Inheritance of Resistance to *Phaeosphaeria* Leaf Spot of Maize

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ABSTRACT

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Phaeosphaeria leaf spot is a potentially important maize disease that has recently appeared in the continental United States in winter breeding nurseries in southern Florida. Inbred lines related to B73 are particularly susceptible to *Phaeosphaeria* leaf spot, whereas inbreds related to Mo17 are highly resistant. The inheritance of resistance to *Phaeosphaeria* leaf spot was studied in the parents, F_1 , F_2 , and backcross generations derived from the cross B73 $\times$ Mo17. A replicated experiment was conducted over two winter nursery seasons in a southern Florida nursery. Individual plants in plots were evaluated for *Phaeosphaeria* leaf spot severity (0 to 9 scale) at the mid-dent stage of kernel development. Variation in segregating generations appeared continuous, so generation mean analysis was used. Additive and, to a lesser extent, dominance gene action were determined to play a role in the inheritance of reaction to *Phaeosphaeria* leaf spot. There was no evidence for epistatic gene interactions. Heritabilities (both narrow and broad-sense) were high (0.70 to 0.85) and the magnitude of genotype $\times$ environment interactions was low. Estimates of the number of effective factors (genes) involved in the inheritance of resistance ranged from three to four. Selection should be highly effective in developing inbred lines with adequate levels of resistance to *Phaeosphaeria* leaf spot.

Phaeosphaeria leaf spot, caused by the ascomycete *Phaeosphaeria maydis* (Henn.) Rane, Payak, & Renfro (anamorph = *Phoma maydis*, synonym = *Leptosphaeria zea-maydis* Saccas; *Metasphaeria maydis* (Henn.) Höhnelt), is a potentially serious foliar disease of maize that is widely distributed in Central and South America, Asia, and Africa (5). The disease is most prevalent in areas of high rainfall and moderate temperatures, such as occur at higher elevations in the tropics.

Phaeosphaeria leaf spot is becoming an increasingly important disease of maize in South Africa and Brazil. It has occurred regularly in southern Florida winter maize breeding nurseries since at least 1990, but has not yet appeared in the rest of the continental United States (2). Although there is little published information about *Phaeosphaeria* leaf spot, its ability to cause damage to maize in other areas of the world make the recent observation of the disease to the U.S. a concern. Initial observations of *Phaeosphaeria* leaf spot in southern Florida nurseries indicated that the inbred line B73 and its derivatives were particularly susceptible (2). These observa-

tions were later confirmed in replicated inoculated trials, although not all B73-derived lines were as susceptible as B73, and some unrelated lines also were found to be quite susceptible to *Phaeosphaeria* leaf spot (1). In the same trials, only a few commercial hybrids were as severely infected as the susceptible hybrid check, indicating that U.S. maize production is not particularly vulnerable to damage from *Phaeosphaeria* leaf spot. However, the susceptibility of several widely used parental inbred lines makes this disease a potential concern to the seed industry should it become established in areas of hybrid seed production.

Resistance to *Phaeosphaeria* leaf spot in the inbred line Mo17 appears to be incompletely dominant and controlled by up to 6 QTLs (quantitative trait loci), based on mapping studies using recombinant inbred lines derived from the cross B73 $\times$ Mo17 (3). This study was undertaken to determine and describe the inheritance of resistance to *Phaeosphaeria* leaf spot in segregating generations of maize derived from the cross B73 $\times$ Mo17.

MATERIALS AND METHODS

The *Phaeosphaeria* leaf spot resistant maize inbred line Mo17 was crossed to the highly susceptible inbred line B73 to develop segregating populations from which to study the inheritance of resistance to the disease. The resulting F_1 cross was selfed and crossed to each parent inbred line to form the F_2 and backcross generations, respectively. All crosses were made in summer nurseries at the Central Crops

Research Station, Clayton, NC. Seeds of the parental inbred lines, the F_1 , F_2 , and backcross generations were planted in field trials near Homestead, FL, in the winters of 1996-97 and 1997-98. Trials were planted in early November, 1996, and early December, 1997. The experimental design consisted of a randomized complete block with three replications. Each block consisted of a single row each of the parental inbred lines and the F_1 generation, three rows of each of the backcross generations, and six rows of the F_2 generation. Rows were 3.1 m long, spaced 0.9 m apart, and were planted with 20 seeds each. The final number of plants per row varied.

