New Sources of Resistance to Southern Corn Leaf Blight from Tropical Hybrid Maize Derivatives

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ABSTRACT

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This study documents several new sources of resistance to *Bipolaris maydis* (= *Helminthosporium maydis, Cochliobolus heterostrophus*) in tropical maize (*Zea mays* L.) germ plasm and presents preliminary data on their mode of inheritance. Three selected inbred lines were intercrossed and were also topcrossed to a known resistant (NC250) and a known susceptible (B73) inbred. Early-generation progenies from these crosses were evaluated in greenhouse and field tests using a mixture of race O isolates as the initial inoculum source. In field studies, natural inoculum levels were high and artificial inoculum was applied once, about 6 wk after planting. Several sources of resistance roughly equivalent to the high level of resistance available from NC250 were identified. Inheritance studies suggest that different genetic systems are involved, with a range of additive and recessive types of gene action. Resistance in these sources appears to be controlled by relatively few genes that combine in a positive epistatic fashion with the resistance genes in NC250.

Additional keywords: exotic germ plasm

Tropical maize (*Zea mays* L.) germ plasm is known to have resistance to *Bipolaris maydis* (Nisik.) Shoemaker (= *Helminthosporium maydis* Nisik. & Miy., *Cochliobolus heterostrophus* (Drechs.) Drechs.)) (6), but relatively few studies have been done to determine what types of resistance are available or how the resistance is inherited.

B. maydis was considered a relatively minor pathogen of corn until the onset of the southern corn leaf blight epiphytotic in the early 1970s. The disease was controlled quickly by avoiding the use of Texas male-sterile cytoplasm (*cms*-T) in the production of hybrids, but the extensive damage caused by the pathogen inspired research into the nature of resistance. Most of the research centered around the new race of the pathogen (race T) and the particular properties of the new race that allowed a previously minor pathogen of maize to destroy some fields in the southern growing region and cause an estimated 15% crop loss on a national basis (1,5). The cytoplasmic nature of the specific susceptibility of *cms*-T maize to race T of *B. maydis* was examined extensively, and research was also done to learn more about the nuclear-genetic component of resistance to the pathogen (7,9,11).

Some research on the inheritance of resistance had been done before the epiphytotic in the United States. In a diallel analysis, Pate and Harvey (10) found a quantitative mode of inheritance with resistance genes partially dominant. In 1968, Craig and Daniel-Kalio (2) published the initial report on a chlorotic lesion type of resistance from a Nigerian maize germ plasm source. Smith and Hooker (12) later determined that resistance from the Nigerian source was controlled by a single recessive gene, designated the *rhm* gene. The *rhm* gene confers a high level of resistance to the pathogen up to anthesis, at which time resistance is lost. The *rhm* gene represents the first documented attempt of breeders in the United States to use resistance to *B. maydis* from tropical maize germ plasm.

Shortly after the 1970 epiphytotic, several researchers began extensive investigations into the nature of resistance to *B. maydis*. Lim and Hooker (5,7–9) studied some quantitative sources of resistance available in corn belt germ plasm by using different combinations of normal and *cms*-T cytoplasms in conjunction with race O and/or race T of the pathogen. Their research demonstrated that resistance genes were partially dominant and that no cytoplasmic-genomic interaction was associated with resistance.

Thompson and Bergquist (13) recently described the inheritance of a new source of resistance from germ plasm related to the Nigerian material from which the *rhm* gene was isolated (12). In contrast to previous work that generally emphasized disease evaluations of seedlings or immature plants, Thompson and Bergquist's work emphasized mature plant resistance, with disease ratings several weeks after flowering. The mode of inheritance of this new source of resistance is not entirely clear, but it

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appears that two complementary recessive genes are the major controlling factors (13). The evaluation of the segregating progeny of crosses of NC250 (resistant line) × B73 (susceptible) and NC250 × B73*rhm* suggested that epistatic interactions among resistance genes may explain some of the confusion as to the number of genes involved and the type of gene action (13).

