Genetic Relationships Between Alleles of the *Rp1* Rust Resistance Locus of Maize

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***Rp1* is a complex resistance locus of maize. Different *Rp1* genes confer resistance to different common rust isolates and vary in their meiotic stability. Susceptible derivatives from *Rp1* heterozygotes, and those with the combined resistance of both parents, are commonly associated with a single nonparental combination of flanking markers—indicating they arise by simple crossing-over, and that one of the genes maps distally or proximally to the other. Susceptible derivatives from lines homozygous for unstable *Rp1* genes exhibit both nonparental combinations of flanking markers—indicating they arise by unequal crossing-over. We analyzed derivatives from 10 different test cross populations to determine if such complex types of recombination events can occur in heterozygotes. The *Rp1* genes used to construct the heterozygotes were known to vary in their meiotic stability. Recombinants with both nonparental combinations of flanking markers were found from three of the heterozygotes. All three hybrids carried at least one unstable gene. The occurrence of these recombinants indicates *Rp1* genes reside on duplicated sequences which, in at least some lines, can mispair during meiosis. Implications for allelism tests for resistance genes, for the generation of a *Rp1* area genetic fine structure map, and for transposon tagging strategies are discussed.**

Additional keywords: complex locus, gene conversion, gene family, gene-for-gene interaction, recombination.

Genes controlling race-specific resistance to plant pathogens are frequently clustered in the genome (Crute 1986; Bennetzen and Hulbert 1992), particularly those for resistance to biotrophic fungi. Multiple genes, each distinguishable by its resistance to a unique spectrum of fungal biotypes, often map to a single locus. These complex loci are assumed to be either clusters of closely linked genes or allelic series at a single locus (Shepherd and Mayo 1972). Little is known of the actual structure of these complex loci. This is due in part to the lack of a cloned DNA fragment with which to probe the plant genomes. It also reflects the difficulty of conducting suitable genetic analysis in most of the species in which complex disease resistance loci have been identified. Such genetic analyses are generally directed at generating recombinants between alleles or closely linked genes in populations derived from a heterozygote. Since complex resistance genes usually exhibit dominant gene action, test cross populations are much more efficient than F_2 populations for identifying genetic recombinants between closely linked genes or alleles. This is particularly true when screening for recombinants with the combined resistance of both parents, since they are phenotypically identical to the heterozygotes in self-fertilized progeny. Large test cross populations are difficult to construct in most plants. Equally important to the mating design is the availability of genetic markers. When screening for rare recombination events it is very important that the parents be adequately marked genetically to rule out pollen or seed contamination. This presents a problem in species where few genetic markers are available. Ideally, genetic markers that closely flank the complex locus can be used to examine the origin of putative recombinants (Hulbert and Bennetzen 1991; Sudupak *et al.* 1993). Individuals that arise by crossing-over will have nonparental combinations of flanking markers, whereas those that arise by mutation will not.

Most of the 25 genes for resistance to the common rust fungus (*Puccinia sorghi* Schwein.) in maize map to a small area on the short arm of chromosome 10 (Hagan and Hooker 1965; Hooker and Russell 1962; Lee *et al.* 1963; Wilkinson and Hooker 1968). Fourteen of these genes (*Rp1*-A–*Rp1*-N) were given the *Rp1* designation when genetic mapping experiments indicated they mapped to a single locus. Two additional genes, *Rp5* and *Rp6*, were found to map about one and two map units from *Rp1* (Wilkinson and Hooker 1968) and about three map units from each other. In addition, a gene for resistance to *P. polysora* Underw. was also mapped about one to two map units from *Rp1* (Ullstrup 1965). The *Rp1* area, therefore, consists of the complex disease resistance locus (*Rp1*) and several other linked genes. Analysis of larger test cross populations (Hulbert and Bennetzen 1991; Saxena and Hooker 1968) revealed that recombinants between various *Rp1* alleles can be obtained and that the locus is composed of multiple genes. While most of the genes mapped within about 0.3 map units from each other, *Rp1*-G mapped one to three map units distally, closer to *Rp5*. The identification of restriction fragment length polymorphism (RFLP) markers that closely flank the area allowed a precise characterization of the types of recombination events that occur in these crosses (Hulbert and Bennetzen 1991). Susceptible individuals were always associated with recombination of the flanking markers verifying their recombinational origin. Most of the events could be explained by simple

