Inheritance of Resistance to *Phytophthora sojae* in the Soybean PI 567574A

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Abstract

Phytophthora root rot of soybean can cause heavy crop losses. Monogenic race-specific resistance to the causal organism, *Phytophthora sojae*, is controlled by 13 dominant alleles at seven loci in soybean. Soybean germplasm lines have been identified with new race resistance patterns. These lines need to be characterized to determine the inheritance of that resistance and compared to known sources of resistance. The objective of this study was to characterize the inheritance of resistance to *P. sojae* in PI 567574A. Results indicate that there are two alleles at independent loci that control resistance in this PI. Allelism studies indicated that the two alleles are *Rps1c* and *Rps3a*.

Introduction

Phytophthora root rot of soybean [*Glycine max* (L.) Merr.], caused by *Phytophthora sojae* (Kaufmann and Gerdemann, 1958), is a destructive disease, causing an estimated yield loss of 560,300 metric tons in 1994 in the United States alone (Wrather et al., 1997). Monogenic race-specific resistance to *P. sojae* has been identified, with 13 identified resistance alleles at seven loci. None of these alleles confer resistance to all known races of *P. sojae*. *Rps1k* confers resistance to the greatest number of races and has been the most widely used allele (Schmitthenner et al., 1994).

Recent investigations in the soybean germplasm collection identified PI 567574A as being resistant to races 1, 3, 4, 5, 7, 10, 12, 13, 17, 25, 28, and 30 and susceptible to race 20 of *P. sojae* after repeated hypocotyl inoculations during 1997, 1998, and from data compiled by Kyle (1997) and Lohnes et al. (1996) (Table 1). The objective of this study was to determine the inheritance of resistance to *P. sojae* in the soybean PI 567574A.

Materials and Methods

Crosses between PI 567574A and selected Rps isolines of 'Williams' (Bernard and Lindahl, 1972) and 'Harosoy' (Weiss and Stevenson, 1955) were made in 1995. F1 plants from these crosses were grown in the field in 1996 for maximum seed production. Seed from each F1 plant was harvested and stored individually. Two hundred F2 seeds from

two F1 plants of each cross were planted in two 3 m rows in the field in 1997 for selection of F2 derived F3 (F2:3) families, while the remaining F2 seed was stored for greenhouse evaluation. Three separate populations, each from a different F1 plant, of the cross PI 567574A x Harosoy were planted to develop F2:3 families. Plants were harvested individually from all populations planted in the field to form the F2:3 families. All seeds were stored in the cold room (4 degrees C) until being planted in the greenhouse for phenotypic evaluation.

Phytophthora sojae races 1, 5, 7, 25, and 30 were used for screening of the populations in the greenhouse. Races 5 and 25 were available at the University of Illinois and were originally obtained from A.F. Schmitthenner (Dept. of Plant Path. Ohio St. Univ.) (Kyle, 1997). Races 1, 7, and 30 were obtained from G.L. Hartman (USDA-ARS, Urbana, Illinois). The three populations of the cross PI 567574A x Harosoy were evaluated with five different races: population #1 with race 1, population #2 with race 25, and population #3 with races 7 and 30, and a subset of 10 F2:3 families was then evaluated with race 5.

All evaluations were conducted in the greenhouse. The differential set of Williams Rps isolines (Bernard and Nelson, 1996; Bernard et al., 1991) (see Table 1) were planted as checks in the greenhouse to confirm the reaction of each race. Eight pots of each parent, 100 F2 individuals, and at least 50 F2:3 families with 60 seeds each of each population were also planted in the greenhouse. All lines were planted at a rate of 10 seeds per pot in 10 cm pots filled with sand. Zoospores were produced according to Moots et al. (1983), with five small pieces of agar (instead of four) with mycelium being transferred to each petri plate (100 x15 mm) containing 8 ml of lima bean agar and then incubated in the dark for 4 days at 25 degrees C (instead of 21 degrees C). The zoospore suspension was inoculated onto 8 to 10 day old plants using the hypocotyl inoculation method with zoospores (Moots et al., 1983; Schwenk et al., 1979). Plants were evaluated 4 days after inoculation for their reaction to *P. sojae* by counting the number of dead and living plants within each pot. Ratios of resistant (R) to susceptible (S) plants within the F2 population and within and among F2:3 families were tabulated and compared using Chi-square goodness-of-fit to the appropriate genetic models.

