Adult Plant Phenotype of the *Rp1-DJ* Compound Rust Resistance Gene in Maize

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ABSTRACT

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The complex structure of the *rp1* rust resistance locus of maize allows two or more resistance genes to be recombined together in coupling phase. The phenotypic effects of the *Rp1-DJ* compound gene, which carries both *Rp1-D* and *Rp1-J*, were analyzed. The *Rp1-DJ* compound gene was associated with a chlorotic spotting phenotype in some genetic

backgrounds. At the seedling stage, lines carrying *Rp1-DJ* are fully susceptible to *Puccinia sorghi* biotype HI1, which is virulent on lines with the two genes singly. At later stages of growth, however, *Rp1-DJ* lines show partial resistance when compared with sibling lines not carrying the compound gene. The *Rp1-DJ* gene also confers partial resistance to *P. polysora* in adult plants.

Additional keywords: durable resistance, lesion mimic.

Two *Puccinia* species cause rust diseases of maize (*Zea mays* L.): *P. sorghi* Schwein. causes common rust and *P. polysora* Underw. causes southern rust. Under favorable conditions, each rust disease can result in heavy yield losses for susceptible genotypes (8,12,17,22,23). Resistance to common rust has been studied fairly extensively and is generally classified as either race-specific or general (8,12). Race-specific resistance is controlled by single genes that are usually dominant and well-expressed at the seedling stage. General resistance is manifested by smaller and fewer pustules (uredinia) and is, therefore, also referred to as partial resistance (16). General resistance is typically most discernible in adult plants, but can also be observed in seedlings (7). It is usually controlled by multiple genes (5,8,12,19), making it difficult to incorporate into breeding programs. General resistance to common rust is thought to be non-race-specific. Although experimental evidence for this view is weak, the resistance of maize lines with high levels of general resistance is consistent from year to year, indicating it is nonspecific resistance that is not dependent on pathogen biotype. Patterns of resistance to southern rust appear similar to common rust resistance, with both race-specific and general forms of resistance (1,31). General resistance to common and southern rust is thought to have played an important role historically in controlling these diseases (8,21,32).

Most of the identified, race-specific genes conferring resistance to *P. sorghi* in maize map to the *Rp1* area (8,24,33), which includes the *rp1* complex, as well as some other linked resistance genes such as *RpG* and *Rp5*. The structure of the *rp1* complex allows two or more different *Rp1* 'alleles' to be recombined into a single haplotype (in coupling phase). A number of these multiple-gene *rp1* haplotypes have been generated and are referred to as compound genes (9).

Several individual *Rp* and *Rpp* genes usually provide high levels of resistance to common or southern rust in the field (6,11,18,31). The effectiveness of resistance based on a single

gene would not last very long, however, if large acreages of lines with the gene were planted, because of the large variation of both common and southern rust biotypes (8). For example, none of the known *Rp* genes provide resistance to all of the common rust biotypes collected in North America in the last several years (11). Durability of resistance may be enhanced by pyramiding different resistance genes into a single line. Common rust resistance in maize may be well-suited to this approach, because genes in the *rp1* complex can be combined and then manipulated as a single gene. Several compound genes have been created that are resistant to all known biotypes (9). The durability of resistance of these pyramids, however, is unpredictable. A more ideal form of resistance may be one that can be manipulated as a single gene, but is non-race-specific.

One of the most interesting aspects of three previously identified *Rp1* variants that had severe lesion mimic phenotypes is that they appeared to have nonspecific resistance to common rust (10). A chlorotic/necrotic-spotting phenotype, similar to a weak lesion mimic phenotype, has been observed on the older leaves of adult plants of maize lines carrying both *Rp1-D* and *Rp1-J* combined together (*Rp1-DJ*), as well as several other compound *Rp1* genes (G. Hu and S. H. Hulbert, *unpublished data*). The present study was undertaken to determine if the spotting phenotype observed in the *Rp1-DJ* line was caused by the compound resistance gene and if lines carrying *Rp1-DJ* had detectable non-race-specific resistance to rust.