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crossing-over; susceptible individuals from test crosses of a given heterozygote usually had only one of two possible combinations of flanking markers. This would be expected if one of the genes maps distally or proximally to the other and suggested it may be possible establish a linear order for all of the *Rp1* genes on the chromosome.

One exception to the unidirectional flanking marker recombination was observed. Three of four susceptible recombinants from a *Rp1*-D/*Rp1*-F heterozygote had the distal flanking marker genotype of the *Rp1*-F parent and the proximal flanking marker of the *Rp1*-D parent; the fourth susceptible individual, however, had the opposite combination of flanking markers. It was postulated that recombinants with bidirectional flanking markers might occur by the same mechanism that generates susceptible recombinants from certain *Rp1* homozygotes. More recently (Sudupak *et al.* 1993), susceptible derivatives from *Rp1*-J and *Rp1*-G homozygotes were demonstrated to be associated with bidirectional recombination of flanking markers. The results indicated that the meiotic instability of *Rp1*-J and *Rp1*-G was caused by unequal crossing-over. It is likely, therefore, that the derivatives from the *Rp1*-D/*Rp1*-F heterozygote also arose by crossing-over following two different types of pairing between duplicated sequences. If the *Rp1* genes frequently pair in different arrangements during meiosis, this would have serious implications for attempts to generate a genetic fine structure map of the *Rp1* area.

The present study was undertaken to determine if recombinants with bidirectional combinations of flanking markers are common in progeny of *Rp1* heterozygotes. Additional test crosses were made with *Rp1*-D and *Rp1*-F, the two genes which were previously observed to recombine in this manner. *Rp1*-A, from the cultivar Golden King was also included in the analysis, since all three of these genes recombined in previous analysis as though they mapped to the distal end of the *Rp1* locus. Other crosses analyzed included those with *Rp1* genes that have been demonstrated to be meiotically unstable, such as *Rp1*-C, *Rp1*-J, and *Rp1*-L, or relatively stable, such as *Rp1*-K and *Rp1*-I.

RESULTS

Analysis of susceptible recombinants from *Rp1* heterozygotes.

The specific virulence phenotypes of isolates of *P. sorghi* used to screen the test cross populations are given in Table 1. Nine test cross populations were screened with single rust isolates to obtain susceptible recombinants (Table 2). The isolate used to screen each population, either IN2 or IN3, was avirulent on both of the *Rp1* genes segregating in the family. All of the susceptible individuals had nonparental combinations of flanking markers indicating they arose by crossing-over. Two of the populations exhibited both possible nonparental combinations of flanking markers.

Isolation of reciprocal recombination events.

The reciprocal products of crossing-over between two linked resistance genes are susceptible individuals and those with the combined resistance of both parents (double resistant types). Isolation of the latter types requires complementary rust isolates—one isolate that is virulent on lines carrying the first gene but avirulent to the second, and another isolate that is avirulent on lines carrying the first gene but virulent on the second. The use of isolate KS1 in combination with isolate IN2 made it possible to select for both types of recombinants in the *Rp1*-J/*Rp1*-C, *Rp1*-J/*Rp1*-F, and *Rp1*-J/*Rp1*-D populations (Table 3). Multiple recombinants of both types were obtained in all three populations. In the *Rp1*-J/*Rp1*-D population, six susceptible progeny and two double resistant progeny were isolated. Four double resistant and two susceptible individuals were recovered from the *Rp1*-J/*Rp1*-F population, and four double resistant and eight susceptible progeny were found in the *Rp1*-J/*Rp1*-C population. All of the recombinants from the *Rp1*-D/*Rp1*-J and *Rp1*-F/*Rp1*-J populations had nonparental combinations of flanking

