

Effects of Powdery Mildew Alleles *Rmd-c*, *Rmd*, and *rmd* on Yield and Other Characteristics in Soybean

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ABSTRACT

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This study was conducted to determine whether soybean (*Glycine max*) yield differs among soybeans nearly isogenic except for reaction to *Microspora diffusa* under a powdery mildew epidemic. Eleven isolines of cvs. Clark and Williams containing the powdery mildew reaction alleles *Rmd-c* (resistant), *Rmd* (adult-plant resistant), and *rmd* (susceptible) were evaluated for yield, lodging, height, seed quality, seed mottling, seed weight, and resistance to powdery mildew and brown stem rot. Isolines containing the resistance alleles *Rmd-c* and *Rmd* yielded 18% more than isolines containing *rmd* when stressed by a powdery mildew epidemic. The *Rmd-c* isolines yielded 7% more than the *Rmd* isolines. This difference suggests that it would be advantageous to use *Rmd-c* as a marker for *Rps2* in a soybean breeding program. There also appears to be a potential linkage between the *Rmd* locus and a gene providing resistance to brown stem rot.

Reaction of soybeans (*Glycine max* (L.) Merr.) to powdery mildew, caused by *Microspora diffusa* Cke. & Pk., is controlled by three alleles at the *Rmd* locus: *Rmd-c*, which confers resistance throughout the life of the soybean plant (7); *Rmd*, which provides resistance at the adult-plant stages (11); and *rmd*, which conditions susceptibility (4). *Rmd-c* was found to be in coupling phase linkage with *Rps2*, which controls reaction to *Phytophthora* root rot, caused by *P. sojae* Kaufmann & Gerdemann, with a recombination frequency of 2.3% (8). Although *Rps2* is a dominant gene providing resistance to 18 of the 27 reported races of *P. sojae*, little breeding work has been done using *Rps2* resistance because screening for the gene requires a liquid culture (6). Since powdery mildew reaction can be easily classified in the greenhouse, the *Rmd* gene would be useful as a genetic marker to indirectly select for resistance to *Phytophthora*, allowing *Rps2* to be used more extensively in breeding programs.

In order to obtain the highest yielding cultivars possible, it is important to know whether *Rmd* or *Rmd-c* differentially affects yield of soybeans infected by powdery mildew before the *Rmd* locus is used as a genetic marker in breeding programs. Yield losses of up to 35% have been reported for susceptible soybean cultivars due to natural infection of powdery mildew in the field (5,12). However, no powdery mildew yield loss studies have been performed comparing cultivars or isolines containing different resistance alleles. This study was conducted to determine whether soybean seed yield differs among soybeans nearly isogenic except for reaction to *M. diffusa* under a powdery mildew epidemic.

MATERIALS AND METHODS

Eleven isolines of cvs. Clark and Williams (1) containing *Rmd-c*, *Rmd*, and *rmd* alleles (Table 1) were evaluated at the University of Illinois Agronomy/Plant Pathology South Farm on Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquoll, pH 5.6-7.8, OM 5-7%) and the Cruse Farm on Flanagan silt loam (fine, montmorillonitic, mesic Aquic Argiudoll, pH 5.6-7.3, OM 4-5%) in Urbana in 1991 and 1992. Both Clark and Williams contain the *Rmd* allele, which confers adult-plant resistance to powdery mildew. All the isolines are released except L89-2037, which is a *rmd*

isoline of Clark still under development. Since all *Rmd-c* isolines contain the *Phytophthora*-resistant gene *Rps2*, several *Rmd* isolines containing *Phytophthora*-resistant genes were included (L61-4222, Williams 79, and Williams 82). Three of the Williams isolines containing *Rmd-c* and *Rmd* were yield-tested in 1978 and 1979 in the absence of powdery mildew. There were no differences in yield among these isolines (mean of eight replicates: L76-1988 = 3,560 kg ha⁻¹, Williams = 3,630 kg ha⁻¹, and Williams 82 = 3,730 kg ha⁻¹; R. L. Bernard, personal communication).