MATERIALS AND METHODS

Genetic materials and analysis of the chlorotic-spotting phenotype. Two lines carrying *Rp1-DJ* compound genes were previously generated (9) by test-crossing *Rp1-D/Rp1-J* heterozygotes to susceptible *rp1/rp1* lines and screening the subsequent progeny with the appropriate *P. sorghi* biotypes for individuals that carried both *Rp1-D* and *Rp1-J*. The two recombinants carrying both genes were recovered from 6,909 test cross progeny. The *rp1* genotypes of the recombinants were designated *Rp1-DJ4* and *Rp1-DJ46*. The genetic background of these original recombinants was highly heterozygous, because of the nature of the families

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from which they were derived. The *Rp1-D* parent had the B14 background, the *Rp1-J* parent had the R168 inbred background, and the *rp1/rp1* tester parent was either OH43 (for *Rp1-DJ46*) or A188 (for *Rp1-DJ4*).

The heterozygous *Rp1-DJ4* recombinant was self-fertilized, and random progeny were self-fertilized again. The resulting families were referred to as F_3 families, although the F_1 was the original recombinant and was selected from a test cross, not a hybrid between two inbred lines. F_3 families were selected that were homozygous for the *Rp1-DJ* compound gene and homozygous for the *rp1* (A188) allele.

The *Rp1-DJ* compound gene was crossed into three different genetic backgrounds to test for genetic background effects. Self-fertilized progeny of the original *Rp1-DJ4* and *Rp1-DJ46* recombinants were selected that carried the *Rp1-DJ* compound gene, and these were then crossed to the inbreds W23, A188, and H95. The resulting F_1 families carrying the compound gene were then backcrossed to their respective inbreds. Single BC_1 plants carrying either *Rp1-DJ4* or *Rp1-DJ46* for each of the three backgrounds were then selected and self-fertilized. The resulting six BC_1F_1 families were segregating for one of the two *Rp1-DJ* compound genes (*Rp1-DJ4* or *Rp1-DJ46*) in each of three different genetic backgrounds.

The six BC_1F_1 families were planted in single rows in the field in 1994 to examine for chlorotic-spotting phenotypes (see below). Each row was thinned to 30 plants, except for the *Rp1-DJ4*-W23 family, where poor germination limited the number of plants to 16. Attempts were made to self-fertilize each plant in each row, to create BC_1F_2 families. Two weeks after pollination, individual plants were scored for the extent of spotting using a 0 to 10 scale. Ratings of 0 to 2 indicated zero to a few noticeable chlorotic spots, whereas ratings of 8 to 10 indicated that most or all of the leaf area below the cob was covered with chlorotic or necrotic spots. The spotting score for each plant that was self-fertilized was recorded directly on the bag used to cover the ear after pollination.

The *rp1* genotype of each field-grown BC_1F_1 plant that produced self-pollinated seed was determined in the greenhouse in the fall of 1994 by testing the progeny for rust resistance. Twenty progeny seedlings from each individual were inoculated with rust biotype IN2, which is avirulent on *Rp1-D* and, therefore, avirulent on the compound gene. If all 20 seedlings were resistant, it was concluded the parental BC_1F_1 plant was homozygous for the *Rp1-DJ* compound gene. If all twenty were susceptible, it was concluded that the parent was homozygous for *rp1*, and it was concluded the parent was heterozygous if the progeny seedlings segregated. Families that were homozygous for the compound gene, or homozygous for the *rp1* allele, were selected for comparisons of resistance to rust biotype HI1 (see below).