Plants were inoculated at the 8- to 10-leaf stage by placing approximately 20 to 30 kernels of sorghum grain cultures of *P. maydis* into each leaf whorl. Inoculum was prepared by soaking sorghum seed overnight in tap water, draining the excess water, placing the soaked seed in 1-liter flasks, and autoclaving for 1 h. After flasks had cooled, they were inoculated with 20 ml of an aqueous suspension of 3-week-old corn leaf agar (CLA, 6) cultures of a Florida isolate (FL-1) of *P. maydis*. Stock cultures were maintained as conidial suspensions in 15% glycerol at -80°C . Sorghum grain cultures were stored at 4°C until used. Individual plants within plots were rated when the inbred line B73 was at the mid-dent growth stage. A 10-class rating system in which 0 = no symptoms, 1 = trace to 2%, 2 = 2 to 4%, 3 = 4 to 7%, 4 = 7 to 15%, 5 = 15 to 35%, 6 = 35 to 65%, 7 = 65 to 85%, 8 = 85 to 95%, and 9 = >95% leaf area spotted, respectively, was used.

The mean *Phaeosphaeria* leaf spot ratings of each generation in each block were analyzed by analysis of variance and generation mean analysis using a multiple regression approach. The variation among generation means was fitted to the model $Y = m + b_1a + b_2d$, where m = overall mean, a = additive genetic effects, d = dominance genetic effects; and b_1 and b_2 = the relative contribution of these effects to the means of each generation (4). The significance of residual variation among generation means was tested to determine the importance of epistasis. Pooled within-plot variances were used to calculate heritabilities and to estimate the numbers of effective factors controlling *Phaeosphaeria* leaf spot resistance in F_2 populations. Broad-sense heritability was calculated as: $h^2(\text{broad}) = [\sigma^2 F_2 - \sigma^2(P_1, P_2, F_1; \text{pooled})] / \sigma^2 F_2$. Estimates of additive and dominance genetic

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variances were calculated as: $\sigma^2_A = 2\sigma^2_{F_2} - (\sigma^2_{BC_1} + \sigma^2_{BC_2})$, and $\sigma^2_D = \sigma^2_{BC_1} + \sigma^2_{BC_2} - \sigma^2_{F_2} - \sigma^2(P_1, P_2, F_1; \text{pooled})$. Narrow-sense heritability was estimated as: $h^2 (\text{narrow}) = \sigma^2_A / \sigma^2_{F_2}$. The minimum number of effective factors (k) controlling resistance in the F_2 population was estimated by: $k = (P_1 - P_2) / 8 [\sigma^2_{F_2} - \sigma^2(P_1, P_2, F_1; \text{pooled})]$ (4).

RESULTS AND DISCUSSION

Phaeosphaeria leaf spot had developed sufficiently by the dent stage in both growing seasons to allow discrimination between the levels of resistance of the two parental inbred lines. Although the genotype $\times$ environment interaction term in the combined ANOVA was significant, the magnitude of this effect relative to the main effects was small (Table 1). Most of the interaction appeared to have resulted from higher (more severe) disease ratings of B73 and the F_1 generation in 1997-98 compared to 1996-97, whereas the disease ratings of the remaining generations were comparable between the two seasons. The ranking of generation means did not change between seasons.

The distribution and means of Phaeosphaeria leaf spot ratings of the different generations resulting from the cross of B73 $\times$ Mo17 closely followed what would be expected for resistance with a mostly additive mode of inheritance (Fig. 1). The mean Phaeosphaeria leaf spot rating of the F_2 generation was slightly, but significantly, higher than that of the F_1 , and the mean ratings of the backcross generations approached those of the recurrent parent inbred lines. Dominance gene action for increased resistance was indicated by the small but significant negative deviation of F_1 means from the mid-parent values. Variation in segregating generations was continuous and approximated a normal distribution, indicating quantitative inheritance for Phaeosphaeria leaf spot.

A simple genetic model including additive and dominance genetic effects adequately described the variation in generation means (Table 1). There was no apparent transgressive segregation in the F_2 generation, indicating that genes for resistance to Phaeosphaeria leaf spot were isodirectionally distributed in the two parents (all resistance genes came from Mo17). No evidence of epistatic gene action was found, as residual effects derived from fitting the additive-dominance model were insignificant. Dominance genetic effects, although significant, accounted for less than 10% of the variation among generation means. The preponderance of additive gene action is further demonstrated by the relatively high estimates of additive genetic variance in relation to the estimates of dominance variance, which were lower than estimates of environmental variance (Table 2). As a result of the low estimates of dominance genetic and environmental variances, estimates of broad- and narrow-

sense heritabilities were high (70 to 85%) and similar to each other. Estimates of the number of "effective factors" (genes) controlling inheritance of resistance to Phaeosphaeria leaf spot are similar to those obtained from QTL mapping of recombinant inbreds from the B73 $\times$ Mo17 cross (3).

