Reactions of Maize Lines Carrying *Rp* Resistance Genes to Isolates of the Common Rust Pathogen, *Puccinia sorghi*

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ABSTRACT

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Isolates of the common rust fungus, *Puccinia sorghi*, were collected from North America, Hawaii, and Africa, and their specific virulence phenotypes were tested using a collection of maize lines carrying different resistance genes. Although all of the isolates collected in 1988 had identical specific virulence phenotypes, the pathotypes collected in 1989 were phenotypically diverse. Maize lines, the resistances of which could not be differentiated by a previous collection of rust isolates of this fungus, usually could not be differentiated by these isolates. One exception was the cultivar Golden King, which was differentiated from other lines carrying $Rp1^A$ by the presence of an additional, previously uncharacterized, resistance factor. This resistance segregated as a single dominant gene independent of the *Rp1* locus.

More than 100 sources of resistance to the common rust fungus, *Puccinia sorghi* Schwein., were identified in maize lines during the 1950s and 1960s (3,5, 6,8,12). In many of these lines, resistance was expressed in the seedling stage and was race specific (4). Twenty-four dominant resistance factors (genes or alleles) were differentiated among these maize lines by the spectrum of rust isolates to which they conferred resistance and by their placement on the maize genetic linkage map. These genes for resistance map to three areas of the maize genome—a cluster of loci on chromosome 10 (*Rp1*, *Rp5*, and *Rp6*) and two other possibly complex loci on chromosomes three (*Rp3*) and four (*Rp4*). These loci have received considerable attention in recent years because of their complexity, instability, and potential for molecular accessibility (1,2,7,10).

Hooker and co-workers (3,5,6,8,12) identified and differentiated the various common rust resistance factors using a diverse collection of *P. sorghi* isolates. In many cases, different cultivars or plant introductions were assumed to carry identical resistance genes because they could not be differentiated with this rust collection. This assumption was probably correct when considering closely related lines. For example, lines B38, B216, and B217, from the Iowa State University breeding program, all carry $Rp1^B$. However, among unrelated cultivars or plant introductions, it is possible that apparently similar resistance genes are not identical and could be differentiated if tested with additional rust isolates.

The collection of rust isolates used by Hooker and co-workers to differentiate the various resistances to common rust no longer exists. To be able to distinguish known resistances to common rust, which is essential for molecular and genetic analysis of loci, and to identify

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new sources of resistance, it is necessary to reestablish a collection of *P. sorghi* pathotypes. Although our initial characterizations of field isolates from the United States corn belt indicated that little variation exists in those populations, subsequent efforts demonstrated that considerable diversity in *P. sorghi* pathotypes can occur in certain years even within small geographical areas. Thus, we were able to establish a new collection of *P. sorghi* pathotypes that can differentiate most of the known resistances to common rust. In addition, we have used this collection to identify a second independently segregating resistance gene present in a maize line previously believed to contain only a single component.

MATERIALS AND METHODS

Collection of rust isolates. Field isolates of *P. sorghi*, collected in 1984, were provided by A. H. Ellingboe, University of Wisconsin, Madison. In 1988 two isolates were collected at the Purdue University, Department of Agronomy Farm, West Lafayette, IN; two were obtained from J. Hawk, University of Delaware, Newark; one from J. K. Pataky, University of Illinois, Urbana; and two from B. A. McDonald, Texas A&M University, College Station. In 1989, isolates were collected on Molokai and Oahu, Hawaii, and in Indiana. Isolates were also received from Texas (obtained from R. A. Frederiksen, Texas A&M University) and from Kenya (collected by L. E. Claflin, Kansas State University) in 1989. A Georgia field population, collected in 1975, was obtained from W. K. Wynn, University of Georgia, Athens. In 1990, an isolate was collected at the Rocky Ford Experimental Farm, Kansas State University, Manhattan.

Many of the field isolates were suspected of being mixtures of different pathotypes because they were collected as bulk field populations. Such populations were inoculated on the differential cultivars (in the R168 inbred background) to distinguish and separate possible component pathotypes. Mixtures of isolates can sometimes be detected by observing both resistant and susceptible reactions on a single plant or cultivar. When this was observed, subpopulations harvested from one or more maize lines with single resistance genes were collected and used to reinoculate the differential series. To obtain pure cultures, susceptible maize seedlings were inoculated at a low inoculum density so that only one or a few infections were established on each seedling. Isolates were then propagated from a single lesion. No more than three isolates were propagated at any given time. When more than a single isolate was examined simultaneously, isolates were propagated in separate greenhouses. As an additional measure to prevent contamination, each isolate being propagated was cultivated on maize lines on which the other isolate(s) being propagated could not grow. Isolates were stored as collections of pure uredospores in gelatin capsules sealed in a capped tube with $CaSO_4$ desiccant; these were kept frozen, at −70 C, for long-term storage or at 4 C for short-term storage.