Table 1. Virulence phenotype of rust isolates on *Rp1* resistance genes

	Rust isolate		
***Rp1* gene**[a]	**IN2**	**IN3**	**KS1**
Rp1-A	–[b]	–	+[c]
Rp1-B	–	+	+
Rp1-C	–	+	+
Rp1-D	–	–	+
Rp1-F	–	–	+
Rp1-I	–	+	–
Rp1-J	+	–	–
Rp1-K	–	+	–
Rp1-L	–	+	+

[a] The source of *Rp1*-A used in this study was a line derived from the cultivar Golden King. Previous analyses have indicated this recombines in a different way from the gene from the maize line GG208R (Hulbert and Bennetzen 1991).
[b] Incompatible interaction (–); gene provides resistance.
[c] Compatible interaction (+); gene does not provide resistance.

Table 2. Identification of susceptible recombinants from *Rp1* heterozygotes by screening with single rust isolates

			Marker[a]	
Test cross	**Isolate**	**Susceptible/ total**	**Proximal**	**Distal**
Rp1-A/*Rp1*-D	IN2	1/8,315	*Rp1*-A	*Rp1*-D
Rp1-A/*Rp1*-F	IN3	4/7,726		
3 recombinants			*Rp1*-A	*Rp1*-F
1 recombinant			*Rp1*-F	*Rp1*-A
Rp1-A/*Rp1*-J	IN3	4/6,298	*Rp1*-A	*Rp1*-J
Rp1-B/*Rp1*-D	IN2	1/5,423	*Rp1*-D	*Rp1*-B
Rp1-B/*Rp1*-I	IN2	1/5,888	*Rp1*-I	*Rp1*-B
Rp1-D/*Rp1*-J	IN3	1/1,946[b]	*Rp1*-D	*Rp1*-J
Rp1-D/*Rp1*-L	IN2	5/5,512[c]	*Rp1*-D	*Rp1*-L
Rp1-F/*Rp1*-J	IN3	3/2,671[b]		
1 recombinant			*Rp1*-F	*Rp1*-J
2 recombinants			*Rp1*-J	*Rp1*-F
Rp1-I/*Rp1*-K	IN2	0/5,185	...	...

[a] The parent from which the restriction fragment length polymorphism (RFLP) marker allele was inherited, e.g. *Rp1*-D, indicates the recombinants have the marker allele of the *Rp1*-D parent. The RFLP loci NPI285, KSU3, and KSU4 were used as proximal markers, and BNL3.04 was used as a distal marker.
[b] Additional recombinants from these populations are reported in Tables 3 and 4.
[c] One of the susceptibles from the *Rp1*-L/*Rp1*-D population died before the flanking markers could be analyzed or susceptibility could be verified by progeny testing.

Table 3. Identification of susceptible and double resistant recombinants from *Rp1* heterozygotes using rust isolates KS1 and IN2

	Test cross family		
	***Rp1*-C/*Rp1*-J**	***Rp1*-J/*Rp1*-D**	***Rp1*-J/*Rp1*-F**
Total progeny screened	3,479	6,909	5,636
Resistant KS1, susceptible IN2	1,770	3,433	2,861
Resistant IN2, susceptible KS1	1,697	3,468	2,769
Susceptible to both isolates	8	6	2
Resistant to both isolates	4	2	4
Distally mapping *Rp1* gene	neither[a]	*Rp1*-D	neither[a]

[a] Both nonparental combinations of flanking markers were observed indicating that recombination was associated with different types of pairing.

