

## Genetic Implications of the Susceptibility of Kent Soybean to *Cercospora sojina*

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### ABSTRACT

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Seven different sources of cultivar Kent soybean (*Glycine max*) seed were obtained from different states in the USA. Separate plants from each source were inoculated with races 2 and 5 of *Cercospora sojina*. Kent was found to be a mixture of reaction types to race 5 in six of the seven sources and to race 2 in two of seven sources. In general, Kent was resistant to race 2 and susceptible to race 5. When plants of Kent and cultivar Bragg were inoculated with race 5 and single-lesion isolates from both cultivars were used to inoculate a group of differential cultivars, the results indicated that the biotype infecting Kent was the same as on Bragg. The understanding of

the reaction of Kent to races 2 and 5 indicated that the *Rcs<sub>2</sub>* gene in Kent for resistance to race 2 cannot condition resistance to race 5. The reactions to races 2 and 5 of F<sub>2</sub> plants of the Blackhawk × Davis cross indicated that the gene in Davis for resistance to race 5 also conditioned resistance to race 2. The segregation ratio among F<sub>3</sub> lines for reaction to race 2 from the Davis × Kent cross indicated the gene in Davis and *Rcs<sub>2</sub>* were at different loci. Thus, the single dominant gene in Davis for resistance to races 2 and 5 should be assigned the symbol, *Rcs<sub>3</sub>*.

*Additional key words:* disease resistance, frogeye leafspot, inheritance.

Frogeye leafspot caused in soybean [*Glycine max* (L.) Merr.] by *Cercospora sojina* Hara was first reported in the USA in the 1920s (4,6,7,15). Yield reductions of up to 21% have been reported (5), but the use of resistant cultivars has prevented frogeye leafspot from becoming a severe problem (1). Five physiological races of the fungus have been identified (2,3,8,13) and others may exist (14). Resistance to races 1 and 2 is conditioned by the genes *Rcs<sub>1</sub>* (2,10) and *Rcs<sub>2</sub>* (12), respectively. The inheritance of resistance to races 3 and 4 has not been reported, and cultures of these races are no longer available. Phillips and Boerma (9) found a dominant gene in cultivar Davis and a separate dominant gene for resistance to race 5 in cultivar Lincoln. Because of the erratic reaction of Kent to inoculation with races 2 and 5 in several studies (3,8,9,13), the relationship of the gene found in Davis for resistance to race 5 and the gene for resistance to race 2 in Kent, *Rcs<sub>2</sub>*, was unknown (9).

The objectives of the present study were to determine the reason(s) for the erratic results from inoculation of Kent with *C. sojina* and to resolve the genetic relationship between *Rcs<sub>2</sub>* and the gene for resistance to race 5 in Davis.

### MATERIALS AND METHODS

In 1981, we obtained seed of soybean cultivar Kent from Illinois (R. L. Bernard), Nebraska (J. H. Williams), Virginia (H. M. Camper, Jr.), Maryland (W. J. Kenworthy), Kentucky (D. B. Egli), and Indiana (J. R. Wilcox). Plants from each seed source plus a Georgia source used in our previous study (8) were grown in the greenhouse and inoculated with either race 2 (obtained from K. L. Athow, Purdue University) or 5 (ATCC 42654) of *C. sojina*. Cultures of both races were maintained, and inoculum was produced on a medium composed of equal parts of soybean stem agar and lima bean agar as previously described (8). A suspension of  $6 \times 10^4$  conidia per milliliter was atomized onto the plants at the two- or three-trifoliolate leaf stage. After inoculation, a clear plastic bag was placed over the plants for 72 hr to maintain high relative humidity. Ratings were made 14 days after inoculation. Plants that showed no lesions or only small lesions or flecks were classified as resistant. Plants that showed numerous large

spreading lesions were classified as susceptible. Plants classified as resistant were reinoculated to eliminate possible escapes.

Seeds were harvested from the inoculated plants at maturity. Progeny of plants resistant to race 2 were inoculated with race 5 by using the previously described inoculation procedure. In addition to the seven sources of Kent, plants of the cultivars Clark, Wabash, and Illini were also inoculated with race 5.

In another study, plants of Bragg and Kent (Illinois source) were inoculated with race 5 of *C. sojina* (ATCC 42654). A single-lesion isolate from each cultivar was obtained and used to inoculate a group of differential cultivars. The inoculation procedure and rating scheme were the same as previously described.