Results and Discussion

Populations from the crosses of PI 567574A with Williams and Harosoy were evaluated with race 1 to determine the number of genes for resistance in PI 567574A. The segregation of F2 plants from the cross PI 567574A x Williams met the expectations of a 15 R to 1 S segregation ratio, indicating that there are two genes for resistance to race 1 (Table 2). The F2:3 family segregation ratio confirmed the two gene model for this population. Since there were only 60 individuals in each family, it was difficult to distinguish between R families and those segregating 15 R to 1 S. Those classes were combined and the segregation pattern fit a 11:4:1 ratio. The data for the F3 plants did not fit the expected 55 R to 9 S ratio, but the difference was slight, with an excess of 66 plants in the resistant class. The F2 plant data from population [c]1 of the cross PI 567574A x Harosoy fit the expected 15:1 ratio, again indicating that two genes were segregating for resistance (Table 3). However, the F2:3 family data did not fit the 7:4:4:1 ratio, with

an excess of families in the susceptible class. Five of these families were approaching 50% resistant plants in the family, and removing these families from the population resulted in a fit to the predicted 7:4:4:1 F2:3 family ratio. From these populations, it appears that there are two genes at independent loci controlling resistance to race 1 of *Phytophthora sojae* in PI 567574A.

A second population from a different F1 plant of the cross PI 567574A x Harosoy was screened with race 25 of *P. sojae* to determine the number of genes for resistance to race 25 in PI 567574A. The F2 plant data fit the 3 R to 1 S ratio expected if PI 567574A has a single gene for resistance to race 25 (Table 4). The F2:3 family data fit a 1 R:2 H:1 S ratio, and the F3 plant data fit the 5 R:3 S ratio, indicating that PI 567574A has a single gene for resistance to race 25. Alleles at the Rps3 locus, *Rps4*, *Rps5*, and *Rps6* all confer resistance to race 25, so populations from crosses of PI 567574A with Williams and Harosoy isolines carrying these alleles were evaluated. The summary of the evaluation of the PI 567574A x L83-570 (*Rps3a*) population with race 25 is shown in Table 5. All F2 plants and all F2:3 families were resistant to race 25. Only 11 out of 2,533 (0.5%) F3 plants were susceptible. These results indicate that the allele for resistance to race 25 is at the Rps3 locus. This was confirmed by evaluating populations from the crosses of PI 567574A with L85-2352 (*Rps4*), L85-3059 (*Rps5*), and Harosoy 62XX (*Rps6*). Summaries of these evaluations are shown in Table 6. All F2 plants from these populations segregated approximately 15 R:1 S, indicating that there were two genes for resistance to race 25 in each of these populations. F2:3 family segregation patterns confirmed these results. These results confirm that the allele for resistance to race 25 is at the Rps3 locus.

In order to ascertain the other gene for resistance in PI 567574A, a third population of the cross PI 567574A x Harosoy was screened separately with races 7 and 30. *Rps1c* and *Rps3b* are the only two resistance alleles that confer resistance to race 30. The results of these experiments are summarized in Table 7. Evaluation of F2 plants indicated a single gene for resistance to races 7 and 30. The F2:3 family segregation pattern confirmed this response. The results from the F2:3 families also revealed that each family gave the same response to both races, indicating that the same gene in PI 567574A controls resistance to races 7 and 30.

One hundred F2 plants from the cross PI 567574A x Harosoy 15XX (*Rps1k*) were screened with race 7, and all plants were resistant (data not shown). *Rps1c*, *Rps1k*, and *Rps3b* confer resistance to race 7, while *Rps3a* confers susceptibility (Table 8). This indicates that an allele for resistance to race 7 is at the Rps1 locus. This same population from the cross of PI 567574A x Harosoy 15XX was also screened with race 5 and the results are summarized in Table 9. *Rps1c* confers susceptibility while *Rps1k*, *Rps3a*, and *Rps3b* all confer resistance to race 5 (Table 8). The F2 plants from this population fit a ratio of 15 R to 1 S, indicating that there are two genes for resistance to race 5 in this population. The F2:3 families also fit this model. Since *Rps1c* is the only allele at the Rps1 locus that confers resistance to race 7 and race 30, and susceptibility to race 5, these results indicate that it is one of the resistance alleles in PI 567574A.