Table 4. Flanking restriction fragment length polymorphism marker genotypes of recombinants from *Rp1*-C/*Rp1*-J, *Rp1*-D/*Rp1*-J, and *Rp1*-F/*Rp1*-J heterozygotes

Parents Recombinants	Resistant or susceptible[a]	Marker[b]	
		Proximal	Distal
Rp1-C × *Rp1*-J			
jc1	Susceptible	*Rp1*-C	*Rp1*-C
cj3a	Susceptible	*Rp1*-C	*Rp1*-J
jc3b	Susceptible	*Rp1*-C	*Rp1*-C
jc4	Susceptible	*Rp1*-J	*Rp1*-C
JC5	Resistant	*Rp1*-C	*Rp1*-J
jc6	Susceptible	*Rp1*-J	*Rp1*-C
JC7	Resistant	*Rp1*-C	*Rp1*-J
jc9	Susceptible	*Rp1*-J	*Rp1*-C
jc11	Susceptible	*Rp1*-J	*Rp1*-J
JC13	Resistant	*Rp1*-C	*Rp1*-J
jc14a	Susceptible	*Rp1*-J	*Rp1*-C
JC14c	Resistant	*Rp1*-C	*Rp1*-J
Rp1-D × *Rp1*-J			
dj3	Susceptible	*Rp1*-D	*Rp1*-J
DJ4	Resistant	*Rp1*-J	*Rp1*-D
dj16	Susceptible	*Rp1*-D	*Rp1*-J
dj18	Susceptible	*Rp1*-D	*Rp1*-J
dj42	Susceptible	*Rp1*-D	*Rp1*-J
DJ46	Resistant	*Rp1*-J	*Rp1*-D
dj47	Susceptible	*Rp1*-D	*Rp1*-J
dj50	Susceptible	*Rp1*-D	*Rp1*-J
Rp1-F × *Rp1*-J			
FJ11	Resistant	*Rp1*-J	*Rp1*-F
FJ51	Resistant	*Rp1*-J	*Rp1*-F
fj55	Susceptible	*Rp1*-F	*Rp1*-J
FJ58	Resistant	*Rp1*-J	*Rp1*-F
jf59	Susceptible	*Rp1*-J	*Rp1*-F
FJ69	Resistant	*Rp1*-J	*Rp1*-F

[a] Only the recombinants derived using two rust isolates (individuals from Table 3) are included.
[b] The parent from which the marker allele was inherited, e.g. *Rp1*-D, indicates the recombinant has the marker allele of the *Rp1*-D parent.

markers as expected if they arose by crossing-over (Table 4). Three susceptible derivatives from the *Rp1*-C/*Rp1*-J population were recovered that did not have recombinant flanking markers. Derivatives jc1 and jc3b had alleles of the *Rp1*-C parent at both proximal and distal markers while jc11 had the marker genotype of the *Rp1*-J parent.

Rp1-D recombined with *Rp1*-J as though it mapped distally to *Rp1*-J in all of the recombinants. Resistant recombinants had the allele of the *Rp1*-D parent at the distal marker BNL3.04 and the allele of the *Rp1*-J parent at the proximal markers. The susceptible recombinants had the opposite combination of markers. In contrast, recombination in the other two populations did not consistently indicate a gene order. Three susceptible recombinants from the *Rp1*-J/*Rp1*-F population were selected using the rust isolate IN3 (Table 2), and two more were isolated using the combination of two rust isolates (Table 3). All five of the recombinants were susceptible to all three rust isolates. Of the five, two had the *Rp1*-J parent allele at the distal marker and the *Rp1*-F parent allele at the proximal marker as would be expected if *Rp1*-F mapped distally. The other three had the marker combination expected if *Rp1*-J mapped distally. All four of the double resistant recombinants had the flanking markers that would be expected if *Rp1*-F mapped distally to *Rp1*-J (Table 4). Susceptible recombinants from the *Rp1*-C/*Rp1*-J heterozygote also exhibited both possible nonparental combinations of flanking markers. Four had the flanking markers expected if *Rp1*-J mapped distally to *Rp1*-C and one had the opposite markers. The double resistant recombinants from the *Rp1*-C/*Rp1*-J cross exhibited only a single combination of flanking markers, those expected if *Rp1*-J mapped distally to *Rp1*-C.

