New Races of *Phytophthora sojae* with *Rps1-d* Virulence

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ABSTRACT

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Hypocotyl inoculations of differential soybean (*Glycine max*) cultivars were used to identify seven new physiologic races of *Phytophthora sojae* (syn. *P. megasperma* f. sp. *glycinea*). Five of the new races were virulent on soybeans with the *Rps1-d* allele, while four of the new races were virulent on soybeans with the *Rps1-k* allele. The *Rps1-k* and *Rps1-d* alleles provide resistance to a majority of the previously described races that cause *Phytophthora* root and stem rot. The seven new races were assigned race numbers 33, 34, 41, 42, 43, 44, and 45 since race numbers 35 through 40 were assigned to other new races recently identified in Ohio, California, Arkansas, and Iowa. The new races identified in this study constituted 14% of the 1993 isolates evaluated from 27 counties in central and northern Indiana. Races 1 and 3 were the most prevalent, representing 31 and 26% of the *P. sojae* isolates identified in 1993. Races 4, 6, 7, 8, 9, 13, 28, and 29 each constituted 2 to 6% of the 1993 isolates. Other races identified among the 1993 isolates included races 19, 21, 25, and 30.

Awareness of *Phytophthora* root rot of soybeans (*Glycine max* (L.) Merr.) has increased since 1990. Several new races of *Phytophthora sojae* M. J. Kaufmann & J. W. Gerdemann (4,6) that infect soybean plants in the Midwest area of the U.S. were recently identified in Indiana (T. S. Abney, unpublished) and Ohio (10). All of these new races isolated from plant samples obtained in 1990 to 1992 were virulent on soybeans with the *Rps1-k* allele, using the hypocotyl inoculation method. Evaluation of the virulence formulas (10) for the *P. sojae* races reported prior to 1990 (i.e., races 1 to 27) demonstrates that *Rps1-k* or *Rps1-d* could be used to control a majority of the races that cause root and stem rot. The *Rps1-k* allele identified in Kingwa and transferred to Williams has been widely used in breeding programs since 1981, whereas the *Rps1-d* allele identified in PI 103091 has rarely been used.

Information on the race situation or inoculum potential for this disease in soybean production areas is limited. Data documenting the races of *P. sojae* isolated

from diseased soybeans in growers' fields are important, but are also somewhat limited. Recovery of the pathogen from field plants in various stages of disease development is problematic, and the selectivity of cultivars and specific *Rps* alleles impose also presents a potential bias on data obtained by sampling diseased plants randomly collected in growers' fields. Therefore, additional isolations of *P. sojae* from soil with the soybean seedling bioassay method (10) are needed to enhance the identification of races. In this report, physiologic races found in 1993 are identified and compared with races previously reported in Indiana. Several new *P. sojae* races are proposed. The reactions of differential cultivars to the new races and frequency of races isolated in Indiana soybean production areas are presented.

MATERIALS AND METHODS

Seventy isolates of *P. sojae* from field plants with symptoms of *Phytophthora* rot (6,11) and 230 isolates from soil were collected in 1993 from producers' soybean fields and experimental soybean nurseries in Indiana. The soil samples from 80 fields in 27 counties were collected primarily in low areas of fields that had a history of *Phytophthora* damage in 1990 to 1992 and/or in areas of fields that had plants with chlorotic foliage and somewhat stunted growth in 1993. Soil samples were collected and *P. sojae* isolated with the Sloan soybean seedling bioassay method, using techniques described by Schmitthen-

ner et al. (10). Isolations were made from the margin of diseased areas of stem or root tissue. Tissue samples were surface disinfected, and transferred to potato dextrose agar (PDA) and to the selective culture media (PRA) as previously described (8). The isolates collected in 1993 were tentatively identified as *P. sojae* (4,6) by comparing colony characteristics and oospores on PDA and oatmeal agar (OA) to known isolates of *P. sojae*.

Inoculum was prepared by growing single oospore isolates on OA in petri plates in an unlighted cabinet at 24°C for 2 to 4 weeks. Inoculations were performed by the standard hypocotyl method (8), using 2 × 2 mm pieces of mycelia with abundant oospores, and the wound was covered with petrolatum to prevent desiccation of the inoculum and host tissue. Ten 7-day-old seedlings of soybean differentials (1,2,10) representing different *Rps* alleles (*Rps1-a*, *Rps1-b*, *Rps1-c*, *Rps1-d*, *Rps1-k*, *Rps3a*, *Rps6*, *Rps7*, and *rps*) were inoculated with each isolate and grown in the greenhouse at 24 to 27°C with supplemental fluorescent and incandescent light. Disease reactions of the differential cultivars were recorded 7 days after inoculation. The races were identified on the basis of a susceptible (plants killed) or a resistant (no effect) reaction of 60 to 70% of the soybean differentials in the tests. Inoculation tests were conducted four times for the new races and two times for the previously reported races.