Inoculation with race 25 had shown that PI 567574A carries an allele at the Rps3 locus. *Rps3a* is the only allele at this locus that would allow for the race reaction pattern of PI 567574A. It confers resistance to race 5, and confers susceptibility to races 7 and 30. This was confirmed by taking 10 selected F2:3 families from population #3 of PI 567574A x Harosoy and screening them with race 5, with the results summarized in Table 10. These selected families had a known response to races 7 and 30, to which both *Rps1c* and *Rps3b* confers resistance, while *Rps1c* confers susceptibility to race 5 and *Rps3b* confers resistance to race 5. If the allele for resistance to races 7 and 30 is *Rps3b*, then each family should respond in a similar fashion to races 5, 7, and 30. However, these families each gave a different response to races 7 and 30 versus race 5, depending on the gene present in the family (Table 10). This experiment confirms that the two alleles for resistance in PI 567574A are *Rps1c* and *Rps3a*.

In summary, PI 567574A has two alleles at separate loci for resistance to *P. sojae*. These alleles appear to be *Rps1c* and *Rps3a*. However, neither of these genes confers resistance to race 12, and PI 567574A was shown to be resistant to race 12 (Kyle, 1997). However, this reaction to race 12 was not confirmed with repeated inoculations. Alternatively, PI 567574A could carry the *Rps7* allele for resistance. An isolate of race 12 was not available for this study, so this possibility was not explored. This study also does not rule out the presence of a new allele at the Rps3 locus, which could carry resistance to race 12. The new allele could have a similar race reaction pattern as *Rps3a* with the addition of resistance to race 12. The race resistance patterns and inheritance studies, however, point strongly to the presence of the *Rps3a* allele in PI 567574A.

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Table 1. Reaction of Williams isolines and PI 567574A to hypocotyl inoculation of selected races of *Phytophthora sojae* used in this study.

Line	Gene	Race												
		1	3	4	5	7	10	12	13	17	20	25	28	30
Williams	*rps*	S[a]	S	S	S	S	S	S	S	S	S	S	S	S
Harosoy	*Rps7*	S	S	S	S	S	S	R	S	S	S	S	S	S
PI 567.574A		R	R	R	R	R	R	R[b]	R	R	S[b]	R	R	R
L75-6141	*Rps1a*	R	S	S	S	S	R	S	R	R	S	S	S	S
L77-1863	*Rps1b*	R	R	R	R	R	S	S	R	S	S	S	S	S
L75-3735	*Rps1c*	R	R	S	S	R	R	S	R	R	S	S	R	R
L93-3312	*Rps1d*	R	R	R	R	S	S[c]	R	R	S	S[c]	R	R	S[c]
Williams 82	*Rps1k*	R	R	R	R	R	R	S	R	R	S	S	S	S
L83-570	*Rps3a*	R	R	R	R	S	S	S	R	S	S	R	R	S
L89-1541	*Rps3b*	R	R	R	R	R	S[c]	R	R	S	S	R	R	R
L92-7857	*Rps3c*	R	R	R	S	S	R	R	R	S	S	R	R	S
L85-2352	*Rps4*	R	R	R	S	S	R	R	S[c]	S	R	R	R	S
L85-3059	*Rps5*	R	R	R	R	S	S	R	R	S	S	R	R[d]	S
L89-1581	*Rps6*	R	R	R	S	S	R	R	S	S	R	R	R	S
L93-3458	*Rps7*	S	S	S	S	S	S	R	S	S	S	S	S	S

[a] S = susceptible, R = resistant.
[b] This reaction was from a single inoculation and was not confirmed.
[c] Original reports indicate that these reactions should be Resistant (R).
[d] Original reports indicate that this reaction should be Susceptible (S).

Table 2. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the cross PI 567574A x Williams to inoculation with race 1 of *Phytophthora sojae*.