All four of the RFLP probes used in this study recognize multiple alleles and were therefore very useful in verifying the origin of both the susceptible and double resistant derivatives. Two progeny from the *Rp1*-J/*Rp1*-D population, one progeny from the *Rp1*-J/*Rp1*-F, and four progeny from the *Rp1*-J/*Rp1*-C population were recovered that were resistant to both IN2 and KS1 but were determined to be the result of self-pollination contamination (*data not shown*). These were easily distinguished from the recombinants using the RFLP markers. The self-fertilized contaminants had alleles from both of the parents of the F_1 at the flanking markers. Furthermore, in progeny from the contaminants, the two *Rp1* genes segregated as though they were linked in repulsion, whereas progeny testing of the true recombinants verified they were linked in coupling. RFLP analysis also revealed another resistant individual from the *Rp1*-J/*Rp1*-D population that was the result of out-cross pollen contamination from a line with a different resistance gene, probably *Rp1*-E. All of the susceptible derivatives had the RFLP banding patterns expected for true recombinants as opposed to contaminants.

DISCUSSION

The recombinational behavior of several different *Rp1* genes was analyzed in heterozygotes. Their recombinational tendencies in heterozygotes were somewhat related to their meiotic instability. *Rp1* genes have been demonstrated to vary considerably in their meiotic stability in homozygotes (Bennetzen *et al.* 1988; Pryor 1987a,b). Lines that are homozygous for *Rp1*-C, *Rp1*-F, *Rp1*-G, *Rp1*-J, or *Rp1*-L have been observed to give rise to susceptible

derivatives at frequencies ranging from 0.5×10^{-3} to 3×10^{-3}, whereas most of the other *Rp1* genes, such as *Rp1*-D and *Rp1*-K, are more stable. Heterozygotes in which at least one of the *Rp1* genes is meiotically unstable usually showed fairly high recombination rates, equivalent to 0.1–0.25 map units of recombination.

Crosses with unstable genes also appear to be the most likely to produce derivatives with both possible nonparental combinations of flanking markers. The heterozygotes *Rp1*-A/*Rp1*-F, *Rp1*-C/*Rp1*-J, and *Rp1*-F/*Rp1*-J recombined in this manner (Tables 2–4). All of these genes are meiotically unstable in homozygotes with the possible exception of *Rp1*-A, which has not been examined. Susceptible recombinants from the *Rp1*-J/*Rp1*-F heterozygote occurred at roughly the same frequency in which they were found by Sudupak *et al.* (1993) in test crosses of an *Rp1*-J homozygote, five of 8,307 for *Rp1*-J/*Rp1*-F (Tables 2 and 3) and five of 9,772 for *Rp1*-J/*Rp1*-J. In both crosses two of the recombinant progeny had one nonparental combination of flanking markers whereas the other three had the opposite combination. The occurrence of both types of cross-overs in homozygotes is evidence of unequal crossing-over. Unequal crossing-over events require duplicated sequences that can mispair during meiosis (Sturtevent 1925; Tartof 1988). Crossing-over events within the duplications while they are mispaired allows genes that map to the same relative position in a series of two or more duplications to recombine.

