Research Note

A Novel Rust Resistance Gene in Maize Showing Overdominance

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The *Rp8* rust resistance locus of maize is unique in that only *Rp8-A/Rp8-B* heterozygotes provide resistance and homozygotes are susceptible. A third allele, *Rp8-C*, provides no resistance in homozygotes or heterozygous combinations. *Rp8* maps to the long arm of chromosome 6, near the restriction fragment length polymorphism marker *umc21*.

In plants, resistance to specific races of plant pathogens is frequently inherited as single dominant or partially dominant genes that correspond to specific genes for avirulence in the pathogen. Exceptions to this general rule have been documented, however, including cases in which resistance segregates as a recessive trait or is controlled by two nonallelic genes that interact epistatically (Crute 1985; Crute and Pink 1996; Roelfs 1988). For example, complementary gene action, in which dominant alleles are required at each of two loci, has been noted for resistance to crown rust in oats (Baker 1966) and leaf rust in wheat (McIntosh et al. 1995). Genes that enhance or inhibit resistance genes at other loci have also been described (Dyck 1987) or identified by mutagenesis (Freialdenhoven et al. 1994; Hammond-Kosack et al. 1994; Salmeron et al. 1994).

The *Rp* genes of maize, which control race-specific resistance to the common rust pathogen *Puccinia sorghi* Schwein., are generally inherited as single dominant genes (Hulbert 1997). Here we describe a new *rp* locus, *Rp8*, with unique patterns of inheritance. The *Rp8-A* allele was identified in a line of unknown pedigree (which we designated AH13) obtained from A. H. Hooker's stocks in the seed storage facility at the University of Illinois. The seed was approximately 30 years old, and we were not able to obtain seed from the few plants we established. However, several *F*₁ seeds were obtained from a cross of AH13 with H95, an inbred line that is susceptible to all known rust biotypes. Surprisingly, both a family from a backcross to H95 and an *F*₂ population made by self fertilizing an *F*₁ plant segregated 1:1 (resistant:susceptible) when inoculated with rust biotype IN1 (Fig. 1). *F*₃ families from resistant plants also segregated 1:1 (Fig. 1). *F*₃ fami-

lies from susceptible *F*₂ plants were all completely susceptible, indicating they were homozygous. We were able to classify the susceptible lines into two different classes (A and B), corresponding to the two different alleles. Class A lines always gave all resistant progeny when backcrossed to H95 and most other inbred lines (Fig. 1 and Table 1). These lines also gave all resistant progeny when crossed to class B *F*₃ families but all susceptible progeny when crossed to other class A *F*₃ families. The class B lines were just the opposite, i.e., all susceptible progeny in crosses with H95, all resistant progeny in crosses with class A *F*₃ families, and all susceptible progeny in crosses with other class B *F*₃ families. These data indicated that resistance was conferred by a single gene with two (or more) alleles, which are both necessary for the expression of resistance. The allele contributed by AH13, and homozygous in the class A *F*₃ families, was designated *Rp8-A*. The allele present in H95, and homozygous in the class B *F*₃ families, was designated *Rp8-B*.

Bulked segregant analysis (Michelmore et al. 1991) was used to determine the map position of the *Rp8* locus. DNAs from each of the homozygous *F*₃ families were pooled according to genotype and compared by gel blot analysis with restriction fragment length polymorphism (RFLP) probes distributed throughout the genome. The probe *umc21* detected a clear difference between the two pools, suggesting the marker was linked to the *rp8* locus. DNAs of the individual homozygous *F*₃ families were then assayed for their RFLP genotype at this locus and several other linked RFLP loci on the long arm of chromosome 6. The markers *umc21* and *umc248* were found to be the most tightly linked markers. Only one and three recombinants were found between *Rp8* and these two loci, respectively, after analysis of 43 individual *F*₃ families. The estimated map distance and gene order of the markers was *Rp8*-(1.4 cM)-*umc21*-(5.9 cM)-*umc248*.