To make a set of families that was more isogenic than the BC_1F_2 families, a single *Rp1-JD4*-A188 BC_1 plant was backcrossed a second time to the inbred A188. A single BC_2 plant heterozygous for *Rp1-DJ4* was self-fertilized in the greenhouse in the fall of 1995. The resulting BC_2F_1 family was grown in a single row (30 plants) in the field in the summer of 1995. Individual BC_2F_1 plants in the row were scored for the intensity of their spotting phenotype and then self-fertilized, as above. Seedlings of the resulting BC_2F_2 families were screened with rust biotype IN2 to determine which were homozygous for the compound gene, segregating, or homozygous for the *rp1* allele. Three families of each homozygote class were selected for comparison of resistance to *P. polysora* (see below). Theoretically, the BC_2F_2 families should have had A188 alleles for roughly 87% of their genes, excluding the *rp1* area where they were selected for either the A188 allele or the compound gene.

Rust inoculation and resistance evaluation of adult plants.

Rust resistance at the adult plant stage was determined for parental lines and lines carrying the compound gene in different back-

grounds. Near-isogenic lines carrying the compound gene are not yet available, so several related lines, with or without the compound gene in a similar genetic background, were compared in each experiment. The first experiment included six F_3 families that were all derived from a single F_2 family, which was generated by self-fertilization of the original *Rp1-DJ4* recombinant. The original recombinant was heterozygous at *Rp1*, enabling selection of F_3 families that were homozygous for either the susceptible *rp1* allele or the *Rp1-DJ4* compound gene. The second experiment compared six BC_1F_2 -W23 families, three that were homozygous for *Rp1-DJ46* and three that carried the *rp1* allele from W23. Experiment three compared five BC_1F_2 -H95 families of which three were *Rp1-DJ46* homozygotes and the other two carried the *rp1* allele from H95. Experiments 4 and 5 compared three BC_1F_2 -A188 families homozygous for *Rp1-DJ46* with three families homozygous for the *rp1* allele from A188. Experiment six compared three BC_2F_2 -A188 families homozygous for *Rp1-DJ4* with three families that were homozygous for the A188 allele.

In each experiment, three replications (blocks) of four plants each for each family were arranged in a randomized complete block design in which a single greenhouse bench was divided into three sections, as blocks. Plants were grown individually in 4-liter pots filled with a soil/peat mixture (3:1, vol/vol) and watered daily with a dilute fertilizer (20-20-20) solution. Greenhouse temperatures were set at 18/27°C (day/night), and supplemental lighting was supplied during the 15-h day.

Plants were inoculated when they had six to seven fully expanded leaves. Plants were sprayed with a suspension of freshly harvested uredospores in solitrol oil. Experiments 1 to 4 used approximately 200 mg of *P. sorghi* biotype HI1 uredospores diluted in 40 ml of oil, while experiment 5 used 400 mg of HI1 in 40 ml of oil. Experiment 6 used 400 mg of *P. polysora* uredospores diluted in 40 ml of oil. After allowing the oil to evaporate, the plants were misted with water, and a plastic tent was constructed over the entire greenhouse bench. Four humidifiers were placed inside the tent to maintain free moisture on the leaves, and the plants were incubated for 16 h. Pustules from inoculations with *P. sorghi* were counted at 10 to 12 days after inoculation, and pustules from the *P. polysora* inoculation were counted 16 days after inoculation. The HI1 biotype of *P. sorghi*, which is virulent on both *Rp1-D* and *Rp1-J*, was used for experiments 1 to 5. The *P. polysora* isolate used in experiment 6 was collected from a field in Manhattan, KS, in 1995 and is virulent on all known *Rp* genes.

Data on the number of pustules per plant were examined by analysis of variance (ANOVA) using the SAS (Statistical Analysis Software) for Windows, release 6.10 (SAS Institute, Inc., Cary, NC). Family means were compared by both Fishers' least significant difference and Duncans' new multiple range test. No differences in groupings were observed between the two statistical tests.