Field plots consisted of four rows, 0.76 m apart, 3.3 m long, with a planting rate of 34 seeds m⁻¹. A randomized complete block design with three replications was used. The planting dates were 1 and 2 May in 1991 and 7 and 8 May in 1992. The middle two rows of the plots were inoculated with *M. diffusa* by brushing infected leaves on the plants when the first trifoliolate leaf had expanded (4). Plots were inoculated on 23 May 1991 and 26 May 1992 with inoculum prepared by growing susceptible cultivars Harosoy and Harosoy 63 in a greenhouse where powdery mildew was endemic.

The inoculated plants were observed weekly throughout the growing season for initial signs of powdery mildew and ultimate severity. Plots were scored from 1 to 5 according to percent leaf tissue showing powdery mildew, with 1 = 0%, 2 = 1-25%, 3 = 25-50%, 4 = 50-75%, and 5 = 75-100%. The middle two rows of each plot were harvested for yield and adjusted to 13% moisture. Other plant traits measured included height (centimeters); lodging, scored from 1 (all erect) to 5 (all prostrate); physiological maturity (days after planting); and incidence of leaf symptoms of brown stem rot, caused by *Phialophora gregata* (Allington & D.W. Chamberlain) W. Gams. Seed traits noted were quality, scored from 1 (no diseased, split, or immature seeds) to 5 (>75% of seeds diseased, split, or immature); mottling, scored from 1

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(no mottling) to 5 (severe mottling); and 100-seed weight (grams). Brown stem rot was scored from 1 to 5 according to percent plants displaying symptoms of brown stem rot, with 1 = 0%, 2 = 1–25%, 3 = 25–50%, 4 = 50–75%, and 5 = 75–100%. The scores reported are the maximum observed at the rating taken closest to physiological maturity.

Analysis of variance was used to cal-

culate variances from the data, with years, locations, and blocks designated as random effects and isolines as fixed effects (9). Several single degree of freedom contrasts were then conducted. The *F* test used to test the significance of each contrast was [MS(Contrast) + MS(Year × Location × Isoline)]/[MS(Year × Isoline) + MS(Location × Isoline)]. Satterthwaite's approximation was used

to calculate degrees of freedom for the complicated *F* tests (9).

RESULTS AND DISCUSSION

A powdery mildew epidemic was successfully produced each year, but disease development differed. Powdery mildew was first observed on the susceptible isolines a month later in 1992 than in 1991 (24 June 1991 and 28 July 1992), and the epidemic was more severe in 1991, with maximum powdery mildew scores of 5 for the *rmd* isolines compared with 4 in 1992. These differences may have been due to the suppression of powdery mildew by 414 cm of rainfall during June and July of 1992 compared with 83 cm in 1991 (Illinois State Water Survey). Increased rainfall has been found to suppress powdery mildew (3). Symptoms of powdery mildew were not observed on any isolines containing the resistance allele *Rmd* or *Rmd-c*. Evidence of *Phytophthora* infection was not seen on either susceptible or resistant isolines. However, symptoms of brown stem rot were observed beginning in late August of each year.

Table 1. Genotype and parentage of soybean isolines

Isoline	Genotype ^a			Parentage ^b
L89-2037	<i>rmd</i>	<i>rps1</i>	<i>rps2</i>	Clark(3) × Harosoy
Clark	<i>Rmd</i>	<i>rps1</i>	<i>rps2</i>	Lincoln(2) × Richland
L61-4222	<i>Rmd</i>	<i>Rps1</i>	<i>rps2</i>	Clark(8) × Blackhawk
L76-2060	<i>Rmd-c</i>	<i>rps1</i>	<i>Rps2</i>	Clark(6) × (Harosoy(5) × D54-2437) ^c
L84-2237	<i>rmd</i>	<i>rps1</i>	<i>rps2</i>	Williams(6) × (Clark 63(2) × PI 86.150)
L88-8226	<i>rmd</i>	<i>rps1</i>	<i>rps2</i>	Williams(6) × PI 86.972-1
Williams	<i>Rmd</i>	<i>rps1</i>	<i>rps2</i>	Wayne × (Clark × Adams)
Williams 79	<i>Rmd</i>	<i>Rps1-c</i>	<i>rps2</i>	Williams(6) × Lee 68
Williams 82	<i>Rmd</i>	<i>Rps1-k</i>	<i>rps2</i>	Williams(7) × Kingwa
L76-1988	<i>Rmd-c</i>	<i>rps1</i>	<i>Rps2</i>	Williams(6) × (Harosoy(5) × D54-2437) ^c
L82-1858	<i>Rmd-c</i>	<i>Rps1-c</i>	<i>Rps2</i>	(Williams 79 × L76-1988) × (Williams × Union) × L76-1988

^a Each locus is homozygous for genes as indicated.