The purpose of this study was to document the presence of several new sources of resistance to *B. maydis* in 100% tropical germ plasm and to gather preliminary data on the type of gene action and the frequency of resistant phenotypes in segregating progeny from resistant × susceptible line crosses. The study was designed to evaluate full-season resistance, with emphasis on mature plants.

MATERIALS AND METHODS

A group of 100% tropical maize inbreds was derived from progeny of a diallel cross of nine tropical hybrids as part of a germ plasm enhancement project. The tropical hybrids used included Pioneer Overseas (Jamaica) hybrids X105A, X304B, and X306B; Agroceres (Brazil) 155 and 504; and CNTA (Centro Nacional de Tecnologia y Agropecuaria, El Salvador) H5 and H101. These hybrids contain germ plasm representing several races of maize, including Coastal Tropical Flint, Cuban Flint, Tusón, Cateto, and Tuxpeño. This germ plasm has been described by Goodman (3) and Holley and Goodman (4). A combination of sib mating followed by selfing was used to develop inbred lines roughly equivalent to S_4's in a standard selfing program. Field observations during the development of the inbred lines indicated a high level of resistance to *B. maydis* among most of the tropical hybrid derivatives.

In a preliminary study, 22 inbred lines representing a wide range of tropical pedigrees were grown in the greenhouse and artificially inoculated with a spore suspension of *B. maydis* race O 6 wk after planting. As in all of the greenhouse studies, a combination of conidial suspensions, ground diseased leaf material, and/or infected sorghum (*Sorghum bicolor* (L.) Moench) seed was used, as needed, in later inoculations to assure a high level of disease pressure. B73 and NC250 were included with the tropical inbreds in a randomized complete block design with two replications with a minimum of seven plants per plot. Each night, water was applied to the plants via overhead sprinklers to promote secondary cycles of infection for maximum disease development. Throughout this study, mature plant resistance was emphasized; plants were evaluated at least 3 wk after anthesis. A rating scale from one to nine was used throughout the study, with 1 = highly resistant and 9 = highly susceptible.

Based on preliminary screening results and general agronomic qualities, three of the most highly resistant tropical inbreds were selected for further study. They were designated as (X105A × H5), (X105A × X306B), and (X306B × H5), using the hybrid pedigrees from which they were derived. The selected lines were intercrossed with each other and crossed as males to B73 and NC250.

The early-generation progeny (F_1, F_2, F_3) from these crosses were used in a preliminary inheritance study. The B73 topcross hybrids were grouped with Pioneer hybrid 3369A, NC250, and NC250 × B73 and evaluated in the greenhouse and in the 1984–1985 winter nursery in Homestead, FL. A randomized complete block design with two replications was used in both locations, with five to 10 plants per plot. In the field studies, inoculum (sorghum seed) was applied only once, about 6 wk after planting, because of the high levels of natural inoculum in Florida.

In a second test, the intercrosses of the tropical inbreds were included with the NC250 crosses, Pioneer 3369A, and NC250 × B73 and evaluated in the 1984–1985 winter nursery in Florida. This test was adjacent to the B73 topcross test just described and received the same treatment. A randomized complete block design with two replications was used.

In 1985–1986, the advanced-generation progenies (individual F_2 plants and F_3 families), intercrosses, and topcrosses of the three highly resistant inbreds were evaluated in both greenhouse and winter nursery environments. In greenhouse tests, about 200 individual F_2 plants were grown from each cross, with three crosses per trial and two trials per year. In each greenhouse experiment, B73 and NC250 were placed at random locations within rows as checks. In addition, 100 F_3 families of each of the three B73 × tropical inbred populations were evaluated in the Homestead winter nursery. A randomized complete block design with two replications was used for all three crosses.