Most public inbred lines produced IN1-susceptible progeny when crossed to H95 (*Rp8-B/Rp8-B*) but resistant progeny when crossed to an *Rp8-A/Rp8-A* line, indicating they carried the *Rp8-B* allele (Table 2). The inbred W23 was exceptional in that crosses with either the *Rp8-A/Rp8-A* line or H95 produced only susceptible progeny. The lack of resistant progeny indicated that W23 either carried a third allele (*rp8-C*) or carried a dominant inhibitor of *Rp8* in addition to either *Rp8-A* or *Rp8-B*. Crosses between W23 and the *Rp8-A* and H95 lines were self pollinated to make *F*₂ families. If W23 carries either *Rp8-A* or *Rp8-B* and an independently segregating dominant

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Fig. 1. Crossing scheme used to characterize the unique inheritance of the *rp8* locus.

inhibitor gene, the *F*₂ from one of these crosses would be expected to segregate 7 susceptible : 1 resistant. However, all crosses produced only susceptible progeny, suggesting that W23 does indeed have a unique *rp8* allele (Table 3).

The *Rp8-A/Rp8-B* heterozygote was highly resistant to biotype IN1 (Table 4; Fig. 2) but all other allelic combinations were completely susceptible. To determine if other alleles or combinations might provide resistance to other rust biotypes, seedlings homozygous for *Rp8-A*, *Rp8-B* (A188 and H95), and *rp8-C* (W23) and seedlings carrying all three possible heterozygous combinations were inoculated separately with the rust biotypes IN1, IN2, IN3, KS1, HI1, and AF1 (Hulbert et al. 1991). All three possible *Rp8* homozygotes were highly susceptible to all six rust biotypes. Similarly, the *Rp8-A/Rp8-C* and *Rp8-B/Rp8-C* heterozygotes were equally susceptible to all the rust biotypes. Only the *Rp8-A/Rp8-B* heterozygote conferred resistance, and only to biotype IN1. The *rp8-C* allele might be a null allele, since it provided no resistance to any of the rust biotypes tested as a homozygote or in heterozygous combination with *Rp8-A* or *Rp8-B*. Alternatively, this allele could possibly confer resistance in combinations with alleles that are yet unidentified or resistance to other rust biotypes not used in the present study.

It is possible that *Rp8-A* and *Rp8-B* are not alleles, but separate resistance genes that are complementary and also closely linked. Attempts to recombine both genes were unsuccessful. No IN1-resistant individuals were identified among 540 progeny from the cross *rp8-C/rp8-C* × *Rp8-A/Rp8-B* (Table 3). Therefore, if they represent complementary genes they must be tightly linked (<1 cM). No evidence of meiotic instability (Sudupak et al. 1993) was observed at either allele; no IN1-susceptible individuals were observed among 2,873 *Rp8-A/Rp8-A* × *Rp8-B/Rp8-B* progeny. Transposon tagging may, therefore, be a viable approach to identifying *Rp8* sequences.

The DNA sequences of most resistance genes that have been characterized to date are consistent with their role as receptors that recognize pathogens by the presence of specific elicitors (Bent 1996; Staskawicz et al. 1995). The exact physical structure and number of different components in these receptors is not yet known. Some have been postulated to exist as dimers or multimers that include a partially homotypic dimer (Dangl 1995). Many well-characterized receptors in animals are dimers or multimers that include two proteins from the same gene or two closely related genes (Fryxell 1996). The *Rp8* gene could code for such a receptor in which two slightly different components, which are provided by two dif-

Table 1. Resistance reaction of progeny of crosses of susceptible F₃ families with several public inbreds and other susceptible F₃ families^a