^b Numbers in parentheses refer to number of times recurrent parent was crossed.

^c D54-2437 parentage is CNS, Lincoln, Ogden, Richland, and Roanoke.

Table 2. Comparison of alleles for reaction to powdery mildew in soybean isolines using single degree of freedom contrasts

Contrasts ^a	Powdery mildew score ^b	Yield (kg ha ⁻¹)	Seed mottling score ^c	Seed weight (g 100 seeds ⁻¹)	Brown stem rot score ^d
<i>Rmd</i> and <i>Rmd-c</i> vs. <i>rmd</i>	1.0 vs. 4.3** ^e	3,865 vs. 3,277**	1.4 vs. 1.8*	17.0 vs. 15.9**	1.9 vs. 1.9
<i>Rmd-c</i> vs. <i>Rmd</i>	1.0 vs. 1.0	4,034 vs. 3,764*	1.3 vs. 1.4	17.2 vs. 16.8	1.6 vs. 2.1*
Williams (<i>Rmd</i> and <i>Rmd-c</i> vs. <i>rmd</i>)	1.0 vs. 4.3**	4,143 vs. 3,426**	1.3 vs. 1.7*	17.3 vs. 15.8**	1.5 vs. 1.7
Williams (<i>Rmd-c</i> vs. <i>Rmd</i>)	1.0 vs. 1.0	4,279 vs. 4,052	1.3 vs. 1.3	17.4 vs. 17.3	1.2 vs. 1.7*
Clark (<i>Rmd</i> and <i>Rmd-c</i> vs. <i>rmd</i>)	1.0 vs. 4.3**	3,402 vs. 2,977*	1.5 vs. 2.0	16.3 vs. 16.1	2.7 vs. 2.4
Clark (<i>Rmd-c</i> vs. <i>Rmd</i>)	1.0 vs. 1.0	3,545 vs. 3,331	1.5 vs. 1.6	16.7 vs. 16.1	2.6 vs. 2.7
Ps (<i>Rmd-c</i> vs. <i>Rmd</i>)	1.0 vs. 1.0	4,034 vs. 3,887	1.3 vs. 1.4	17.2 vs. 16.8	1.6 vs. 2.1
Williams Ps (<i>Rmd-c</i> vs. <i>Rmd</i>)	1.0 vs. 1.0	4,279 vs. 4,200	1.3 vs. 1.2	17.4 vs. 17.4	1.2 vs. 1.7

^a *Rmd* = adult-plant resistant, *Rmd-c* = resistant, and *rmd* = susceptible to powdery mildew; Ps = *Phytophthora* resistant.

^b From 1 = no symptoms to 5 = 75–100% infected leaf tissue.

^c From 1 = no mottling to 5 = severe mottling.

^d From 1 = no symptoms to 5 = 75–100% incidence.

* = Significant at *P* = 0.05, ** = significant at *P* = 0.01.

Table 3. Mean response of soybean isolines under field conditions at the University of Illinois Agronomy/Plant Pathology South Farm and the Cruse Farm in Urbana in 1991 and 1992 following inoculation with *Microsphaera diffusa*

Recurrent parent Isoline	Allele	Seed yield (kg ha ⁻¹)	Powdery mildew score ^a	Maturity ^b (dap)	Lodging score ^c	Height (cm)	Seed quality score ^d	Seed mottling score ^e	Seed weight (g 100 seeds ⁻¹)	Brown stem rot score ^f
Clark										
L89-2037	<i>rmd</i>	2,977	4.3	110.5	2.8	110	1.5	2.0	16.1	2.4
Clark	<i>Rmd</i>	3,401	1.0	111.5	2.3	107	1.9	1.6	16.2	2.5
L61-4222	<i>Rmd</i>	3,261	1.0	107.7	2.7	104	1.6	1.6	16.0	2.8
L76-2060	<i>Rmd-c</i>	3,545	1.0	110.7	2.6	105	1.6	1.5	16.7	2.6
Williams										
L84-2237	<i>rmd</i>	3,319	4.3	107.3	1.5	105	1.8	2.1	15.5	2.1
L88-8226	<i>rmd</i>	3,533	4.3	104.0	1.8	103	1.3	1.4	16.0	1.3
Williams	<i>Rmd</i>	3,757	1.0	109.2	1.5	105	1.5	1.2	17.1	1.8
Williams 79	<i>Rmd</i>	4,255	1.0	105.5	2.1	105	1.3	1.3	17.5	1.5
Williams 82	<i>Rmd</i>	4,145	1.0	110.0	1.9	105	1.4	1.3	17.3	1.8
L76-1988	<i>Rmd-c</i>	4,402	1.0	106.8	2.1	103	1.5	1.3	17.2	1.2
L82-1858	<i>Rmd-c</i>	4,156	1.0	106.0	2.0	106	1.4	1.3	17.7	1.1