In a separate study, F<sub>2</sub> plants of the Blackhawk × Davis cross were inoculated with race 2 of *C. sojina*. A different group of F<sub>2</sub> plants from this cross were first inoculated with race 5 on a single, expanding leaf (second or third trifoliolate) and 14 days later inoculated with race 2 on a different expanding leaf (previous results have indicated no effect on reaction to one race from prior inoculation with a different race [D. V. Phillips and H. R. Boerma, unpublished]). Plants that were susceptible to race 5 were moved to a separate greenhouse prior to inoculation of the race 5-resistant plants with race 2. Progeny of race 5-susceptible F<sub>2</sub> plants were inoculated with race 2. Also, 25 F<sub>3</sub> lines (20–60 plants per line) from the Davis × Kent cross were inoculated with race 2. The inoculation procedure and rating scheme were the same as previously described, except the F<sub>2</sub> plants were inoculated with both races 2 and 5 on the same plant, and small plastic bags were placed over the inoculated leaf for 72 hr after inoculation.

### RESULTS AND DISCUSSION

The inoculation of plants grown from seed obtained from the different Kent seed sources with races 2 and 5 of *C. sojina* indicated that Kent was a mixture of reaction types to race 5 in six of seven sources and to race 2 in two of seven sources (Table 1). In general, Kent would be described as resistant to race 2 and, with the exception of the Georgia source, susceptible to race 5. All inoculated plants were phenotypically similar in flower, pubescence, hilum, and pod wall color. It is possible that the two reaction types to race 5 originated from the F<sub>6</sub> parent plant of Kent, being heterozygous for a gene controlling resistance (11). Since selection for resistance to race 5 was not practiced during the development of Kent, the two reaction types would not have been

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observed. The two reaction types to race 2 in the Virginia and Maryland seed sources is more difficult to explain because Kent was reported to be resistant to race 2 when released (11). This mixture of reaction types may explain the resistant reaction of Kent to race 2 reported by Athow et al (3) and the susceptible reaction reported by Ross (13).

Plants grown from seed from the Georgia seed source and inoculated with race 5 had 11 resistant plants and four susceptible plants (Table 1). This seed source originated from seed obtained from R. L. Bernard, Urbana, IL, in 1978. Three plants were grown in the greenhouse for crossing purposes and the seed from each was composited to create the Georgia source. With unequal seed

TABLE 1. Reaction of Kent soybean from seven different sources to *Cercospora sojina* races 2 and 5 and reaction of the progeny of race 2-resistant plants to race 5

Seed source	No. of plants inoculated with :				No. of plant lines-resistant to race 2 inoculated with race 5	
	Race 5		Race 2		Susc.	Res.
	Susc. <sup>a</sup>	Res. <sup>b</sup>	Susc.	Res.		
Nebraska	12	1	0	11	3	1
Illinois	15	0	0	16	5	0
Virginia	11	1	3	15	2	0
Maryland	11	2	6	13	4	0
Georgia	4	11	0	11	3	2
Indiana	39	5	0	36	...	...
Kentucky	25	7	0	41	...	...

<sup>a</sup>Susceptible, with numerous large spreading lesions.

<sup>b</sup>Resistant, with either no lesions or a few small lesions or flecks.

TABLE 2. Reaction of cultivars inoculated with *Cercospora sojina* race 5 and single lesion isolates from plants of Bragg and Kent

Cultivar	ATCC 42654	ATCC 42654 reisolated from:	
	Race 5	Bragg	Kent <sup>d</sup>
Lee	R <sup>a</sup>	R	R
Davis	R	R	R
Blackhawk	S <sup>b</sup>	S	S
Hood	S	S	S
Bragg	S	S	S
Flambeau	S	S	S
Comet	S	S	S
Lincoln	R	R	R
Hill	R	R	R
Ga Soy 17	S	S	S
Ransom	R	R	R
Wright	R	R	R
Kent <sup>c</sup>	R	R	R
Clark	R	...	...
Wabash	R	...	...
Illini	R	...	...

<sup>a</sup>Resistant, with either no lesions or a few small lesions or flecks.

<sup>b</sup>Susceptible, with numerous large spreading lesions.

<sup>c</sup>Georgia source.

<sup>d</sup>Illinois source.

production from each plant and only three plants composited, a change in the frequency of the gene for race 5 resistance could have occurred.

Since Kent is the only proven source of the *Rcs2* gene for resistance to race 2 of *C. sojina*, it was assumed Kent should be resistant to race 2 (12). To determine if any race 2-resistant plants were susceptible to race 5, the progeny of some race 2-resistant plants from five seed sources were inoculated with race 5 (Table 1). In plantings from both the Nebraska and Georgia seed sources, individual plants resistant to race 2 produced progeny all susceptible or all resistant to race 5. These results indicate that plants of the genotype *Rcs2* could be either resistant or susceptible to race 5.