The occurrence of susceptible recombinants from *Rp1* heterozygotes, such as *Rp1*-J/*Rp1*-F, with both nonparental combinations of flanking markers is also evidence of a form of unequal exchange; duplications in one (or both) of the parents are capable of pairing in two different arrangements with sequences from the other parent. The nature of the duplications that mispair and recombine has bearing on models of the structure of the *Rp1* area. One possibility is if dispersed repetitive sequences, capable of ectopic recombination, lie between the *Rp1* genes. A second possibility is that the *Rp1* genes themselves reside on duplications that retain synaptic homology. Sudupak *et al.* (1993) argue that the latter model is more likely, since ectopic recombination between repetitive DNA is usually very infrequent and because crossing-over in maize appears to usually involve low-copy sequences. Furthermore, recombination in *Rp1* heterozygotes has indicated that the area carries more than a single *Rp* locus, since multiple genes can be combined in a single haplotype. For example, we have now constructed haplotypes that carry *Rp1*-J, *Rp1*-F, and *Rp1*-G linked in *cis*.

Figure 1 shows possible structures for four of the *Rp1* differential lines. The arrows represent duplications (possibly tandem) that carry *Rp* genes. The model is similar to that originally presented by Saxena and Hooker (1968) in that different lines have different numbers and arrangements of dominant and recessive genes; where recessive genes could be nonfunctional or functional genes that are undetectable with the current collection of rust isolates. Lines with different numbers of duplications can be generated by recombination events occurring while the duplications are paired in different arrangements. The model is also similar to that proposed for the *R* locus in maize, a locus that controls pigmentation of different tissues and also carries duplications that can recombine unequally (Dooner and Kermicle 1971; Robbins *et al.* 1991). Genetic and molecular analysis of the *R* locus has indicated that the duplications may be large; the physical extent of the duplication in the standard *R-r* allele is not known but it is large enough to carry a second unrelated gene (*Isr*). Analysis of the *R* locus has also indicated that duplications may mispair as frequently as they as they pair correctly. Similar pairing between duplications carrying *Rp1* genes would make a resistance gene recombine as if it mapped distally to another *Rp1* gene if it had one or more duplications located centromere-proximally to it, and if the second gene did not have the proximal duplication(s). The recombinational tendencies of the *Rp1*-D and *Rp1*-A lines suggest that they have duplicated sequences that lie proximal to the resistance gene (Fig. 1). Both *Rp1*-A and *Rp1*-D generally recombine as though they map distally to other *Rp1* genes (Table 2; Hulbert and Bennetzen 1991). The recombinational properties of *Rp1*-F and *Rp1*-J suggest that these lines carry one or more duplications on each side of their respective gene. Both genes have, in most cases, recombined as though they map proximally to *Rp1*-D and *Rp1*-A, but at least *Rp1*-F maps distally to many of the other *Rp1* genes (Hulbert and Bennetzen 1991). These arrangements of duplicated sequences would also explain why *Rp1*-D or *Rp1*-A can occasionally recombine with *Rp1*-J or *Rp1*-F as though *Rp1*-D and *Rp1*-A map proximally. This presumably results from a less frequent type of mispairing; for example, if the duplication proximal to *Rp1*-J paired with the duplication carrying the *Rp1*-D gene. The proposed arrangements could explain why *Rp1*-D recombined with *Rp1*-J as though it mapped distally in nine out of nine recombinants. The proposed arrangements could also explain why *Rp1*-F and *Rp1*-J are among the most unstable genes as homozygotes. Other unstable genes such as *Rp1*-C and *Rp1*-L may also have duplicated sequences on each side of the gene. It is possible that the more stable genes such as *Rp1*-K have fewer duplications, but other explanations are possible. For example, it is possible that the more stable genes reside on duplications that are less recombinogenic or that these lines carry two detectable resistance genes linked in *cis*.