The one to nine rating system was used to assign genotypes to one of three classes designed to reflect relative frequencies of extreme phenotypes in both lesion type and quantity: 1–2 = highly resistant, 3–5 = moderately resistant, and 6–9 = susceptible. Phenotypes with a rating of 1 or 2 had very little or no necrotic lesion development, whereas phenotypes with ratings of 6–9 had large, necrotic, rapidly developing lesions. Observations of the randomly placed plants of NC250 and B73 were part of the data base used in determining the range of values assigned to the three classes.

RESULTS AND DISCUSSION

Several new sources of resistance to *B. maydis* were identified (Table 1), shown to be roughly equivalent to NC250 in level of resistance, and shown to be equal or superior to NC250 in hybrid combination with a susceptible tester (B73) (Table 2). Resistance rankings for inbreds and hybrids were consistent across greenhouse and field evaluations. Resistant genotypes show a hypersensitive reaction, with small chlorotic flecks appearing after inoculation with the pathogen. The level of full-season resistance in these genotypes was much greater than resistance conditioned by the *rhm* gene (Table 1). No residual heterozygosity for resistance was observed within any of the inbred lines.

When the tropical inbreds were crossed to the susceptible tester (B73), the hybrids ranged from moderately resistant to susceptible (Table 2). The range of resistance indicated additive and recessive types of gene action, in contrast with the recessive type found in NC250. The inbreds generated from the crosses of (X105A × H5) and (X105A × X306B) both appeared to have genes that act additively, although a combination of dominant and recessive genes could produce the same results. In contrast, the (X306B × H5) inbred appears to have genes that act in a recessive manner similar to NC250.

The results of the B73 topcross test supported previous findings of

Table 1. Mean disease ratings for selected inbred lines grown in the greenhouse and inoculated with *Bipolaris maydis*

Pedigree[a]	Disease rating[b]
NC250	1.0
B73	9.0
B73*rhm*	7.5
(H101)	1.0
(X105A × H5)	1.0
(X105A × X306B)	2.0
(X304B × H101)	2.0
(X306B × H5)	2.0
(X105A × Ag 155)	2.0

[a] The hybrid designations within parentheses refer to the pedigrees from which inbreds were derived.

[b] Resistant = 1, susceptible = 9. The least significant difference ($P = 0.05$) = 2.2.

Table 2. Mean disease ratings for selected B73 topcross hybrids inoculated with *Bipolaris maydis*, based on greenhouse and field data

Pedigree[a]	Disease rating[b]
NC250	1.0
NC250 × B73	8.0
Pioneer 3369A	4.8
B73 × (X105A × H5)	5.5
B73 × (X105A × X306B)	6.0
B73 × (X306B × H5)	7.4

[a] The hybrid designations within parentheses refer to pedigrees from which inbreds were derived.

[b] Resistant = 1, susceptible = 9. The least significant difference ($P = 0.05$) = 1.6.

Table 3. Mean disease ratings for NC250 topcross hybrids and tropical resistant line intercrosses, based on field data from Florida

Pedigree[a]	Disease rating[b]
NC250	1.4
NC250 × (X105A × H5)	1.8
NC250 × (X105A × X306B)	2.8
NC250 × (X306B × H5)	5.6
(X105A × X306B) × (X105A × H5)	2.1
(X105A × H5) × (X306B × H5)	3.1
(X105A × X306B) × (X306B × H5)	1.8

[a] The hybrid designations within parentheses refer to the pedigree from which inbreds were derived.

[b] Resistant = 1, susceptible = 9. The least significant difference ($P = 0.05$) = 1.1.