RESULTS

Association of the chlorotic-spotting phenotype with the compound *Rp1* gene. When self-fertilized progeny from the recombinant *Rp1-DJ4* were grown in the field in 1992, a chlorotic-spotting phenotype was observed on some progeny. Spots were slightly yellow-green and were easily visualized with some back-lighting (Fig. 1A). The spots were usually 1 to 2 mm in diameter and were not noticeable until the plants had at least five to six fully expanded leaves, and, in some cases, were not noticeable until the flowering stage. The lower leaves always had more severe spotting than the upper leaves on the same plant. The extent or severity of spotting appeared nearly continuous in distribution. Of 28 plants that were self-fertilized from this family, six were determined to be homozygous for *rp1* by progeny testing. None of these six showed a chlorotic-spotting phenotype. The remaining 22 plants were either homozygous or heterozygous for the com-

pound gene. Of these 22, 13 showed a noticeable chlorotic-spotting phenotype. Since some of the plants that carried the compound gene showed a noticeable chlorotic-spotting phenotype, but none of those homozygous for the *rp1* allele did, we postulated that the compound gene contributed to the phenotype.

To test the association of the *Rp1* genotype with the chlorotic-spotting phenotype, six different BC₁F₁ families segregating for the *Rp1-DJ* compound gene were planted in the field in 1994. Several individuals in each of the families carrying either the *Rp1-DJ46* or *Rp1-DJ4* recombinant genes in A188 and H95 backgrounds expressed the chlorotic-spotting phenotype very well (Table 1). Plants in each of these four families had ratings as high as 8 to 10, indicating that most or all of the leaves below the ear were covered with chlorotic or necrotic spots at maturity. All individuals with a rating of 6 or higher were either homozygous or heterozygous for the compound gene. On the average, plants that were homozygous for *Rp1-DJ4* or *Rp1-DJ46* showed the highest chlorotic-spotting scores, while *rp1* homozygotes showed the lowest. For example, plants in the *Rp1-DJ46* BC₁F₁-A188 family that were homozygous for the compound gene had average ratings of 5.4, while heterozygotes had average ratings of 3.1 and *rp1* homozygotes averaged 0.7 (Table 1).

Expression of the chlorotic-spotting phenotype was less noticeable in BC₁F₁ families carrying *Rp1-DJ46* or *Rp1-DJ4* in the W23 background. None of 25 plants examined in the *Rp1-DJ46* family had a chlorotic-spotting score greater than 4. Similarly, no plants

in the *Rp1-DJ4* family had a score higher than 4, but only 16 plants were available for analysis.

A BC₂F₁ family segregating for *Rp1-DJ4* in the A188 background was examined in the field in 1995. The chlorotic-spotting phenotype was generally not as severe as in the families grown in 1994, and the highest ratings were 4. However, as with the families examined in 1994 in the A188 and H95 backgrounds, the chlorotic-spotting phenotype appeared dependent on the *Rp1* genotype. Of 21 individuals scored and progeny tested, eight were homozygous for *Rp1-DJ4* and had an average rating of 3.6, nine were heterozygous and averaged a rating of 1.9, and four were homozygous for *rp1* and averaged a rating of 0.8.

Adult plant resistance to common rust from the compound gene. Lines carrying the *Rp1-DJ* compound gene are generally free of common rust in the field in Kansas, because of the lack of rust biotypes that are virulent on both the *Rp1-D* and *Rp1-J* genes. To measure non-race-specific effects of the compound gene, it was necessary to use the biotype H11, which is virulent on both genes. The *Rp1-DJ* compound gene had no apparent resistance to the H11 biotype at the seedling (two to three leaf) stage. Hyper-sensitive reactions were not observed, and no differences in pustule number were observed when three F₃ families homozygous for the compound gene were compared with three families without the gene (data not shown). However, when plants from the same families were inoculated after they had six to seven fully expanded leaves, significant differences in pustule number were observed, with three families that carried the *Rp1-DJ4* compound gene showing fewer pustules than three that did not (Table 2). It also was clear from the analysis, however, that the *rp1* genotype was not the only genetic factor affecting the segregation of rust resistance among the families. Resistance expressed by the families that carried the *rp1* allele also varied significantly, as did that of the families carrying the compound gene. Since these genetic background effects complicated estimation of the effects of the compound gene on adult plant resistance, families with more homogeneous genetic backgrounds (BC₁F₂ or BC₂F₂) were used for subsequent experiments.

