Inheritance of Resistance to *Peronospora manshurica* Races 2 and 33 in Soybean

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ABSTRACT

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This study determined the inheritance of resistance to races 2 and 33 of *Peronospora manshurica* in the soybean cultivars Fayette and PI 88.788 and identified the gene (or genes) conferring this resistance. F_2 progeny from the susceptible cultivar Williams 82 × Union and from Williams 82 × Fayette segregated in a ratio of 3 resistant:1 susceptible when inoculated with race 2. F_3 families from Williams 82 × Fayette segregated in a ratio of 1 resistant:2 segregating for resistance:1 susceptible, confirming that resistance in Fayette is conferred by a single dominant gene. The F_2 and F_3 progenies from Union × Fayette and Union × PI 88.788 segregated in a ratio of 3 resistant:1 susceptible when inoculated with race 33 and in a ratio of 15 resistant:1 susceptible when inoculated with race 2, indicating that the gene for resistance in the both Fayette and PI 88.788 segregates independently from the *Rpm* gene of Union. Fayette was developed from Williams × PI 88.788 for resistance to the soybean cyst nematode. Williams is susceptible to both races of *P. manshurica*. The resistance to these races in Fayette was probably transferred from PI 88.788. The gene symbol Rpm_2 was assigned to the gene in PI 88.788. The resistant parents were symptomless, but in crosses of a parent susceptible to race 2 or race 33 with a parent resistant to that race, the F_1 progeny developed small flecks. The segregation ratios in the F_2 and F_3 progenies indicate that homozygous resistant plants are symptomless and heterozygous resistant plants develop small flecks. The F_1 progeny from resistant × resistant crosses were symptomless when inoculated with race 2, indicating an epistatic effect of the two resistance genes at different loci.

Downy mildew of soybean (*Glycine max* (L.) Merr.), caused by *Peronospora manshurica* (Naum.) Syd. ex Gäum., occurs throughout the world. Currently, 33 races of the pathogen are recognized (6,8–10). Various sources of resistance to *P. manshurica* have been identified in soybean (1,4,7,10), but little is known about the genetics of the resistance. Most sources are resistant to some but not all of the known races of *P. manshurica* (4,7). Prior to the occurrence of race 33 (10), soybean plants carrying the gene *Rpm* were reported to be resistant to all of the known races of the fungus (1,2); however, race 33 is virulent on soybean plants with the *Rpm* gene (10). Since race 33 was detected in the soybean disease-monitoring plots throughout Illinois in 1981 (10), it has also been observed on the soybean cultivar Union, carrying the *Rpm* gene, which was transferred from the cultivar Kanrich (2). The severity of downy mildew on Union varied from 5 to 10% (S. M. Lim, *unpublished*). Currently, several soybean cultivars are known to be resistant to race 33 and race 2, which occurs commonly in Illinois (10).

The objectives of this study were to determine the inheritance of resistance to races 2 and 33 of *P. manshurica* in the soybean cultivars Fayette and PI 88.788 and to determine whether the gene (or genes) conferring the resistance in these two genotypes are allelic with the gene *Rpm*, which confers resistance to race 2 in Union.

MATERIALS AND METHODS

Races 2 and 33 of *P. manshurica* were isolated from the soybean-monitoring plots in Illinois (10,11). Sporangia collected from a single lesion on leaves of Williams 82 infected with race 2 and of Union infected with race 33 were increased by repeated inoculation of seedlings of Williams 82 with race 2 and seedlings of Union with race 33. The plants inoculated with race 2 and those inoculated with race 33 were kept in separate rooms of a greenhouse. Seed of both cultivars was planted at biweekly intervals in clay pots (15 cm in diameter) filled with a sterilized mixture of clay-loam and sand (2:1, v/v). Sporangial suspensions (3,000–5,000 sporangia per milliliter) of each race were sprayed on seedlings of Williams 82 and Union when the first trifoliolate leaves were completely expanded. The inoculated seedlings were covered with plastic bags and sealed for 16–20 hr at 22–24 C in the greenhouse to induce infection. Ten to 14 days after inoculation, seedlings with downy mildew lesions were sprayed with water and covered with the plastic bags overnight (for 16 hr) to induce sporulation. Sporangia produced on the lower side of leaves were collected by gently brushing lesion surfaces with a number 3 camel hair brush and dipping the brush into a 250-ml beaker of water with 1 ml of 0.1% Tween 20. This procedure was repeated to obtain fresh inocula for each inoculation. Seedlings of Union inoculated with race 2 did not produce downy mildew lesions, and seedlings inoculated with race 33 produced downy mildew lesions that served as checks for the races.

