crosses were inoculated with OH1 for a preliminary analysis. Populations PI 273483D, 274456, 398440, 398643, 398666, 398693, 398694, 398697, 399036, 407974B, 408029, 408097, 408211B, 408319C, 416840, 417229, and 424247A did not have segregation ratios consistent with a single *Rps* gene as confirmed by chi-square analyses (data not shown). The number of susceptible plants ranged from 0 to 14 for all populations. While the number of plants inoculated was too small to completely distinguish between two and three *Rps* genes, these results indicate that each of these PIs have more than one independent *Rps* locus. For all inoculations the differential checks responded as expected (data not shown).

$F_{2:3}$ populations. Data for the resistance response were collected from 22 PI populations following inoculation with *P. sojae* OH17 and OH25 (Tables 2 and 3). In all populations, resistance to *P. sojae* was inherited as a dominant trait (Tables 2 and 3). For PI398440, resistance to both OH17 and OH25 was conferred by a single gene, based on maximum likelihood and chi-square analyses (Tables 2 and 3). However, some individual $F_{2:3}$ and $F_{2:4}$ families were homozygous resistant to OH17 and homozygous susceptible to OH25, while others were homozygous resistant to OH25 and homozygous susceptible to OH17. This indicates that PI398440 has two *Rps* genes: one that confers resistance to *P. sojae* OH17, and a second that confers resistance to *P. sojae* OH25.

Resistance to *P. sojae* OH17 was conferred by a single gene in 18 populations (Table 2) and resistance to *P. sojae* OH25 was conferred by two or three genes in 8 and 11 populations (Table 3),

respectively. For two of the populations resistant to OH17, the chi-square value *P* value was less than or equal to 0.05 (Table 2). Similarly, the chi-square *P* value for two genes conferring resistance to OH25 was less than 0.05 in two populations. Results for eight populations with resistance conferred by one gene to *P. sojae* OH17 and two genes to OH25 indicate that these populations may have one of the four three-gene combinations proposed in a previous study (11). Many of the $F_{2:3}$ populations resistant to *P. sojae* OH25 had no or very few (<3) homozygous susceptible families.

Resistance to *P. sojae* OH17 expressed in PIs 399079 and 424354 was conferred by two genes, and resistance to OH25 by two or three genes, based on chi-square and maximum likelihood analyses. *P. sojae* OH17 has virulence to soybeans with genes that exist at all *Rps* loci except *Rps8*; thus, resistance conferred by two genes is a strong indication of a novel gene.

$F_{2:4}$ populations. Twenty-two $F_{2:4}$ populations were inoculated with OH17 and OH25, respectively (Tables 4 and 5). Results from the maximum likelihood and chi-square analyses indicate that all but two of the populations (PI408029 and 417229) had consistent segregation ratios for *P. sojae* OH17 between the $F_{2:3}$ and $F_{2:4}$ generations (Tables 2 and 4). For these two PIs, there was not a good fit to a single gene segregation ratio in the $F_{2:4}$ generation (Table 4). In addition, four additional populations, 398295, 398697, 408097, and 424354 had the same 3:1 segregation ratios, but the chi-square *P* values were ≤ 0.05 and were primarily skewed toward the resistant and heterozygous classes. For *P. sojae* OH25 (Tables 3 and 5), 16 populations segregated in the same ratio as their respective $F_{2:3}$ counterparts, confirming those results. PI populations 274456, 398666, 398693, 398697, 408029, and 408319C had different segregation ratios between the $F_{2:3}$ and $F_{2:4}$ populations. For PI398666, 90% or more of the plants developed lesions in seven of the families in the susceptible class of the $F_{2:3}$, while in the corresponding $F_{2:4}$ families only 30 to 50% of the plants developed lesions following inoculation, thus classifying them as heterozygous. For PI398693, the discrepancy between generations may be that fewer families were evaluated in the $F_{2:3}$ compared with the $F_{2:4}$ generation (35 and 56 families, respectively) (Tables 3 and 5). For example, in 398693, 0 of the