Families in three different backgrounds were analyzed (Table 2, experiments 2 to 5). Families with the *Rp1-DJ46* gene always had fewer pustules than other families in the same experiment that did not carry the gene. In the W23 background, three families homozygous for *Rp1-DJ46* (K, F, and G) had significantly fewer pus-

Fig. 1. Comparisons of individuals from two different BC₂F₂ families in the A188 background. Leaves on the left are from individuals that are homozygous for the *Rp1-DJ4* compound gene and those on the right are from individuals that are homozygous for the *rp1* allele. **A**, Sections of the ninth leaf of uninoculated, field-grown plants harvested 1 week after pollen shed. **B**, Sections of the eighth leaf 20 days after inoculation with *Puccinia polysora*.

TABLE 1. Severity of adult plant chlorotic-spotting phenotypes in field-grown BC₁F₂ families segregating for the *Rp1-DJ* compound common rust resistance gene in different genetic backgrounds

Rating ^x	<i>Rp1-DJ46</i>			<i>Rp1-DJ4</i>	
	A188 ^y	H95 ^y	W23 ^y	A188	H95
10	...	...	...	3 RR, 3 Rr	...
9	1 RR ^z , 1 Rr	1 RR	...	2 RR, 1 Rr	...
8	1 Rr	1 RR	...	4 Rr	2 RR
7	1 RR	...	...	1 Rr	1 RR, 1 Rr
6	1 Rr	2 RR	...	...	1 Rr
5	1 RR	...	...	1 Rr, 1 rr	1 Rr
4	3 Rr	1 Rr	1 Rr	1 rr	1 Rr, 1 rr
3	2 RR, 2 Rr	1 Rr	2 Rr	...	1 RR, 1 Rr, 1 rr
2	2 Rr, 2 rr	1 Rr	2 Rr	2 rr	2 RR, 4 Rr, 1 rr
1	1 Rr, 1 rr	6 Rr, 1 rr	3 RR, 1 Rr, 2 rr	...	...
0	4 Rr, 4 rr	1 RR, 6 Rr, 1 rr	2 RR, 4 Rr, 2 rr	2 rr	4 Rr, 3 rr
Average rating for each <i>Rp1</i> genotype					
Mean RR	5.4	5.8	0.6	9.6	5.0
Mean Rr	3.1	1.0	1.5	8.3	2.5
Mean rr	0.7	0.5	0.5	2.7	1.5

^x Degree of chlorotic spotting was rated on a 0 to 10 scale before *Rp1* genotype was determined by progeny testing.

^y Refers to the genetic background into which the *Rp1-DJ* compound gene was crossed.

^z RR = homozygous for *Rp1-DJ* compound gene, rr = homozygous for *rp1* allele, and Rr = heterozygous. Numbers indicate the number of individuals of that genotype that had that phenotypic score.

tules than did three families homozygous for the *rp1* allele (M, H, and I). In the H95 background, three families homozygous for the compound gene (A, B, and C) had significantly fewer pustules than did two *rp1/rp1* families (L and P). In the A188 background (experiment 4), three families homozygous for the compound gene (B, D, and F) had fewer pustules than did three *rp1/rp1* families (M, Q, and S), but the differences in pustule numbers were not significantly different between family M and family F. However, when the same families were again tested in experiment 6, the rankings were similar, but families carrying the compound gene were all significantly more resistant than those without the gene. In the latter experiment, a higher concentration of inoculum was used (approximately 400 mg of uredospores), and it was conducted in the fall of 1995, whereas experiment 4 was conducted in the summer. The cooler temperatures were probably more conducive to both rust infection and resistance expression.