Generation		15:1	3:1		Theoretical Ratio	Chi-square
	Res.[a]	Het.[b]	Het.[c]	Susc.[d]		

	no. plants					Value	Prob.
F2 plants	91			5	15:1	0.18	0.673
F2:3 families	21	13	12	4	7:4:4:1	0.32	0.956
F2:3 families[e]	34		12	4	11:4:1	0.27	0.874
F3 plants	2257			293	55:9	13.96	0.0002
Williams	1			76			
PI 567574A	77			0			

[a] Observed resistant plants or families.
[b] Observed 15:1 heterogeneous families.
[c] Observed 3:1 heterogeneous families.
[d] Observed susceptible plants or families.
[e] Resistant and 15:1 heterogeneous families combined.

Table 3. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the cross PI 567574A x Harosoy to inoculation with race 1 of *Phytophthora sojae*.

Generation		15:1	3:1		Theoretical Ratio	Chi-square	
	Res.[a]	Het.[b]	Het.[c]	Susc.[d]			
	no. plants					Value	Prob.
F2 plants	126			13	15:1	2.28	0.131
F2:3 families	33	19	18	15	7:4:4:1	18.87	0.0003
F2:3 families[e]	52		18	15	11:4:1	18.87	0.0001
F3 plants	5816			1472	55:9	227	0.0001
Harosoy	1			180			
PI 567574A	176			0			

[a] Observed resistant plants or families.
[b] Observed 15:1 heterogeneous families.
[c] Observed 3:1 heterogeneous families.
[d] Observed susceptible plants or families.
[e] Resistant and 15:1 heterogeneous families combined.

Table 4. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the cross PI 567574A x Harosoy to inoculation with race 25 of *Phytophthora sojae*.

Generation	Res.[a]	Het.[b]	Susc.[c]	Theoretical Ratio	Chi-square	
	no. plants				Value	Prob.

F2 plants	58		31	3:1	4.59	0.032
F2:3 families	9	30	11	1:2:1	2.16	0.340
F2:3 families[d]	39		11	3:1	0.24	0.624
F3 plants	1575		901	5:3	1.30	0.254
Harosoy	8		65			
PI 567574A	70		1			

[a] Observed resistant plants or families.
[b] Observed 3:1 heterogeneous families.
[c] Observed susceptible plants or families.
[d] Resistant and heterogeneous families combined.

Table 5. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the cross PI 567574A x L83-570 (*Rps3a*) to inoculation with race 25 of *Phytophthora sojae*.

Generation	Res.[a]	Susc.[b]	Theoretical Ratio
	no. plants		
F2 plants	85	0	All R
F2:3 families	50	0	All R
F3 plants	2522	11	All R
L83-570	77	0	
PI 567574A	74	1	

[a] Observed resistant plants or families.
[b] Observed susceptible plants or families.

Table 6. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the crosses of PI 567574A x L85-2352 (*Rps4*), L85-3059 (*Rps5*), and Harosoy 62XX (*Rps6*) to inoculation with race 25 of *Phytophthora sojae*.

Generation		15:1	3:1		Theoretical Ratio	Chi-square	
	Res.[a]	Het.[b]	Het.[c]	Susc.[d]			
	no. plants					Value	Prob.
PI 567574A x L83-2352 (*Rps4*)							
F2 plants	88			11	15:1	3.99	0.046
F2:3 families	13	14	18	5	7:4:4:1	7.32	0.062
F2:3 families[e]	27		18	5	11:4:1	5.12	0.077
F3 plants	2213			512	55:9	50.4	0.0001

L85-2352	78			0			
PI 567574A	76			2			
PI 567574A x L85-3059 (*Rps5*)							
F2 plants	86			10	15:1	2.84	0.092
F2:3 families	16	16	12	6	7:4:4:1	5.22	0.156
F2:3 families[e]	32		12	6	11:4:1	2.83	0.243
F3 plants	2039			416	55:9	16.9	0.0001
L85-3059	77			4			
PI 567574A	76			0			
PI 567574A x Harosoy 62XX (*Rps6*)							
F2 plants	81			5	15:1	0.03	0.867
F2:3 families	20	13	14	3	7:4:4:1	0.36	0.947
F2:3 families[e]	33		14	3	11:4:1	0.24	0.887
F3 plants	2337			349	55:9	2.54	0.111
Harosoy 62XX	72			5			
PI 567574A	76			2			

[a] Observed resistant plants or families.
[b] Observed 15:1 heterogeneous families.
[c] Observed 3:1 heterogeneous families.
[d] Observed susceptible plants or families.
[e] Resistant and 15:1 heterogeneous families combined.