To determine if the lesions on Kent were caused by the same biotype as the lesions on Bragg (source of race 5), single-lesion isolates from Bragg and Kent inoculated with race 5 (ATCC 42654) were used to inoculate a group of differential cultivars (Table 2). With all cultivars, the reaction of the original race 5 was the same as the isolates obtained from Bragg and Kent. Thus, Kent was not differentially selecting a unique biotype from a mixture in the original isolate of race 5.

The results of these experiments indicate that most seed sources of Kent are mixtures of reaction types for race 5. The predominant reaction of Kent is resistant to race 2 and susceptible to race 5. This heterogeneity would make Kent a poor choice as a differential cultivar for *C. sojina* race determinations. The change in classification of Kent from resistant to susceptible to race 5 is a correction of the conclusions from our earlier work (8).

In our previous work, we reported that plants of soybean cultivars Lincoln and Davis each contained a different single dominant gene for resistance to race 5 (9). We could not determine if the gene for resistance to race 1, *Rcs1*, in Lincoln was the same gene conditioning resistance to race 5. Inoculation of plants of soybean cultivars Wabash, Illini, and Clark, the other known sources of the *Rcs1* gene, indicated that all were resistant to race 5 (Table 2). Thus, the relationship of *Rcs1*, and the gene for resistance to race 5 in Lincoln cannot be resolved, since no culture of race 1 is available.

Davis was previously found to be resistant to both races 2 and 5 (3,8). The F<sub>2</sub> plants of the Blackhawk × Davis cross that were inoculated with race 2 segregated in a 3 resistant: 1 susceptible ratio (observed numbers: 98 resistant and 35 susceptible) indicating that Davis has a single dominant gene conditioning resistance to race 2. Reactions from inoculations with races 2 and 5 on different leaves of the same F<sub>2</sub> plant from the Blackhawk × Davis cross indicated all 74 plants resistant to race 5 were resistant to race 2. Also, 10 F<sub>3</sub> plants (progeny of F<sub>2</sub> plants susceptible to race 5) were all susceptible to race 2. Thus, the dominant gene in Davis conditioning resistance to race 5 (9) also conditions resistance to race 2.

To determine the genetic relationship between *Rcs2* and the gene in Davis for resistance to races 2 and 5, F<sub>3</sub> lines from the Davis × Kent cross were inoculated with race 2 (Table 3). The F<sub>3</sub> lines segregated in a 7:4:4:1 ratio (7/16 of the lines homozygous resistant: 4/16 of the lines segregating 3 resistant to 1 susceptible: 4/16 of the lines segregating 15 resistant to 1 susceptible: 1/16 of the lines homozygous susceptible), indicating the *Rcs2* gene and the

TABLE 3. Segregation for reaction to *Cercospora sojina* race 2 among F<sub>3</sub> soybean lines of the Davis × Kent cross

	Homozygous resistant	Segregating			Chi-square probability
		3 Resistant 1 Susceptible	15 Resistant 1 Susceptible	Homozygous susceptible	
Expected ratio	7	4	4	1	0.81
Observed no.	9	6	8	2	
Expected no.	10.94	6.25	6.25	1.56	
F <sub>2</sub> genotypes	<i>Rcs2 Rcs2 Rcs3 Rcs3</i> <i>Rcs2 Rcs2 Rcs3 Rcs3</i> <i>Rcs2 Rcs2 rcs3 rcs3</i> <i>Rcs2 rcs2 Rcs3 Rcs3</i> <i>rcs2 rcs2 Rcs3 Rcs3</i>	<i>Rcs2 rcs2 rcs3 rcs3</i> <i>rcs2 rcs2 Rcs3 rcs3</i>	<i>Rcs2 rcs2 Rcs3 rcs3</i> <i>Rcs2 rcs2 Rcs3 rcs3</i>	<i>rcs2 rcs2 rcs3 rcs3</i>	

gene in Davis for resistance to races 2 and 5 were at different loci.

Since the *Rcs<sub>2</sub>* gene does not condition resistance to race 5 and this gene is at a different locus than the gene in Davis for resistance to races 2 and 5, the dominant gene in Davis should now be designated *Rcs<sub>3</sub>*. Since Davis is resistant to all known races of *C. sojina* (14), the possibility that *Rcs<sub>3</sub>* is conditioning resistance to additional races exists. This possibility is currently being evaluated.

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