Adult plant resistance to southern rust from the compound gene. The effect of the compound gene on southern rust resistance was examined using six BC₂F₂ families in the A188 background. Three of the families were homozygous for *Rp1-DJ4*, and the other three were homozygous for the A188 (*rp1*) allele. No differences in resistance to *P. polysora* were noticeable at the three-leaf stage. Seedlings of all six families were highly susceptible.

TABLE 2. Comparisons of number of pustules in related families, with and without the compound *Rp1-DJ* gene, after inoculation with *Puccinia sorghi* biotype HI1^s

Experiment (genotypes)	Mean no. of pustules	Grouping ^t
Experiment 1 ^u		
E ^v (<i>rp/rp</i>)	521.5	a
I (<i>rp/rp</i>)	465.4	a
D (<i>rp/rp</i>)	234.8	b
H (<i>Rp1-DJ4/Rp1-DJ4</i>)	134.8	c
A (<i>Rp1-DJ4/Rp1-DJ4</i>)	39.6	d
B (<i>Rp1-DJ4/Rp1-DJ4</i>)	21.3	d
Experiment 2 ^w		
M (<i>rp/rp</i>)	88.4	a
H (<i>rp/rp</i>)	81.9	a
I (<i>rp/rp</i>)	64.6	a
K (<i>Rp1-DJ46/Rp1-DJ46</i>)	25.4	b
F (<i>Rp1-DJ46/Rp1-DJ46</i>)	18.1	b
G (<i>Rp1-DJ46/Rp1-DJ46</i>)	2.3	b
Experiment 3 ^x		
L (<i>rp/rp</i>)	105.6	a
P (<i>rp/rp</i>)	83.2	a
B (<i>Rp1-DJ46/Rp1-DJ46</i>)	49.8	b
A (<i>Rp1-DJ46/Rp1-DJ46</i>)	33.0	b
C (<i>Rp1-DJ46/Rp1-DJ46</i>)	27.0	b
Experiment 4 ^y		
S (<i>rp/rp</i>)	194.6	a
Q (<i>rp/rp</i>)	178.5	a
M (<i>rp/rp</i>)	97.3	b
F (<i>Rp1-DJ46/Rp1-DJ46</i>)	38.8	bc
B (<i>Rp1-DJ46/Rp1-DJ46</i>)	9.2	c
D (<i>Rp1-DJ46/Rp1-DJ46</i>)	9.2	c
Experiment 5 ^z		
S (<i>rp/rp</i>)	980.0	a
Q (<i>rp/rp</i>)	770.0	a
M (<i>rp/rp</i>)	518.8	b
B (<i>Rp1-DJ46/Rp1-DJ46</i>)	147.0	c
F (<i>Rp1-DJ46/Rp1-DJ46</i>)	140.5	c
D (<i>Rp1-DJ46/Rp1-DJ46</i>)	85.1	c

^s The HI1 biotype is virulent on lines carrying either *Rp1-J* or *Rp1-D*.

^t Genotype means with different letters are significantly different at *P* = 0.05.

^u *Rp1-DJ4* F₃ families. Least significant difference = 94.6.

^v Letters are designations of different F₃ or BC₁F₂ families.

^w *Rp1-DJ46* BC₁F₂, W23 background. Least significant difference = 24.7.

^x *Rp1-DJ46* BC₁F₂, H95 background. Least significant difference = 28.4.

^y *Rp1-DJ46* BC₁F₂, A188 background. Least significant difference = 63.1.

^z *Rp1-DJ46* BC₁F₂, A188 background. Least significant difference = 244.8.

Adult plant resistance was examined in the six BC₂F₂ families by inoculating plants that had six to seven fully expanded leaves. Significant differences in pustule number were observed, with the *Rp1-DJ4* families averaging approximately half as many pustules as those without the compound gene. Families A, E, and F (*Rp1-DJ4/Rp1-DJ4*) averaged 1,572, 1,810, and 1,603 pustules/plant, respectively, whereas families B, C, and D (*rp1/rp1*) averaged only 814, 873, and 867 pustules/plant (Table 3).