Table 7. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the cross PI 567574A x Harosoy to inoculation with races 7 and 30 of *Phytophthora sojae*.

Generation	Res.[a]	Het.[b]	Susc.[c]	Theoretical Ratio	Chi-square	
	no. plants				Value	Prob.
Race 7						
F2 plants	54		14	3:1	0.71	0.401
F2:3 families	9	25	16	1:2:1	1.96	0.375
F2:3 families[d]	34		16	3:1	1.31	0.253
F3 plants	1561		1048	5:3	7.93	0.005
Harosoy	0		75			
PI 567574A	76		0			
Race 30						
F2 plants	49		21	3:1	0.93	0.334

F2:3 families	9	25	16	1:2:1	1.96	0.375
F2:3 families[d]	34		16	3:1	1.31	0.253
F3 plants	1575		901	5:3	13.9	0.0002
Harosoy	1		75			
PI 567574A	76		1			

[a] Observed resistant plants or families.
[b] Observed 3:1 heterogeneous families.
[c] Observed susceptible plants or families.
[d] Resistant and heterogeneous families combined.

Table 8. Reaction of PI 567574A and Williams isolines containing resistance alleles *Rps1c*, *Rps1k*, *Rps3a*, and *Rps3b* to hypocotyl inoculation of selected races of *Phytophthora sojae* used in this study.

Line	Allele	Race												
		1	3	4	5	7	10	12	13	17	20	25	28	30
PI 567.574A		R[a]	R	R	R	R	R	R[b]	R	R	S[b]	R	R	R
L75-3735	*Rps1c*	R	R	S	S	R	R	S	R	R	S	S	R	R
Williams 82	*Rps1k*	R	R	R	R	R	R	S	R	R	S	S	S	S
L83-570	*Rps3a*	R	R	R	R	S	S	S	R	S	S	R	R	S
L89-1541	*Rps3b*	R	R	R	R	R	S[c]	R	R	S	S	R	R	R

[a] S = susceptible, R = resistant.
[b] This reaction was from a single inoculation and was not confirmed.
[c] Original reports indicate that these reactions should be Resistant (R).

Table 9. Reaction of parents, F2 plants, F2:3 families, and F3 plants from the cross PI 567574A x Harosoy 15XX (*Rps1k*) to inoculation with race 5 of *Phytophthora sojae*.

Generation		15:1	3:1		Theoretical Ratio	Chi-square	
	Res.[a]	Het.[b]	Het.[c]	Susc.[d]			
	no. plants					Value	Prob.
F2 plants	81			7	15:1	0.44	0.509
F2:3 families	20	12	14	4	7:4:4:1	0.61	0.895
F2:3 families[e]	32		14	4	11:4:1	0.59	0.745
F3 plants	2114			411	55:9	10.2	0.001

Harosoy 15XX	77			0			
PI 567574A	76			1			

[a] Observed resistant plants or families.
[b] Observed 15:1 heterogeneous families.
[c] Observed 3:1 heterogeneous families.
[d] Observed susceptible plants or families.
[e] Resistant and 15:1 heterogeneous families combined.

Table 10. Response of selected F2:3 families from the cross of PI 567574A x Harosoy to inoculation with race 5 of *Phytophthora sojae*.

Number[a]	F2:3 family				Gene in F2:3 family for resistance to	
	Race 7 & 30	Reaction to race 5				
	Reaction[b]	R[c]	S[c]	Rxn.	Race 7 & 30	Race 5
		no. plants				
1	S[c]	8	36	S[c]	none	none
2	H	54	1	H	*Rps1c*	*Rps3a*
3	H	53	7	H	*Rps1c*	*Rps3a*
6	H	8	42	S	*Rps1c*	none
7	R	58	0	R	*Rps1c*	*Rps3a*
8	H	32	2	R	*Rps1c*	*Rps3a*
9	S	55	2	R	none	*Rps3a*
11	R	49	14	H	*Rps1c*	*Rps3a*
12	H	7	40	S	*Rps1c*	none
13	H	44	12	H	*Rps1c*	*Rps3a*

[a] F2:3 Family designation.
[b] Reaction of selected F2:3 families to distinct inoculations with races 7 and 30.
[c] S=susceptible, H=3R:1S heterogeneous, and R=resistant.