The results of this study are probably representative of how Phaeosphaeria leaf

spot resistance is inherited in most germplasm currently used in U.S. maize breeding programs. While, strictly speaking, they are applicable only to the cross B73 $\times$ Mo17, the two main heterotic groups used in current U.S. hybrids are largely derivatives of these two lines. Although most commercial hybrids tested to date are resistant to Phaeosphaeria leaf spot, most in-

Table 1. Combined analysis of variance of mean Phaeosphaeria leaf spot ratings of parent inbred lines, F_1 , F_2 , and backcross generations derived from the cross of maize inbred lines B73 and Mo17 during the 1996-97 and 1997-98 winter nursery seasons near Homestead, FL

Source of variation	df	Mean squares
Replications/years	4	0.2564
Years	1	1.3147
Generations	5	12.2325***
Additive ^b	1	56.0710***
Dominance	1	4.8482**
Residual	3	0.0811
Generations $\times$ years	5	0.2512*
Pooled error	19	0.0810

a ***, **, * indicate the effect is significant at the 0.001, 0.01, 0.05 levels of probability, respectively. Plants were rated on a 0 to 9 scale where 0 = no symptoms and 9 = 95 to 100% leaf area spotted.

b The variation among generation means was further partitioned into that attributable to additive and dominance genetic effects by fitting generation means to the model $Y = m + b_1a + b_2d$, where m = overall mean, a = additive genetic effects, d = dominance genetic effects, and b_1 and b_2 are coefficients describing the relative contributions of the genetic effects to the mean of each generation (4).

Fig. 1. Frequency distributions of Phaeosphaeria leaf spot (PLS) ratings of individual plants of maize parental inbred lines and the F_1 , F_2 , and backcross generations derived from the cross, B73 $\times$ Mo17. Solid and cross-hatched bars represent data from the 1996-97 and 1997-98 winter nursery seasons, respectively, in Homestead, FL. Plants were rated on a 0 to 9 scale where 0 = no symptoms and 9 = 95 to 100% leaf area spotted.

Table 2. Estimates of genetic variances, broad- and narrow-sense heritabilities, and numbers of effective factors (genes) controlling resistance to *Phaeosphaeria* leaf spot^a in the F₂ generation of the cross of the maize inbred lines B73 and Mo17

Estimated effect ^b	1996-97	1997-98	Combined years
σ^2_a (additive variance)	1.80	2.75	2.30
σ^2_d (dominance variance)	0.07	0.43	0.31
h^2_B (broad-sense heritability)	0.85	0.81	0.82
h^2_N (narrow-sense heritability)	0.82	0.70	0.72
K (# effective factors)	4.3	3.6	3.1

^a Resistance to *Phaeosphaeria* leaf spot was measured on a 0-9 scale where 0 = no symptoms and 9 = 95 to 100% leaf area spotted following artificial inoculation with a sorghum grain culture of *Phaeosphaeria maydis*.

^b Estimates of additive and dominance genetic variances were calculated as: $\sigma^2_A = 2\sigma^2 F_2 - (\sigma^2 BC_1 + \sigma^2 BC_2)$, and $\sigma^2_d = \sigma^2 BC_1 + \sigma^2 BC_2 - \sigma^2 F_2 - \sigma^2(P_1, P_2, F_1; \text{pooled})$; broad-sense heritability was calculated as: $h^2_B = [\sigma^2 F_2 - \sigma^2(P_1, P_2, F_1; \text{pooled})] / \sigma^2 F_2$ narrow-sense heritability was estimated as: $h^2_N = \sigma^2_A / \sigma^2 F_2$ the minimum number of effective factors controlling resistance in the F₂ generation was estimated by: $K = (P_1 - 2) / 8 * [\sigma^2 F_2 - \sigma^2(P_1, P_2, F_1; \text{pooled})]$ (4).

bred lines related to B73 and commonly used as female (seed) parents in hybrid seed production are quite susceptible (1). Should it be deemed desirable to develop *Phaeosphaeria* leaf spot-resistant inbreds of the B73 type, results from this study indicate the process should not be particularly difficult given the very high heritabilities and few genes involved. However, because most sources of resistance to *Phaeosphaeria* leaf spot found to date belong to the "Lancaster" heterotic group, transferring

Phaeosphaeria leaf spot resistance from these sources to B73-type inbreds from the "Stiff Stalk Synthetic" heterotic group could potentially interfere with the heterotic pattern for grain yield used in U.S. maize hybrids. *Phaeosphaeria* leaf spot has yet to be found in the U.S. outside southern Florida, and there is no indication that it represents an immediate threat to the central maize belt. However, the lack of knowledge concerning the epidemiology of this disease means it is difficult to make

accurate predictions of the potential for *Phaeosphaeria* leaf spot to spread and cause widespread damage. Should the genetic base of U.S. hybrids shift unexpectedly toward increased susceptibility to *Phaeosphaeria* leaf spot, there would be an increased risk to U.S. maize production from this disease.

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