Differential cultivars. Hooker and coworkers crossed the *Rp* resistance genes into the R168 inbred background and many of them into the B14 background to create an isogenic series of differentials. All of the previously characterized dominant genes for resistance are available in the R168 background, except *Rp6* and $Rp1^B$. In addition to the *Rp* lines in the R168 background, we used the following maize lines as our differentials. Our source of *Rp6* was PI 172597 because no inbred line carrying this resistance gene was available. We used lines derived from the cultivar Golden King as an additional source of $Rp1^A$. Our differentials for $Rp1^B$ were the inbred lines B216 and B217, as well as $Rp1^B$ from B38 in the B14 background. Of the three sources of $Rp1^C$ used, $Rp1^C$ from K148 was in the R168 background and that from Blacks Yellow Dent and Synthetic A were in the B14 background. Of the three sources of $Rp1^D$ used, $Rp1^D$ from Cuzco was in the R168 background and that from Njoro and Kitale were in the B14 background. When possible, we compared the resistance of the available differential lines and the resistance of the lines from which the resistance genes were first extracted (4) to determine if the lines carried the genes intended. In this manner, we found that some of the stocks of $Rp1^A$, $Rp1^C$, $Rp1^L$, $Rp1^M$, and $Rp1^N$ had lost the resistance gene they were thought to carry. Homozygous lines carrying all of these genes were established.

Inoculation and resistance ratings. Ratings of the interactions of the various *Rp* resistance genes with the *P. sorghi* isolates were made in the greenhouse. Seedlings were grown in 38 × 61 × 8 cm flats with 10 rows per flat. Each row consisted of eight to 10 seedlings of a differential line. Freshly harvested uredospores of *P. sorghi* were diluted roughly 1:20 (v/v) with talc and rubbed onto the second and third leaves of the seedlings when they were 7–10 days old. Seedlings were then placed in a dew chamber overnight (16 hr) and scored as resistant or susceptible after observation at 7 and 10 days after inoculation.

Resistance was commonly expressed as small chlorotic or necrotic flecks with no sporulation. Lines with small pustules surrounded by chlorosis or necrosis were also considered resistant. Lines that formed well-developed pustules were considered susceptible. Each of the differential lines was tested with each of the rust isolates on at least two separate occasions. Many of the isolate/differential line combinations were observed several times. Interactions were labeled intermediate if resistant and susceptible reaction types were observed on a single leaf or if reaction type was inconsistent between the seedlings of a single line. Although most of the disease reactions of differential lines and specific isolates were repeatable, a few of the intermediate interactions varied in reaction type between inoculations.

RESULTS AND DISCUSSION

Virulence phenotypes of *P. sorghi* isolates. The seven isolates collected in 1988 were indistinguishable with respect to specific virulence on the various *Rp* differential lines. This suggests that a single pathotype can occupy large geographical areas and diverse environments because these isolates were collected from several states (Delaware, Illinois, Indiana, and Texas). Furthermore, our 1988 isolates had virulence phenotypes identical to the 1984 rust isolates obtained from A. Ellingboe (1-2, 1-4, and 1-5), which we also found were indistinguishable from each other (see isolate 1-4, Table 1). This indicates a single pathotype may be prevalent in the corn belt in more than one growing season. A pathotype similar to those from 1984 and 1988 may have been prevalent in 1986 as well; Pataky (9) tested isolates from several regions of North America and found that maize lines containing $Rp1^D$, $Rp1^E$, $Rp1^F$, $Rp1^G$, $Rp1^I$, $Rp1^K$, and $Rp3^C$ were resistant to all isolates. The 1-4 pathotype and our 1988 isolates are also avirulent on all of these resistance genes.

In contrast, isolates collected in 1989 showed considerable diversity. Three different pathotypes were isolated from a single field at the Purdue Agronomy Farm (IN1, IN2, and IN3). An additional isolate collected from Texas (TX1) was also different. Two isolates selected from collections made in 1975 from Georgia (GA1 and GA2) were also different from the other isolates. All of these isolates differed in virulence from the 1988 pathotype on five or more of the differential lines. At least one of these six isolates was virulent on each of the identified *Rp* resistance genes except $Rp1^D$ and $Rp3^C$.