In addition to having fewer pustules, plants carrying *Rp1-DJ4* generally had noticeably smaller, less-well-developed pustules (Fig. 1B). To quantify this, individual plants were given a rating for pustule development at 4 weeks after inoculation. A 0 to 10 scale was used for pustule development rating, and individual plant scores ranged from 2 to 9. A score of 2 indicated that most pustules on the plant remained small (approximately 1 mm), were surrounded by chlorosis, and were no longer producing spores by 4 weeks after inoculation. Most pustules on plants with ratings of 9 continued to expand and were 2 to 3 mm in length. Such pustules continued to produce spores and were not associated with a chlorotic reaction.

The *Rp1-DJ4* families (B, C, and D) had significantly (*P* < 0.05; least significant differences = 1.45) lower pustule development ratings than did families (A, F, and E) not carrying the compound gene. Families B, C, and D had average ratings of 4.4, 4.3, and 4.0, respectively, and the A, E, and F families had average ratings of 8.1, 8.4, and 7.9.

Common rust resistance in lines carrying single *Rp1* genes or no known *Rp1* genes. Levels of adult plant resistance to common rust were analyzed in four *Rp1* near-isogenic lines, *Rp1-D*, *Rp1-J*, *Rp1-F*, and *rp1*, in the R168 background. No differences were noticeable between these lines when inoculated with *P. sorghi* biotype HI1 at the seedling stage; all four lines were highly susceptible to this biotype. When the four lines were inoculated at the six- to seven-leaf stage with HI1 in the greenhouse, no significant (*P* > 0.05) differences in pustule number were observed. The R168 inbred averaged 756 pustules/plant, and the *Rp1-D*, *Rp1-F*, and *Rp1-J* lines averaged 623, 798, and 686 pustules/plant, respectively.

DISCUSSION

The *Rp1-DJ* compound gene provided significant levels of adult plant resistance to a common rust biotype that is virulent on lines carrying either parental gene. This indicates that certain *Rp1* gene combinations, which can be manipulated as single genes once they are recombined together, can have beneficial effects even if rust biotypes have specific virulence to the individual genes.

In the A188 and H95 genetic backgrounds, the *Rp1-DJ* compound gene appeared associated with an adult plant chlorotic-spotting phenotype. The effect was apparently background specific, because it is less noticeable in a W23 background. This is also indicated by the fact that several lines have been established that are homozygous for the compound gene, but express no noticeable chlorotic spotting.

TABLE 3. Comparisons of pustule numbers in BC₂F₂ families, with and without the compound *Rp1-DJ4* gene, 16 days after inoculation with *Puccinia polysora*

Experiment 5 ^x	Mean no. of pustules	Grouping ^y
B ^z (<i>rp/rp</i>)	1,572	a
C (<i>rp/rp</i>)	1,810	a
D (<i>rp/rp</i>)	1,603	a
A (<i>Rp1-DJ4/Rp1-DJ4</i>)	814	b
E (<i>Rp1-DJ4/Rp1-DJ4</i>)	873	b
F (<i>Rp1-DJ4/Rp1-DJ4</i>)	867	b

^x BC₂F₂ families. Least significant difference = 277.

^y Genotype means with different letters are significantly different at *P* = 0.05.

^z Letters are designations of different BC₂F₂ families.