The soybean cultivars Fayette, Union, Williams 82, and PI

88.788 were used as parents in the following crosses: Williams 82 × Union, Williams 82 × Fayette, Union × Fayette, and Union × PI 88.788. Williams 82 is susceptible to races 2 and 33 of *P. manshurica*. Union has the *Rpm* gene, which confers resistance to race 2, but is susceptible to race 33. Fayette and PI 88.788 are resistant to both races. Crosses were made during the 1984 growing season at the Agronomy and Plant Pathology Farm, Urbana, Illinois. F_1 progeny, ranging from seven to 17 plants per cross, were grown in the greenhouse in clay pots (20 cm in diameter) filled with a sterilized mixture of clay-loam and sand (2:1, v/v) to produce the F_2 progeny during the winter of 1984 and the spring of 1985. Five to nine F_1 seedlings from each cross were inoculated with each of the two races; 20 seedlings of each parent were also inoculated with each race. Ten to 12 days after inoculation, the reactions of the seedlings were classified on the basis of the development of lesions on unifoliolate leaves: symptomless seedlings and those that developed flecks were classified as resistant, and those with chlorotic lesions were classified as susceptible. F_2 seeds from individual inoculated F_1 plants of each cross were harvested separately to maintain individual F_2 populations. A portion of the seed harvested from the F_1 plants was planted in the field in 1985 to produce F_2 plants. Seed from these F_2 plants was harvested to form F_3 families within each cross.

During the winter of 1985, a series of experiments was performed in the greenhouse to evaluate reactions of F_2 plants and F_3 families to races 2 and 33. In each experiment, F_2 progeny from each cross were evaluated for their reactions to each of the two races. Twenty-four to 30 seedlings from each F_3 family were also evaluated for their reactions to races 2 and 33. Seeds were planted in clay pots (15 cm in diameter) filled with a sterilized mixture of clay-loam and sand (2:1, v/v). Each pot contained two or three seedlings. The number of F_2 seedlings evaluated in these experiments varied from 98 to 242, and the number of F_3 families varied from 55 to 106. The seedlings in each experiment were inoculated when the first trifoliolate leaves were partially expanded. Sporangial suspensions of each race were sprayed on leaves in separate moist chambers (100% relative humidity), and the plants were kept in the chambers overnight (for 16 hr) at 22–24 C without light. The seedlings were then moved to the greenhouse and placed on separate benches on the basis of the race with which they had been inoculated. Ten to 12 days after inoculation, the reactions of the inoculated seedlings were classified as described above. The chi-square (χ^2) test was used to determine the goodness of fit of the observed F_2 and F_3 segregations to the expected ratios for one- and two-dominant-gene models, respectively.

RESULTS AND DISCUSSION

The reactions of the four parents to races 2 and 33 of *P. manshurica* were as described previously (10). Williams 82 was susceptible to both races, Union was resistant to race 2 (symptomless) but susceptible to race 33, and Fayette and PI 88.788 were resistant to both races (symptomless) (Tables 1 and 2). However, one plant of 20 Fayette seedlings was susceptible to race 2, and two of 20 seedlings were susceptible to race 33. The Fayette seedling that was susceptible to race 2 was also susceptible to race 33, and the two seedlings that were susceptible to race 33 were also susceptible to race 2, when newly grown leaves were inoculated with these two races. Prior to these cross-inoculations of new leaves, leaves infected from the first inoculations were removed. Thus, it appeared that Fayette is not homogeneous for downy mildew resistance. Fayette was developed from Williams × PI 88.788 in order to develop resistance to the soybean cyst nematode (*Heterodera glycines* Ichinohe) (3). Williams is susceptible to races 2 and 33 of *P. manshurica*, and thus the resistance to these races in Fayette was probably transferred from PI 88.788. Nevertheless, resistance to race 33 of *P. manshurica* was not selected for in Fayette, because this cultivar was released prior to the identification of this race. It has previously been observed that downy mildew occurs on Fayette at a low incidence (about 5–10% incidence in susceptible plants) in fields in central and southern Illinois (S. M. Lim, *unpublished*), which also indicates that Fayette is heterogeneous for reaction to downy mildew.