Two identical isolates collected from Molokai and Oahu in 1989 (HI1) were virulent on both $Rp1^D$ and $Rp3^C$. An isolate collected from Kenya (AF1) was virulent on $Rp1^D$ but not on $Rp3^C$, as was an isolate collected in Kansas in 1990. This is the first isolate from the United States that we have observed that is virulent on $Rp1^D$. Therefore, $Rp3^C$ is the only *Rp* allele that is resistant to all of the North American isolates tested. Nearly all of the *Rp* resistance genes provided resistance to at least one of the rust isolates. The only lines that did not provide resistance to any isolates were those carrying $Rp4^A$ and *Rp6*.

The origin of the diversity in the recent

populations of *P. sorghi* in North America is not certain, but the multiple differences between each of the isolates is suggestive of sexual recombination. The frequencies of certain virulence factors may have changed in North America since the 1960s. For example, $Rp1^M$ provided resistance to all 10 of the isolates used by Hagan and Hooker (3), whereas most of the isolates in our collection are virulent on this resistance gene (Table 1).

Independence of resistance factors in the *Rp1* differential lines. Most or all of the resistances of the differential lines listed in Table 1 have been differentiated by isolates used in previous studies (3,6,8,12). It is possible, however, that $Rp1^C$ and $Rp1^N$ or $Rp1^F$ and $Rp1^A$ are not distinct alleles. Published data include only single isolates that differentiate these pairs of lines, and the recorded differences in resistance are intermediate vs. resistant (3) or susceptible (12) responses. Furthermore, these comparisons were made before isogenic lines were available, so it is possible that the differences observed were attributable to other factors such as genetic background. Neither of these pairs of lines could be differentiated by our current collection of rust isolates.

Evidence from recombination studies indicates that the *Rp1* area is composed of several distinct, closely linked loci (*Rp1*, *Rp5*, and *Rp6*) and that the *Rp1* "locus" itself is composed of multiple loci (7,11). It is possible, therefore, that the resistance of certain *Rp1* alleles to some *P. sorghi* isolates are actually controlled by two distinct genes closely linked in the *cis* configuration, each of which could provide resistance by itself. It follows that two *Rp* differentials may carry a common resistance gene but still show a differential response to some rust isolates if one of the differentials carries an additional gene. In this case, the differential line with the extra gene would be resistant to every isolate that was avirulent on the other differential line and would also be resistant to additional isolates. The resistance reactions of several differentials on both the previous and current collection of rust isolates indicates there are alleles that may not be unique. For example, $Rp1^L$ or $Rp1^N$ could be $Rp1^C$ plus another allele, and $Rp1^I$ could be either $Rp1^E$ or $Rp1^K$ plus another allele. Therefore, it should not be assumed that the resistances of these differentials to some of the isolates are controlled by independent alleles. The interactions of other alleles, such as $Rp1^M$, with the current collection of rust isolates indicates they are unique genes or alleles. It was not previously clear whether the resistance provided by $Rp1^M$ to the previous collection of rust isolates was attributable to a unique gene or to a combination of two other *Rp1* genes (e.g., $Rp1^A$ plus $Rp1^B$).

A novel resistance factor. Some maize lines have consistently shown identical patterns of resistance or susceptibility to previous collections of rust isolates and may carry identical *Rp* alleles. Cultivars or lines with resistances that have not been differentiated in previous studies are listed as separate sources of the same *Rp1* allele in Table 1. As with the previous collections of isolates, we could not differentiate the resistances of the three lines thought to carry $Rp1^C$ (K148, Synthetic A, or Blacks Yellow Dent) nor could we differentiate the three sources of $Rp1^D$ (Cuzco, Njoro, or Kitale). Similarly, we could not differentiate a line derived from the cultivar Golden King and the $Rp1^A$ line derived from GG208R. However, another line derived from Golden King showed a different resistance reaction indicating the presence of more than one resistance gene in Golden King.

Progeny derived by self-fertilization of the hybrid cultivar Golden King segregated for the $Rp1^A$ rust resistance gene and also for another resistance factor that provided resistance to the African isolate AF1 (virulent on $Rp1^A$). One individual that carried the gene providing resistance to AF1 and not $Rp1^A$ was outcrossed to the inbred line R168 carrying $Rp1^F$ ($Rp1^F$-R168). This hybrid was then test-crossed to the susceptible inbred A188. The resulting test-cross population segregated approximately 1:1 for resistance to isolate 1-4 (resistance provided by $Rp1^F$) and for resistance to isolate AF1 (provided by the gene from Golden King).