The chlorotic-spotting phenotype associated with *Rp1-DJ* is similar to, but less severe than, two previously identified *Rp1* recombinants, *Rp1-NC3* and *Rp1-D21*, which show necrotic lesions in the absence of rust (10). Instead of showing the race-specific response of their parents, these *Rp1* lesion mimic recombinants gave the same highly necrotic reaction to all nine *P. sorghi* biotypes tested. Lines carrying *Rp1-NC3* and *Rp1-D21* gave hypersensitive-like necrotic spots even when inoculated with *P. polysora* or several nonhost *Puccinia* species. If the resistance to *P. sorghi* biotype HI1 exhibited by lines carrying the *Rp1-DJ* compound gene results from a similar phenomenon as that controlling the reactions of the *Rp1* lesion mimics, this may be a non-race-specific resistance. Only a single *P. sorghi* biotype, HI1, was available that was virulent on both *Rp1-J* and *Rp1-D*. The HI1 isolate, however, is fully virulent on lines carrying these parental genes at the seedling and adult plant stage and fully virulent on lines with *Rp1-DJ* at the seedling stage. Furthermore, lines carrying *Rp1-DJ4* were more resistant than *rp1/rp1* plants to southern rust, supporting the idea that the compound gene has nonspecific effects.

There is some evidence that the resistance provided by certain other genes is non-race-specific. Barley lines that are homozygous for recessive alleles at the *mlo* locus have remained resistant to all known powdery mildew biotypes for many years (29,34). The *Lr34* leaf rust resistance gene of wheat also is thought to have non-race-specific effects (14). Resistance conferred by *Lr34* is generally best expressed at the adult plant stage (27). In spite of its wide use, no *P. recondita* biotypes have been identified in North America that are fully virulent on it (3,4,26,28). In addition, the *Lr34* gene is associated with adult plant resistance to stripe rust (15,26) and stem rust (2), either due to effects of the gene itself or due to genes that are closely linked in coupling.

Like *Rp1-DJ*, the *mlo* (13,29,34) and *Lr34* (25) resistance genes are also both associated with phenotypes that are visible in the absence of the pathogen. It is likely that this association is not purely coincidental and that there is a causal connection between at least some forms of nonspecific resistance and physiological alterations that are conferred by these genes before inoculation with the pathogen.

It is not known why *Rp1-DJ* confers resistance to the HI1 *P. sorghi* biotype or the *P. polysora* biotype. It could be due to the specific combination of the two detectable *Rp1* genes, *Rp1-J* and *Rp1-D*, or possibly these along with other linked genes in the *rp1* complex. Since recombination in the *rp1* complex is frequently unequal (30), the cross-overs that generated the *Rp1-DJ4* and *Rp1-DJ46* compound genes may have changed the actual number of *Rp1* genes in the complex. Since the resistance was observed in both *Rp1-DJ* recombinants, it was probably not due to a novel *Rp1* gene (20) created by the recombination event. Therefore, it is likely a combination of genes that gives the effect. It is possible that the *Rp1-J* and *Rp1-D* genes (or genes linked in coupling to them) have small effects on adult plant resistance that are more noticeable when they are combined. In the present study, we observed that our genetic material is probably not appropriate for looking for small differences in resistance, because many of the *Rp1* near-isogenic lines in the R168 background are not highly isogenic. A preliminary analysis of the near-isogenic lines using random amplified polymorphic DNAs indicated that polymorphisms between some of them were frequent and that the *Rp1-D* line was one of the least isogenic (T. E. Richter, G. Hu, and S. H. Hulbert, unpublished data). Construction of a more isogenic set of *Rp1* differentials, so that more careful comparisons can be made, is underway.

A number of compound genes have now been constructed (9), some of which contain up to four characterized *rp1* area genes. Most of these are resistant at the seedling and adult plant stage to all *P. sorghi* biotypes in our collection, because of the race-specific effects of the multiple genes they carry. This impedes analy-

ses of non-race-specific (general) resistance using *P. sorghi* biotypes. If resistance to *P. polysora* implies general resistance to *P. sorghi*, this may be an alternative method of searching for this type of resistance. It is also possible that an association with a chlorotic-spotting phenotype in some backgrounds may indicate which compound genes confer general resistance. Further experiments with compound *Rp1* genes should examine the relationship between the chlorotic-spotting phenotype, general resistance to *P. sorghi*, and resistance to *P. polysora*.

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