TABLE 3. Results of maximum likelihood and chi-square (χ^2) analyses of soybean $F_{2:3}$ families to determine number of genes conferring resistance to *Phytophthora sojae* OH25 (vir *Rps1a*, 1b, 1c, 1k, 7) in plant introductions (PI) crossed to the susceptible cv. Williams

PI	$F_{2:3}$ family ^a reactions for <i>P. sojae</i> OH25							χ^2	<i>P</i>
	R	H	S	Log L1 ^b	Log L2	Log L3	Ratio		
273483D	24	22	4	-23.5	-20.1	-21.5	15:1	0.26	0.61
274456	77	54	16	-72.2	-63.2	-68.3	15:1	5.39	0.02
398295	42	55	2	-43.0	-34.0	-35.1	63:1	0.135	0.71
398440	32	35	14	-38.2	-38.9	-46.6	3:1	2.57	0.11
398643	29	11	1	-21.4	-14.9	-13.0	15:1	1.02	0.31
398666	26	54	11	-38.5	-38.8	-47.2	15:1	5.29	0.02
398693	95	1	0	-57.5	-34.4	-23.0	63:1	1.52	0.22
398694	16	30	3	-20.5	-18.4	-21.0	15:1	0.001	0.98
398697	60	16	5	-44.0	-32.4	-29.6	15:1	0.001	0.98
399036	38	12	0	-26.5	-17.3	-13.7	63:1	0.79	0.37
399079	98	40	2	-72.2	-49.6	-42.6	63:1	0.02	0.90
404159	65	22	0	-45.8	-30.0	-24.1	63:1	1.38	0.24
407861C	95	46	2	-72.2	-50.4	-44.2	63:1	0.03	0.87
407974B	37	57	6	-43.0	-37.7	-41.9	15:1	0.01	0.92
408029	20	14	0	-16.3	-11.4	-10.2	63:1	0.54	0.46
408097	52	37	5	-45.5	-35.8	-35.9	15:1	0.14	0.71
408211B	33	33	0	-29.8	-21.8	-20.8	63:1	1.05	0.46
408319C	55	32	0	-42.7	-29.4	-25.6	63:1	1.38	0.24
416840	76	44	5	-62.0	-46.6	-44.3	15:1	1.08	0.30
417229	45	55	2	-44.9	-35.1	-35.8	15:1	3.20	0.07
424247A	108	105	3	-98.4	-74.0	-72.2	63:1	0.04	0.84
424354	56	49	1	-49.1	-36.1	-34.3	63:1	0.26	0.61

^a Families were classified as R (homozygous resistant) if 0 to 20% developed susceptible lesions, H (heterozygous or segregating) if 21 to 79% developed susceptible lesions, and S (homozygous susceptible) if 80 to 100% developed susceptible lesions.

^b Log Ln refers to the logarithm of the likelihood associated with a model positing *n* segregating *Rps* genes. L1 = one gene, L2 = two genes, and L3 = three genes.

TABLE 4. Results of maximum likelihood and chi-square (χ^2) analyses of soybean $F_{2:4}$ families to determine number of genes conferring resistance to *Phytophthora sojae* OH17 (vir *Rps*1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) in plant introductions (PI) crossed to the susceptible cv. Williams