Table 4. Segregation ratios of individual F_2 plants (greenhouse) and F_3 families (field-grown) for topcross and intercross progeny

Pedigree	Class[a]		
	Susceptible	Intermediate	Resistant
(B73 × (X105A × H5))F_2	20	134	29
(B73 × (X105A × H5))F_3	4	88	8
(B73 × (X105A × X306B))F_2	9	136	21
(B73 × (X105A × X306B))F_3	6	84	10
(B73 × (X306B × H5))F_2	126	52	3
(B73 × (X306B × H5))F_3	92	8	0
(NC250 × (X105A × H5))F_2	2	20	154
(NC250 × (X105A × X306B))F_2	0	43	152
(NC250 × (X306B × H5))F_2	10	77	99
((X105A × H5) × (X105A × X306B))F_2	0	4	190
((X105A × H5) × (X306B × H5))F_2	0	33	149
((X306B × H5) × (X105A × X306B))F_2	0	30	167

[a] A disease rating above 5 was classified as susceptible, 3–5 was classified as intermediate, and below 3 was classified as resistant.

Thompson and Bergquist (13) describing the recessive behavior of the resistance genes in the NC250 genome. However, the results of the NC250 topcross and tropical inbred intercross evaluations reflect a more complicated mode of inheritance than the B73 topcross results suggested. Specifically, the cross of a recessive resistant genotype with an additive resistant genotype is expected to produce hybrids with moderate levels of resistance, as in the case of a susceptible × additive resistant cross, assuming there is little or no epistasis. When NC250 was crossed with (X105A × H5) or (X105A × X306B), the hybrids were highly resistant (Table 3). The hybrid of NC250 × (X105A × H5) was statistically equivalent in resistance to NC250 and significantly more resistant than two of the tropical inbred intercross hybrids, including (X105A × H5) × (X105A × X306B). In addition, the cross of the two "recessive" sources of resistance (NC250 × (X306B × H5)) was moderately resistant. Some of these inconsistencies could be explained by different resistance sources having some genes in common. However, this line of evidence, combined with the fact that the topcross progeny show different types of gene action, indicates that at least some of the genes differ among sources. In the case of the cross of the two "recessive" sources of resistance (NC250 × (X306B × H5)), the moderate level of resistance can be explained only on the basis of an accumulation of minor modifying genes or some form of epistasis.

Evaluation of later-generation progenies from the topcrosses and intercrosses did not provide sufficient information to fully determine the genetic basis of the resistance source interactions that appeared in the F_1 generation hybrids. The segregation ratios of the F_2 generation progeny from the NC250 topcrosses with tropical inbreds all showed high frequencies of resistant genotypes (Table 4). The high frequency of resistant genotypes is strong evidence of some positive epistasis similar to that found by Thompson and Bergquist (13) in the cross of NC250 × B73*rhm*.

At first glance the data in Table 4 appear to suggest that the resistance from (X105A × H5) is not significantly different from that of (X105A × X306B); however, the segregation of a few (2%) moderately resistant progeny from the cross of highly resistant parents suggests that the two parents differ at least slightly. The intermediate level of resistance of the F_1 generation of the two inbreds (Table 3) also argues against the two genotypes having the same set of resistance genes. In addition, the frequencies of resistant and susceptible progeny among the F_2's when the two lines were crossed to NC250 were much different (Table 4).

Because of observed differences in gene action, there can be little doubt that the genes carried in the (X306B × H5) inbred are different from those in the other tropical inbreds. With this in mind, the high frequencies of resistant progeny in the F_2 generation of (X306B × H5) × (X105A × H5) and (X306B × H5) × (X105A × X306B) support the hypothesis of positive epistasis.

Some caution is warranted in the interpretation of the data from the segregating generations of resistant × resistant crosses that were evaluated only in the greenhouse experiment, where disease pressure was lower than in the field. The minor differences between the ratios of individual F_2 plants in the greenhouse and F_2 families in the field probably partly reflect differences in disease pressure.

The frequency of fully resistant segregates among the F_2 and F_3 progeny of the resistant × susceptible line crosses suggests that backcross transfer of resistance can be accomplished using relatively small populations (Table 4). This resistance is retained for at least 4 wk after flowering and can still be seen at harvest in the most resistant genotypes.

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