An interesting aspect of both the *Rp1*-F/*Rp1*-J and *Rp1*-C/*Rp1*-J recombinations is that the susceptible recombinants showed both nonparental combinations of flanking markers, but the recombinants with both resistance genes showed only one. Additional recombination events will

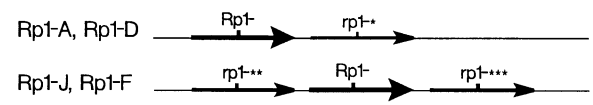

Fig. 1. Possible arrangements of duplicated sequences at the *Rp1* locus in maize lines carrying four different *Rp1* genes. Thick arrows represent sequences which carry detectable resistance genes. Thin arrows indicate the presence of one or more duplicated sequences located proximally or distally to the duplication carrying the detectable resistance gene. The physical size and numbers of the duplications are not known. Recombination between the repeats following pairing in different arrangements would give patterns of recombination similar to those observed in crosses between these four lines.

have to be isolated to determine if the double resistant class of recombinants is more limited in the arrangements in which they can pair and recombine to recover the double resistant phenotype. This might be expected if most of the cross-over events on the duplications occur within the resistance genes themselves. Robbins *et al.* (1991) found that most of the unequal exchanges between duplications carrying the *R* locus occurred within the *R* gene sequences.

Recombination events at *Rp1* are usually interchromosomal, as evidenced by flanking marker recombination. In a previous study (Hulbert and Bennetzen 1991), all of the susceptible derivatives from *Rp1* heterozygotes in which susceptibility was verified by progeny testing had recombinant flanking markers. Similarly, most of the susceptible and double resistant progeny identified herein were also associated with crossing-over. Three susceptible derivatives were identified from the *Rp1*-C/*Rp1*-J cross, however, which were not associated with crossing-over. The origin of these non-cross-over (NCO) derivatives is unknown. Three such derivatives were observed out of 3,479 *Rp1*-C/*Rp1*-J test cross progeny indicating they may occur frequently in certain genotypes. A previous study found one NCO type out of 20 susceptible derivatives from an *Rp1*-G homozygote (Sudupak *et al.* 1993). *Rp1*-C, *Rp1*-G, and *Rp1*-J generally recombine frequently in heterozygotes and homozygotes, indicating the event that generates the NCO derivatives is probably associated with recombination as opposed to mutation. One possibility is gene conversion, but intrachromosomal crossing-over events, such as recombination between sister chromatids or between adjacent duplications on the same chromatid (Laughnan 1961), could also result in susceptible derivatives with parental flanking marker genotypes.

The meiotic instability of *Rp1* genes has hindered their molecular isolation by transposon tagging approaches (Bennetzen *et al.* 1988; Pryor 1987b). Susceptible derivatives from most *Rp1* genes arise by recombination at a higher frequency than those due to insertional inactivation, and the two types of derivatives are difficult to distinguish from one another. Our results indicate that susceptible derivatives arising spontaneously in most *Rp1* heterozygotes and homozygotes (Sudupak and Hulbert 1992) generally have nonparental combinations of flanking markers. The use of heterozygous flanking markers may, therefore, enable one to distinguish between recombinants and derivatives from transposon insertion. The latter events would not be expected to result in flanking marker exchange. A possible tagging strategy would be to construct lines that were homozygous for a relatively stable *Rp1* gene but heterozygous for flanking markers in a background with an active transposable element system. An alternative approach would use hybrids between different *Rp1* genes that recombine infrequently and whose flanking marker alleles can be distinguished. In either case, susceptible test cross progeny from such lines could be assayed for flanking marker recombination to distinguish recombinants from transposon-induced mutants.

It is generally difficult to determine if two resistance genes that map to the same location represent two distinct loci or two closely linked genes. Shepherd and Mayo (1972) described the "modified *cis-trans* test" as a means of distinguishing between these two possibilities. The test, however, relies on the assumption that a single gene product cannot express the combined specificities of both parental genes. The results herein present an additional reason the test may be equivocal; genes may be allelic but able to generate derivatives with copies of both genes linked in *cis* by mispairing and recombination. Two resistance genes that are allelic, or even identical, may map to different relative positions after an unequal exchange event. A possible example of this is the resistance genes in the maize lines Golden King and GG208R. Both are designated *Rp1*-A, but one maps roughly 0.2 map units distal to the other (Hulbert and Bennetzen 1991). Although it is currently impossible to determine if they are actually identical genes, the two genes remain phenotypically indistinguishable even though their resistance has been compared by Lee *et al.* using 47 rust isolates and using 11 isolates from our current collection (Hulbert *et al.* 1991; T. Richter and S. H. Hulbert, *unpublished*). The results imply that classical allelism terminology is not appropriate when considering complex resistance genes which, in at least some lines, carry duplicated sequences that can mispair and recombine in ways that simple loci do not.