PI	$F_{2:4}$ family ^a reactions for <i>P. sojae</i> OH17							χ^2	<i>P</i> value
	R	H	S	Log L1 ^b	Log L2	Log L3	Ratio		
273483D	31	54	25	-50.0	-57.5	-73.7	3:1	0.30	0.58
274456	25	62	22	-47.0	-54.1	-69.9	3:1	1.35	0.25
398295	33	94	23	-62.0	-67.8	-86.2	3:1	7.48	0.006
398440	24	48	22	-42.1	-49.6	-64.2	3:1	0.13	0.72
398643	30	61	36	-58.1	-72.5	-96.0	3:1	0.76	0.38
398666	14	41	24	-35.2	-46.3	-62.7	3:1	1.22	0.27
398693	25	26	23	-36.7	-44.5	-57.7	3:1	1.46	0.23
398694	35	53	24	-51.5	-57.4	-72.4	3:1	0.76	0.38
398697	42	34	14	-17.9	-19.0	-25.8	3:1	4.28	0.04
399036	28	52	31	-51.2	-63.0	-83.0	3:1	0.51	0.48
399079	39	40	6	-13.5	-9.4	-11.4	15:1	0.09	0.76
404159	41	33	20	-46.7	-48.7	-58.8	3:1	0.70	0.40
407861C	33	36	15	-39.7	-40.7	-49.0	3:1	2.29	0.13
407974B	23	50	17	-19.4	-22.5	-31.2	3:1	1.79	0.18
408029	36	44	10	-16.1	-15.4	-20.4	15:1	5.29	0.02
408097	36	31	12	-15.6	-16.3	-22.1	3:1	4.05	0.05
408211B	17	50	23	-39.1	-48.8	-65.1	3:1	0.02	0.90
408319C	20	56	24	-43.3	-52.9	-70.0	3:1	0.05	0.82
416840	6	25	10	-17.2	-21.7	-29.3	3:1	0.01	0.93
417229	55	44	14	-20.8	-19.6	-26.0	15:1	7.27	0.01
424247A	26	46	18	-19.8	-23.7	-33.0	3:1	1.20	0.27
424354	68	48	14	-63.8	-55.7	-60.2	15:1	4.53	0.03

^a Families were classified as R (homozygous resistant) if 0 to 20% developed susceptible lesions, H (heterozygous or segregating) if 21 to 79% developed susceptible lesions, and S (homozygous susceptible) if 80 to 100% developed susceptible lesions.

^b Log Ln refers to the logarithm of the likelihood associated with a model positing n segregating *Rps* genes. L1 = one gene, L2 = two genes, and L3 = three genes.

TABLE 5. Results of maximum likelihood and chi-square (χ^2) analyses of soybean $F_{2:4}$ families to determine number of genes conferring resistance to *Phytophthora sojae* OH25 (vir 1a, 1b, 1c, 1k, 7) in plant introductions (PI) crossed to the susceptible cv. Williams

PI	$F_{2:4}$ family ^a reactions for <i>P. sojae</i> OH25							χ^2	<i>P</i> value
	R	H	S	Log L1 ^b	Log L2	Log L3	Ratio		
273483D	27	80	3	-15.2	-6.6	-6.2	15:1	2.33	0.13
274456	79	29	1	-14.1	-4.2	-2.5	63:1	0.29	0.59
398295	27	18	0	-5.6	-1.3	-0.3	63:1	0.71	0.41
398440	24	49	15	-18.2	-20.1	-27.6	3:1	2.97	0.08
398643	45	45	6	-14.9	-9.7	-11.5	15:1	0.0	1.0
398666	35	42	0	-9.6	-2.2	-0.5	63:1	1.22	0.27
398693	36	44	17	-20.2	-22.7	-31.3	3:1	2.89	0.09
398694	46	62	6	-17.1	-10.3	-11.6	15:1	0.19	0.66
398697	61	32	0	-11.6	-2.6	-0.6	63:1	1.48	0.22
399036	64	35	3	-50.9	-37.1	-34.3	63:1	1.23	0.26
399079	54	28	1	-10.8	-3.5	-2.4	63:1	0.07	0.79
404159	64	25	0	-46.1	-30.5	-25.0	63:1	1.41	0.23
407861C	57	30	2	-44.6	-31.9	-28.9	63:1	0.27	0.60
407974B	48	32	1	-10.6	-3.4	-2.4	15:1	3.48	0.06
408029	48	40	5	-14.0	-8.5	-9.6	15:1	0.12	0.73
408097	59	24	3	-12.2	-5.9	-6.0	15:1	1.36	0.24
408211B	39	46	1	-11.2	-3.6	-2.4	63:1	0.09	0.77
408319C	42	47	8	-15.9	-12.1	-15.1	15:1	0.66	0.41
416840	18	17	2	-5.6	-3.4	-3.9	15:1	0.04	0.83
417229	66	44	4	-16.2	-7.9	-8.0	15:1	1.46	0.23
424247A	53	33	3	-12.6	-6.0	-6.0	63:1	1.89	0.17
424354	75	33	2	-14.7	-5.4	-4.4	63:1	0.05	0.83