The F_1 seedlings from Williams 82 × Union and Williams 82 × Fayette were resistant but developed small flecks on infected

TABLE 1. Seedling reactions to *Peronospora manshurica* race 2 for the parents and progeny of crosses of susceptible and resistant soybeans

Parent or cross	Expected ratio R:Seg:S[a]	Observed reactions[a] (no. of plants or families) R	Seg	S	χ^2	*P*
Williams 82	All S	0	0	20		
Union	All R	20	0	0		
Fayette	All R	19	0	1		
PI 88.788	All R	20	0	0		
Williams 82 × Union						
F_1	All R	0 (7)	0	0		
F_2	3:0:1	46 (74)	0	32	1.26	0.27
Williams 82 × Fayette						
F_1	All R	0 (6)	0	0		
F_2	3:0:1	36 (55)	0	27	0.28	0.62
F_3[b]	1:2:1	22	37	16	0.97	0.62
Union × Fayette						
F_1	All R	7 (0)	0	0		
F_2	15:0:1	97 (29)	0	8	0.28	0.61
F_3	7:8:1	26	29	2	0.74	0.69
	(7:4:4:1)[c]	26	12 17	2	2.15	0.55
Union × PI 88.788						
F_1	All R	9 (0)	0	0		
F_2	15:0:1	179 (52)	0	11	1.20	0.28
F_3	7:8:1	52	54	4	1.53	0.47
	(7:4:4:1)	52	21 33	4	4.15	0.25

[a] R = resistant—no symptoms or small flecks (less than 2 mm in diameter and irregular in shape), the latter indicated in parentheses; Seg = segregating for resistance; S = susceptible—large chlorotic lesions (more than 2 mm in diameter and angular).
[b] Twenty-six to 30 seedlings of each F_3 family were evaluated for resistance.
[c] A ratio of 7 resistant:4 segregating (15 resistant:1 susceptible):4 segregating (3 resistant:1 susceptible):1 susceptible.

TABLE 2. Seedling reactions to *Peronospora manshurica* race 33 for the parents and progeny of crosses between susceptible and resistant soybeans

Parent or cross	Expected ratio R:Seg:S[a]	Observed reactions[a] (no. of plants or families) R	Seg	S	χ^2	*P*
Williams 82	All S	0		20		
Union	All S	0		20		
Fayette	All R	18		2		
PI 88.788	All R	20		0		
Williams 82 × Fayette						
F_1	All R	0 (5)		0		
F_2	3:0:1	33 (42)		23	0.12	0.74
F_3[b]	1:2:1	28	43	19	1.97	0.38
Union × Fayette						
F_1	All R	0 (6)		0		
F_2	3:0:1	38 (61)		29	0.38	0.55
F_3	1:2:1	17	26	12	1.07	0.59
Union × PI 88.788						
F_1	All R	0 (8)		0		
F_2	3:0:1	31 (56)		22	1.57	0.46
F_3	1:2:1	18	25	13	1.53	0.47

[a] R = resistant—no symptoms or small flecks (less than 2.0 mm in diameter and irregular in shape), the latter indicated in parentheses; Seg = segregating for resistance; S = susceptible—large chlorotic lesions (more than 2.0 mm in diameter and angular).
[b] Twenty-four to 30 seedlings of each F_3 family were evaluated for resistance.

leaves when inoculated with race 2 (Table 1). Sporangia were not produced on the lower side of leaves with small flecks enclosed in moist plastic bags for 16 hr. This was considered a resistant reaction. The F_2 progeny from these crosses segregated in a ratio of 3 resistant:1 susceptible when inoculated with race 2 (Table 1). The chi-square values for testing the goodness of fit to an expected ratio of 3:1 were acceptable ($P \geq 0.27$). The F_2 progeny segregated for symptomless plants, plants with small flecks, and plants with large chlorotic lesions in a 1:2:1 ratio ($\chi^2 = 2.68$ and $P = 0.27$ for Williams 82 × Union; $\chi^2 = 1.91$ and $P = 0.40$ for Williams 82 × Fayette), indicating that small flecks are characteristic of heterozygous resistant plants. The F_3 families from Williams 82 × Fayette segregated in a ratio of 1 resistant:2 segregating (in a ratio of 3 resistant:1 susceptible):1 susceptible, confirming that the resistance in Fayette to race 2 is conferred by a single dominant gene, similar to the *Rpm* gene, which confers resistance to race 2 in Union.