Susceptible F ₃ family	Class (rp8 allele)	Crosses to rp8-B/rp8-B inbreds ^b	Crosses to rp8-A/rp8-A F ₃ lines	Crosses to rp8-B/rp8-B F ₃ lines	Crosses to W23
161	A	72:0	0:83	41:0	0:16
162	A	41:0	0:126	39:0	0:33
163	A	78:0	0:57	44:0	0:21
164	A	59:0	0:75	50:0	—
165	B	0:76	104:0	0:12	0:22
171	A	4:0	0:20	—	—
172	A	9:0	0:10	—	—
174	A	31:0	—	—	0:19
175	A	12:0	—	—	—
176	B	0:23	—	—	—
177	B	0:37	27:0	—	0:7
178	A	8:0	—	—	0:10
179	B	0:18	—	—	—
1710	A	21:0	—	—	0:15
1711	A	50:0	—	—	—
1712	B	0:25	—	—	—
181	A	27:0	0:8	—	0:10
182	A	18:0	—	—	0:10
183	B	0:22	41:0	—	—
184	B	0:15	—	—	—
185	B	0:39	9:0	—	0:17
186	B	0:19	—	—	0:10
187	A	31:0	—	8:0	—
188	B	0:40	—	—	0:24
189	A	23:0	—	13:0	—
1810	A	7:0	0:12	—	—
1811	B	0:40	14:0	0:12	—
1812	B	0:9	—	—	—

^a Ratio of resistant plants : susceptible plants.

^b All families were crossed to H95, most were also crossed to several other Rp8-B/Rp8-B inbreds.

Table 2. Alleles of *rp8* present in various maize inbreds

rp8 allele	Inbred lines tested
Rp8-A	None
Rp8-B	A188, A619, B37, CM37, CO159, H95, MO17, MO20w, OH28
rp8-C	W23

Table 3. Additional crosses tested with biotype IN1 to test the monogenic overdominance model

Cross	Segregation (# resistant : # susceptible)
[Rp8-C/Rp8-C X Rp8-B/Rp8-B] self ^a	0:232
[Rp8-C/Rp8-C X Rp8-A/Rp8-A] self	0:219
Rp8-C/Rp8-C X Rp8-A/Rp8-B	0:540
Rp8-A/Rp8-A X Rp8-B/Rp8-B (A188) ^b	1,569:0
Rp8-A/Rp8-A X Rp8-B/Rp8-B (Mo17)	636:0
Rp8-A/Rp8-A X Rp8-B/Rp8-B (A619)	668:0

^a Self indicates the F₁ was self fertilized to make an F₂ family.

^b Rp8-B parent was either H95 or is listed in parentheses; the Rp8-C parent is W23; and the Rp8-A parent was from a homozygous AH13 × H95 F₃ family.

ferent *Rp8* alleles, are required to interact with the ligand. If *Rp8* is a complex locus, the two alleles might be members of the same gene family but not be actual alleles in a strict sense. An alternative possibility is that the two components required for the *Rp8* resistance are unrelated gene products. *Rp8* could be a complex locus with two or more different types of genes, similar to the *Pto-Prf* gene cluster in tomato (Salmeron et al. 1996).

Table 4. Reactions of various *rp8* allelic combinations with six different rust pathotypes^a

rp8 genotype	Rust biotype					
	AF1	HI1	IN1	IN2	IN3	KS1
Rp8-A/Rp8-A	+	+	+	+	+	+
Rp8-B/Rp8-B	+	+	+	+	+	+
Rp8-C/Rp8-C	+	+	+	+	+	+
Rp8-A/Rp8-B	+	+	—	+	+	+
Rp8-A/Rp8-C	+	+	+	+	+	+
Rp8-B/Rp8-C	+	+	+	+	+	+

^a +, compatible interaction (susceptible); —, incompatible interaction (host highly resistant).

Fig. 2. Resistance phenotype of the *Rp8-A/Rp8-B* heterozygote compared with the parental homozygotes. The third leaf of each seedling was inoculated with common rust biotype IN1 and photographed after 8 days.

The *Rp8* rust resistance locus is unusual in that only certain heterozygous allelic combinations confer rust resistance. The requirement for two different alleles of a single locus for disease resistance has not been previously reported. Such resistance genes may not be uncommon, since few plant programs make efforts to screen heterozygous material when testing germplasm for useful disease resistances. Future screening of hybrid material may result in additional reports of resistance genes that require heterozygosity to confer resistance. If common, such overdominant resistance genes, in which only the heterozygote confers resistance, may be a major contributor to hybrid vigor.

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