^a Families were classified as R (homozygous resistant) if 0 to 20% developed susceptible lesions, H (heterozygous or segregating) if 21 to 79% developed susceptible lesions, and S (homozygous susceptible) if 80 to 100% developed susceptible lesions.

^b Log Ln refers to the logarithm of the likelihood associated with a model positing n segregating *Rps* genes. L1 = one gene, L2 = two genes, and L3 = three genes.

35 families were homozygous susceptible in the $F_{2:3}$ compared with two homozygous susceptible families out of the 56 evaluated in the $F_{2:4}$.

Based on the best fit to both the maximum likelihood and chi-square analyses, the putative number of *Rps* genes is summarized in Table 6. Novel genes or *Rps*8 may exist in 7 of the 22 PIs evaluated in this study. However, many of these are in combinations with known genes, and the potential for pre-existing virulence may also exist. In addition, PIs with the three known gene combinations of *Rps*1c and *Rps*2, 3a, 3b, or 4 were also identified.

DISCUSSION

In soybeans, 14 *Rps* genes have been described across eight loci (13). In this study, two *P. sojae* isolates with complex pathotypes and virulence to soybeans with all of the described *Rps* genes were selected in order to identify novel genes. One of the hypotheses developed from an earlier study (11) was that these PIs harbor three *Rps*-gene combinations. *Rps*1c confers resistance to *P. sojae* OH17, while *Rps* genes 2, 3a, 3b, and 4 confer resistance to OH25. For six of the PIs in this study, a 3:1 ratio to OH17 and a 15:1 ratio for OH25 was found.

TABLE 6. Putative *Rps* gene models for combinations of *Rps* genes and the response of 13 F_{2:3} and F_{2:4} segregating soybean populations for resistance to *Phytophthora sojae* isolates OH17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and OH25 (vir 1a, 1b, 1c, 1k, 7)

<i>Rps</i> gene model ^a	Segregation ratio (R:S ^b) <i>P. sojae</i> isolate		Plant introduction (PI) populations that fit the proposed gene model
	OH17	OH25	
One novel gene	3:1	3:1	None (PI 399073, [7])
Two novel	15:1	63:1	399079, 424354
One novel plus known gene S to OH25	15:1	3:1	None
1b plus novel gene	3:1	3:1	None
<i>Rps</i> 1c, 3a, 4	3:1	15:1	273483D, 398643, 398694, 407974B, 408097, 416840
<i>Rps</i> 1c, 3b, 4			
<i>Rps</i> 1c, 2, 3a			
<i>Rps</i> 1c, 2, 3b			
<i>Rps</i> 1c, 2, 3 (a or b), 4, or novel plus known	3:1	63:1	399036, 404159, 408211B, 424247A
Two novel ^c	3:1	3:1	398440

^a *Rps*8 is considered a novel gene in this study primarily due to its recent discovery.

^b R:S indicates ratio of the number of families with resistant/susceptible response following inoculation.

^c May be due to undescribed virulence, since the families did not respond similarly to both isolates.