RESULTS AND DISCUSSION

The differential virulence data for seven new physiologic races of *P. sojae* with eight different *Rps* alleles are listed in Table 1. Differential responses of the new physiologic races are distinctly different from the responses of races 1 to 32 (1,2,7,10) and responses of the Ohio isolate (race 35) discussed by A. F. Schmitthenner at the 1995 University of Wisconsin *Phytophthora* Workshop, the two Japanese isolates (races 36, 37) reported by Förster et al. (3), the two Arkansas isolates (races 38, 39) reported by Henry and Kirkpatrick (5), and the Iowa isolate reported by Yang et al. (12). Since the Iowa isolate was not assigned a race number in the initial publication, it is being designated as race 40 (virulence

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formula = 1a,1c,1d,1k,7) in this report. Race numbers 33 and 34 were reserved for the responses of two Indiana isolates also discussed at the University of Wisconsin *Phytophthora* Workshop. The results of repeated inoculation tests with each of the new races from Indiana were similar. Seven new race phenotypes are presented in Table 1. Isolates with the virulence formula 1a,1b,1c,1d,1k,7 were designated race 33 and isolates with the virulence formula 1a,1k,7 were designated race 34. Other unique isolates A41 (1a,1b, 1d,1k,7), A42 (1a,1d,3a,7), A43 (1a,1c, 1d,7), A44 (1a,1d,7), and A45 (1a,1b,1c, 1k,6,7) were designated races 41, 42, 43, 44, and 45, respectively.

Four of the seven new races (races 33, 34, 41, and 45) were virulent on soybeans with the *Rps1-k* allele, whereas five were virulent on soybeans with the *Rps1-d* allele. In each instance of a new race capable of killing soybeans with the *Rps1-d* allele, the virulence formula for a previously identified race in Indiana was quite similar except for the 1-d reaction. The virulence phenotype for race 33 (1a,1b,1c,1d,1k,7), based on four isolates from Madison and Wabash counties, was most similar to race 25 (1a,1b,1c,1k,7). Race 41 (1a,1b,1d,1k,7), based on five isolates from Adams and Madison counties, was most similar to race 28 (1a,1b,1k,7). Race 42 (1a,1d,3a,7), based on four isolates from Boone County,

was most similar to race 21 (1a,3a,7). Race 43 (1a,1c,1d,7), based on eight isolates from Allen, Boone, Carroll, Randolph, Tipton, and Wabash counties, was most similar to race 4 (1a,1c,7). Race 44 (1a,1d,7), based on 15 isolates from Blackford, Carroll, Cass, Clinton, Jay, Randolph, Tipton, Wabash, and Wells counties, was most similar to race 3 (1a,7). Race 34 (1a,1k,7), based on five isolates from Delaware, Hamilton, Jasper, Madison, and Tipton counties, was most similar to race 28 (1a,1b,1k,7) and race 3 (1a,7), except for the *Rps* 1-b and 1-k reactions, respectively. Isolates of races 34, 43, and 44 are also similar to phenotypes (H29, H19, and H17, respectively) identified in Ohio as early as 1981 (10). The H29 (1a,1k), H19 (1a,1c,1d), and H17 (1a,1d) isolates were not saved and were not named as races since the *Rps7* reaction was not evaluated. Race 45 (1a,1b,1c,1k,6,7) was isolated only from Wells County and was most similar to race 29 (1a,1b,1k,6,7) and race 25 (1a,1b,1c,1k,7), except for the *Rps* 1-c and 6 reactions, respectively.

At least two different *Rps* alleles were identified that confer resistance to each of the new *P. sojae* races. Soybean cultivars with the *Rps3a* or *Rps6* allele were resistant to races 33, 34, 41, 43, and 44, whereas only one of these alleles conferred resistance to races 42 and 45. The *Rps1-d* and *Rps3a* alleles conferred resistance to races 34 and 45. The *Rps1-b*, *Rps1-k*, and *Rps6* alleles were resistant to races 42, 43, and 44. Races 34, 41, 42, and 44 were also controlled by the *Rps1-c* allele.

P. sojae races previously reported in Indiana and races identified in 1993 from diseased field plants collected in 11 counties and from Sloan seedlings growing in soil collected in 27 central and northern Indiana counties are listed in Table 2. All races previously reported in Indiana except for races 5 and 22 were identified among the 300 isolates collected in 1993. Races 19, 28, 29, and 30 have not been reported in Indiana previously. Among the 1993 plant and soil isolates, races 1 and 3 were the most prevalent, representing 31 and 26% of the isolates, respectively. Races 4, 6, 7, 8, 9, 13, 28, and 29 each constituted 2 to 6% of the isolates. The new races constituted 14% of the isolates.