It has previously been reported (1) that plants in segregating F_3 lines from backcross populations of susceptible × resistant soybeans carrying the gene *Rpm* segregated in a ratio of 1 resistant:2 intermediate:1 susceptible when inoculated with isolates of *P. manshurica* obtained from seeds encrusted with downy mildew that were produced in Illinois. Resistant plants were symptomless, intermediate plants developed small chlorotic spots, and susceptible plants developed large chlorotic lesions. Plants with the intermediate reaction were heterozygous for downy mildew resistance. Plant age, the stage of development of the infected leaf, inoculum concentration, and environment also influenced the development of downy mildew (12). Thus, a wide range of reactions can occur in a single soybean genotype. In this study, small flecks on resistant plants and chlorotic lesions on susceptible plants were distinctive in size and shape. The small flecks were usually less than 2 mm in diameter and irregular in shape, whereas the chlorotic lesions were more than 2 mm in diameter and mostly angular. Often, chlorotic lesions on infected leaves of susceptible plants merged and developed large, coalescent chlorotic areas that were unrestricted by leaf veins. The reaction of unifoliolate leaves to *P. manshurica* was the most consistent measure for classifying resistance and susceptibility, because the distinctive size and shape of lesions were maintained longer on unifoliolate leaves.

The F_1 progeny from Union × Fayette and Union × PI 88.788 were symptomless when inoculated with race 2 (Table 1). The F_2 progeny from these two crosses segregated in a ratio of 15 resistant:1 susceptible when inoculated with race 2, with acceptable χ^2 values ($P \geq 0.28$). If the resistant F_2 progeny were separated into those with symptomless reactions and those with small flecks, the ratio of segregation was 11 symptomless (at least two dominant alleles for resistance):4 small flecks (only one dominant allele for resistance):1 large chlorotic lesions (double-recessive homozygote) for both crosses ($\chi^2 = 0.28$ and $P = 0.61$ for Union × Fayette; $\chi^2 = 3.23$ and $P = 0.20$ for Union × PI 88.788). This segregation ratio indicates that plants heterozygous dominant at both loci develop no downy mildew symptoms. This is the first report in which segregation patterns of resistant × resistant crosses have indicated an epistatic effect for heterozygous resistance genes at different loci. In reaction to race 2, the F_3 families derived from individual F_2 plants from the crosses between two resistant parents segregated in a ratio of 7 homozygous resistant:8 segregating for resistance (4 segregating in a ratio of 15 resistant:1 susceptible and 4 segregating in a ratio of 3 resistant:1 susceptible):1 homozygous susceptible. These ratios confirm that different, unlinked genes control resistance in Union and PI 88.788. The gene symbol Rpm_2 is assigned to the gene in PI 88.788.

In the F_1, F_2, and F_3 progeny from the susceptible × resistant crosses (Williams 82 × Fayette, Union × Fayette, and Union × PI 88.788), the reactions to race 33 were similar to the reactions to race 2. All of the F_1 seedlings developed small flecks, and the ratios of segregation in the F_2 and F_3 progeny fit ratios of 3 resistant:1 susceptible and 1 resistant:2 segregating:1 susceptible, respectively (Table 2). These results indicate that resistance to race 33 in Fayette and PI 88.788 is conferred by a single dominant gene, probably the same gene that confers resistance to race 2. Also, the resistance in Fayette and PI 88.788 is probably conferred by the same gene. The reaction of F_2 progeny from Fayette × PI 88.788 to the two races would confirm whether the same gene confers the resistance in both soybean lines. However, this cross was not made, since Fayette was developed from Williams × PI 88.788. Also, the reaction of the same individual F_2 plants to both races through sequential inoculations with the two races should clarify whether the same gene confers resistance to both races. However, it is difficult to inoculate separate unifoliolate leaves of a large number of single plants with the two races sequentially.

Downy mildew usually does not significantly reduce soybean yield, but severe infection can cause yield reduction and poor seed quality (1,5,8,9). The prevalence of downy mildew over a wide geographic range and the diversity of the races in the United States indicate its potential significance in soybean production. Several studies (1,4,7,10) indicate that other genes conferring resistance to races of *P. manshurica* are yet to be identified.

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