This is consistent with the hypothesis that these PIs contain three independent *Rps* loci. In order to determine exactly which *Rps* loci these PIs contain, crosses to differentials containing characterized single *Rps* genes are required. In order to accurately classify each PI in this manner, each of these populations that fit the three-gene hypothesis must be crossed to at least three different isolines. This would result in a total of 18 very large populations with three to four segregating *Rps* genes that could be used to characterize the response to the appropriate *P. sojae* isolates. In addition, results for the four populations in which three genes confer resistance to OH25 and one gene confers resistance to OH17 could indicate the presence of novel loci or of a four-*Rps* gene combination of *Rps*1c, 2, 3 (a or c), and 4. These four PIs originate from three different regions in South Korea and one in Georgia, Russia.

In addition, based on a genetic diversity analysis of this germ plasm, the PIs from each province clustered separately (8). Thus, it would seem unlikely that this diverse group of soybean germ plasm would all contain the same four-*Rps* gene combination. Another possibility is that the PIs contain novel, uncharacterized *Rps* alleles at these loci (11). Two PIs in this study have potentially novel *Rps* loci or genes, including *Rps*8, based on segregation ratios of their F₂-derived progeny. These include 399079 and 424354 based on their 15:1 segregation for resistance to *P. sojae* OH17 and 63:1 to OH25. *P. sojae* OH17 is virulent to soybeans with all *Rps* genes except for *Rps*8 and three alleles at a single locus: *Rps*1a, 1c, and 1k. For two independent loci to be segregating in these populations, one of the loci must be novel or a novel allele of a known locus.

One of the populations, PI398440, segregated for a single gene to both OH17 and OH25 in both the F_{2:3} and F_{2:4} populations. Since *P. sojae* isolates OH17 and OH25 together have virulence to all known *Rps* genes, it is likely that PI398440 contains a novel gene (Table 4). However, since the families did not respond the same to these two isolates, undescribed virulence corresponding to novel *Rps* genes may be present. If unidentified virulences are present in these isolates to undescribed *Rps* genes, there are two questions. First and foremost, are these *Rps* genes worth pursuing for incorporation and deployment in new germ plasm? The answer is probably “no” since populations of *P. sojae* may already exist that are virulent to the new genes. This is not an unusual finding. *P. sojae* isolates that had a susceptible interaction with Harosoy were widespread, as this cultivar was used as the susceptible check long before *Rps*7 was actually identified. It was not until *P. sojae* isolates were identified that had a resistant reaction on Harosoy that this resistance gene was discovered (9). The second question, then, is “are these *Rps* genes at novel loci or are they alleles of previously described loci?” A molecular

analysis which maps the resistance genes in each of these populations can aid in addressing this question (12).

This subset of South Korean soybean PIs has great potential for novel *Rps* genes to deploy in order to continue to effectively manage *Phytophthora* root and stem rot. Vavilov (25) proposed that sources of resistance to highly specialized plant pathogens are often found in regions of the greatest differentiation within the host species. In theory, both host and pathogen have co-evolved in these regions, and this protracted association has led to diversity. The mountainous regions of central and western China, together with the adjacent lowlands, were first proposed as the center of origin for soybeans (25). Secondary and tertiary centers have also been described that include a much larger geographic region encompassing almost all of China and North and South Korea (18,25). The presence of multiple *Rps* genes, which confer different responses to *P. sojae* as well as the high degree of partial resistance in many of these PIs previously identified (11), suggests that this region is a center for *P. sojae*–soybean interactions.

It will be necessary to continue to identify novel *Rps* genes as seed companies and universities continue to deploy resistant commercial cultivars and germ plasm, and as *P. sojae* populations continue to adapt to these genes and to increase in diversity and complexity. Deployment of single *Rps* genes has been a very effective means to manage *Phytophthora* root and stem rot. Genetic mapping in both the F_{2:3} and F_{2:4} populations of these PIs is in progress as is the development of additional populations for allelism studies and germ plasm for future use in deployment of soybean cultivars with novel resistance to *P. sojae*.

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