Race 3 was the most prevalent (48%) among the 1973 to 1979 isolates in Indiana (8). Races 1, 7, and 9 constituted 17, 18, and 12% of the isolates, respectively. Races virulent on *Rps1-k* were not reported in the midwestern states until 1984 (10). The *Rps1-k* allele identified in Kingwa has been widely used in breeding programs since 1981 and could therefore constitute a selection pressure for *P. sojae* races virulent on soybeans with the *Rps1-k* allele. The *Rps1-d* allele identified in PI 103091 has rarely been used as a source of resistance and would therefore produce very little selection pressure

Table 1. Virulence formulas and reaction of differential soybean cultivars to new races of *Phytophthora sojae* found in Indiana

Race ^a	Virulence formula ^b	Susceptibility of cultivars with different <i>Rps</i> alleles ^c							
		1-a	1-b	1-c	1-d	1-k	3-a	6	7
A33	1a,1b,1c,1d,1k,7	S ^d	S	S	S	S			S
A34	1a,1k,7	S				S			S
A41	1a,1b,1d,1k,,7	S	S		S	S			S
A42	1a,1d,3a,7	S			S		S		S
A43	1a,1c,1d,7	S		S	S				S
A44	1a,1d,7	S			S				S
A45	1a,1b,1c,1k,6,7	S	S	S		S		S	S

^a A series from T. S. Abney (*unpublished*), sequence starts with the last published race by Schmitthenner et al. (10), but skips the following numbers: 35 (number reserved for new race from Ohio, A. F. Schmitthenner, *unpublished*); 36 and 37 (new races reported by Forster et al. from Japan [3]); 38 and 39 (new races reported by Henry and Kirkpatrick from Arkansas [5]); and 40 (number assigned in this paper for new race from Iowa, reported by Yang et al. [12]).

^b Listing of defeated *Rps* alleles (1a,1b,1c,1d,1k,3a,6,7).

^c *Rps* 1-a in Haro12, 1-b in Haro13, 1-c in Harosoy isolate L85-129, 1-d in Haro16, 1-k in cv. Williams-82 and Resnik, 3-a in PI 171442, 6 in cv. Altona, 7 in cv. Harosoy, and 1-a,7 in cv. Harosoy 63.

^d S = >60% of hypocotyl inoculated plants killed.

Table 2. *Phytophthora sojae* races reported in Indiana between 1973 and 1979 and in 1993

Race ^a	Virulence formula ^b	1st year reported ^c	Races isolated	
			1973 to 1979 (%) ^c	1993 (%) ^d
1	7	1958	17	31
3	1a,7	1978	48	26
4	1a,1c,7	1980	3	6
5	1a,1c,6,7	1978	<1	0
6	1a,1d,3a,6,7	1975	0	2 ^e
7	1a,3a,6,7	1976	18	3
8	1a,1d,6,7	1978	2	2
9	1a,6,7	1978	12	3
13	6,7	1978	<1	2
19	1a,1b,1c,1d,1k,3a	1993	0	<1
21	1a,3a,7	1978	<1	<1
22	1a,1c,3a,6,7	1980	<1	0
25	1a,1b,1c,1k,7	1984	<1	1
28	1a,1b,1k,7	1993	0	6
29	1a,1b,1k,6,7	1993	0	4
30	1a,1b,1k,3a,6,7	1993	0	<1
New	Variable	1993	0	14

^a Numerical races according to Schmitthenner et al. (10). All races are virulent on universal suspects (*rps*) such as Sloan or Haro(1-7)1. New races are other phenotypes with virulence patterns different from races 1-32.

^b Listing of defeated *Rps* alleles (1a,1b,1c,1d,1k,3a,6,7).

^c Year first isolated in Indiana and percentage reported 1973 to 1979 according to Laviolette and Athow (8), except for race 25 according to Layton et al. (9) and races 19, 28, 29, and 30, which were identified by the authors.

^d Percentage for races in 1993 based on 300 isolates from plant and soil samples collected in central and northern Indiana.

^e Identification of race 6 was not verified due to the inconsistent reaction involving *Rps1-d*.

for races virulent on soybeans with the *Rps1-d* allele.

The 1993 isolates of races previously reported in Indiana had reaction patterns on differentials that were in agreement with previous race identifications (2,10,11). All of the races except race 6 (1a,1d,3a,6,7) were highly pathogenic on susceptible cultivars, and generally seedlings were 90 to 100% healthy or 90 to 100% dead. The susceptible reactions of soybean seedlings with the *Rps1-d* allele were inconsistent when inoculated with race 6 isolates. Laviolette and Athow (8) suggested race 6 was an invalid race because of the inconsistent reaction involving the *Rps1-d* allele.

This and previous research (1,8,10,12) demonstrate the presence of at least 28 physiologic races of *P. sojae* that attack soybeans in the Midwest. Several of the races reported in Mississippi and other southern states have not been reported in the Midwest. Awareness of the presence of these races and the identification of parental lines resistant to the new races should

be used in soybean breeding programs to minimize soybean yield losses.

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