^a From 1 = no symptoms to 5 = 75–100% infected leaf tissue.

^b dap = Days after planting.

^c From 1 = all erect to 5 = all prostrate.

^d From 1 = no diseased, split, or immature seeds to 5 = >75% of seeds diseased, split, or immature.

^e From 1 = no mottling to 5 = severe mottling.

^f From 1 = no symptoms to 5 = 75–100% incidence.

There were no significant isolate \times year, isolate \times location, or isolate \times year \times location interactions observed for yield, indicating that the isolines performed consistently among years and locations. Isolines containing the resistance alleles *Rmd-c* and *Rmd* yielded 18% more than the isolines containing *rmd* when infected by *M. diffusa* (Table 2). Part of this difference was due to a 7% decrease in seed weight in the *rmd* isolines (Table 2). The *Rmd-c* and *Rmd* isolines also had a 0.4 lower seed mottling score than the *rmd* isolines (Table 2). Since seed mottling has been associated with soybean mosaic virus (14), this suggests there may be more soybean mosaic virus infection of susceptible isolines. Roane and Roane (13) and Buss et al (2) have observed an association between the disease incidence of powdery mildew and viruses in virus nurseries and the surrounding virus-free soybean fields. It is not known if this association is due to genetics, interaction of the diseases, or mechanical spreading of powdery mildew from greenhouses into the virus nurseries when soybean plants are inoculated with the virus. The lack of differences in other traits measured is a good indication that the isolines are near-isogenic.

When resistance alleles are compared, *Rmd-c* isolines yielded 7% more than *Rmd* isolines, possibly due to latent infection of the *Rmd* isolines (Table 2). There were no differences among the isolines for other traits measured, except in the brown stem rot score. Because all the *Rmd-c* isolines contain a *Phytophthora*-resistant gene from the cultivar CNS (Table 1), a single degree of freedom contrast was made between *Rmd-c* and the *Rmd* isolines that have genes for *Phy-*

tophthora resistance. Within these *Phytophthora*-resistant isolines there was no yield difference.

A confounding factor in these contrasts is the difference in the level of brown stem rot resistance between the *Rmd-c* and *Rmd* alleles in the Williams background. One of the *rmd* and both of the *Rmd-c* Williams isolines were more resistant to brown stem rot than the *Rmd* isolines (Table 3). With symptoms of brown stem rot showing up in late August of each year, some of the yield difference could have been due to brown stem rot. However, Mengistu and Grau (10) calculated a yield loss curve for brown stem rot which found that a 30% reduction in leaf area is needed for a 5% reduction in yield, and none of the *Rmd* isolines had sufficient reduction in leaf area to cause a 5% reduction in yield.

The 7% yield advantage of *Rmd-c* over *Rmd* indicates that in a soybean breeding program it would be advantageous to use *Rmd-c* as marker for *Rps2*. Since symptoms of powdery mildew were not seen on adult-plant resistant isolines in the field, it is necessary to classify lines in the greenhouse to differentiate those containing *Rmd* from those containing *Rmd-c*. The association of brown stem rot resistance with *Rmd-c* should be studied to see if there is any linkage involved. Since the three Williams isolines that have either the *Rmd-c* or the *rmd* allele display an increased level of brown stem rot resistance, there is a potential linkage between *Rmd* and a brown stem rot reaction gene. The association of mottling with powdery mildew is also interesting, since it has been observed previously in virus nurseries and now in association with a field inoculated with powdery mildew. The breeding value of

this region of the genome would increase if more disease resistance genes are found to be tightly linked to the *Rmd* locus.

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