MATERIALS AND METHODS

Generation of segregating populations.

Maize stocks carrying *Rp1*-F, *Rp1*-I, *Rp1*-J, *Rp1*-K, and *Rp1*-L were in the R168 inbred background. The line carrying *Rp1*-A was derived from the hybrid cultivar Golden King. This cultivar was found to carry two separate resistance genes, only one of which mapped to the *Rp1* area (Hulbert *et al.* 1991). Hooker and LeRoux (1957) gave the *Rp1*-A designation to resistance genes from two different maize stocks, Golden King and GG208R, because they could not be distinguished on the basis of their resistance reaction to various rust isolates. Previous mapping experiments (Hulbert and Bennetzen 1991) indicated that the gene from Golden King mapped distally to the gene from the line GG208R. The *Rp1*-D lines used were in either the R168 or B14 background. *Rp1*-D in the R168 background was crossed to the *Rp1*-A, *Rp1*-B, and *Rp1*-L lines, and *Rp1*-D in the B14 background was crossed to the *Rp1*-J line to make their corresponding test cross populations. Ongoing experiments in our lab have indicated that most, or all, of these maize lines carry only a single resistance gene which is detectable with the rust isolates employed (T. Richter and S. H. Hulbert, *unpublished*).

Test cross populations were constructed by crossing F_1 hybrids of different *Rp1* lines to the tester cultivars H95 or OH43, which carry no known *Rp* genes. F_1 hybrids were used as females in these crosses so that rare self-fertilization events from contaminant pollen would not result in susceptible individuals.

Isolation of *Rp1* recombinants.

Three isolates of *P. sorghi* were used to screen the test cross populations (Hulbert *et al.* 1991). Their specific virulence phenotypes on the *Rp1* genes analyzed are given in Table 1. Test cross populations were screened with either

a single rust isolate, to identify susceptible recombinants only, or with two complimentary isolates, to identify both susceptible and double resistant recombinants. When screening with a single rust isolate, 8- to 10-day-old seedlings were inoculated by rubbing *P. sorghi* uredospores diluted with talc onto the first and second leaves and incubated in a moist chamber for 16 hr. When two isolates were used, the first isolate was inoculated as above, then 3 days later the second isolate was used to inoculate the third, or third and fourth leaves, depending on the stage of growth of the seedling. Resistance reactions were scored 8–10 days after inoculation. DNA was isolated from leaf material from each susceptible or double resistant derivative to assay flanking RFLP markers. Each derivative was grown to maturity and self-fertilized. The resulting progeny were rescreened with the rust isolates to verify the genetic change that occurred.

Analysis of flanking DNA markers.

RFLPs that mapped to each side of the *Rp1* locus were used to assay recombination. BNL3.04, which maps two to four map units from most *Rp1* genes, was used as the distal flanking marker (Hulbert and Bennetzen 1991). At least two of three proximally-mapping RFLP markers were assayed for each putative recombinant. NPI285 maps roughly four to 11 map units proximally to *Rp1*, depending on the cross, whereas KSU3 and KSU4 map about a single map unit proximal to *Rp1* in most crosses. The KSU3 and KSU4 probes were isolated from a genomic library using NPI422 as a probe (Hong *et al., in press*). They are superior probes for most purposes because they hybridize more strongly than NPI422 to the DNA sequences that map near *Rp1*, and they identify additional polymorphic restriction fragments that map to the *Rp1* area.

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