To test the linkage relationships of the two resistance genes, individual seedlings were scored for resistance to both isolates on separate leaves. The two resistance genes segregated independently of each other. Of 135 test-cross progeny, 35 were resistant to both isolates, 33 were susceptible to both isolates, 27 were resistant to AF1 but susceptible to 1-4, and 40 were susceptible to AF1 but resistant to 1-4 (χ^2 1:1:1:1 = 2.57, $0.25 > P > 0.50$). The resistance gene from Golden King is, therefore, not allelic to any of the resistance genes previously mapped to chromosome 10 of maize (*Rp1*, *Rp5*, or *Rp6*). Furthermore, this gene is not identical to any of the characterized alleles at *Rp3* or *Rp4*. It does not provide resistance to isolate 1-4, whereas all of the characterized *Rp3* alleles do, and it provides resistance to the AF1, whereas the alleles at *Rp4* do not.

The identification of a new resistance gene in the cultivar Golden King illustrates the utility of a diverse group of pathotypes in identifying new resistance genes, even in material that has been screened previously. These isolates have also proven useful for testing the independence of the factors controlling the

Table 1. Virulence phenotypes of *Puccinia sorghi* isolates on maize *Rp* differentials

Rp gene	Source[x]	Common rust reaction by isolate[y]									
		1-4	HI1	IN1	IN2	IN3	GA1	GA2	TX1	AF1	KS1
$Rp1^A$	GG208R, G. King	–[z]	+	+	–	–	–	–	–	+	+
^{B}	B38	I	–	+	–	–	–	I	–	I	+
^{C}	K148, Syn. A, B.Y. Dent	+	–	+	–	+	+	+	+	+	+
^{D}	Cuzco, Kitale, Njoro	–	+	–	–	–	–	–	–	+	+
^{E}	B49	–	–	–	–	+	–	+	+	–	–
^{F}	PI 172332	–	+	+	–	–	–	–	–	+	+
^{G}	PI 163558	–	–	–	+	–	+	+	+	+	+
^{H}	Guanajuato 29-157A	+	+	+	+	–	+	+	I	I	–
^{I}	PI 163558	–	–	–	–	+	–	+	+	–	–
^{J}	Queretaro VI 366	+	+	+	+	–	+	+	+	I	–
^{K}	Queretaro V 231-5	–	–	–	–	+	–	+	+	–	–
^{L}	PI 163558	+	–	+	–	+	+	+	+	+	+
^{M}	PI 163563	I	–	+	+	+	+	+	+	+	–
^{N}	BZU-20	+	–	+	–	+	+	+	+	+	+
$Rp3^A$	25	–	+	–	I	–	+	+	+	–	–
^{B}	M16	–	+	–	I	–	+	+	+	–	–
^{C}	NN14	–	+	–	–	–	–	–	–	–	–
^{D}	Leon I 27-4-1	–	+	–	I	–	+	+	+	–	–
^{E}	Hidalgo 3-5-1	–	+	–	I	–	+	–	+	–	–
^{F}	PI 251653	–	+	–	I	–	+	+	+	–	–
$Rp4^A$	Queretaro V 260-1	+	+	+	+	+	+	+	+	+	+
^{B}	PI 193906	+	+	I	I	I	+	+	+	+	+
Rp5	PI 186191	I	+	–	+	+	+	+	+	–	–
Rp6	PI 172597	+	+	+	+	+	+	+	+	+	+

[x]The maize line from which the designated *Rp* allele was originally obtained.

[y]Isolate 1-4 had an identical virulence phenotype to two other isolates, 1-2 and 1-5, collected in 1984 and to seven isolates collected from four states in 1988. HI1 corresponds to two phenotypically similar isolates collected in Hawaii in 1989. IN1, IN2, IN3 were collected in Indiana in 1989. GA1 and GA2 were isolated from a population that was collected in 1975 in Georgia. TX1 was collected in Texas in 1989 and AF1 was collected in Kenya in 1989. KS1 was collected in Kansas in 1990.

[z]– = Isolate was avirulent, expressed as chlorotic or necrotic flecks or as small pustules surrounded by chlorosis or necrosis; + = isolate was virulent and formed well-developed pustules; and I = intermediate interaction with inconsistent reaction type.

resistance of the various *Rp1* differential lines. The resistance reactions of these lines to the current collection of isolates verified that some carry unique *Rp* genes or alleles but raised the possibility that others may share a common gene. The possibility that certain *Rp1* alleles are actually composed of two closely linked genes will be investigated by testing with additional isolates and by genetic fine-structure analysis of the *Rp1* area.

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