Genetic control of resistance to gray leaf spot of maize in tropical germplasm

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Abstract - The main goal of this study was to assess the nature and magnitude of gene effects for resistance to Cercospora leaf spot. A randomized block design with three replications was used. The data were obtained at the plant level by assessing the disease severity. The data were analyzed per experiment, using the average data per plot. A dominant-additive genetic model without epistasis was considered, with estimation of the components of means and variance. The genetic control of resistance to gray leaf spot is polygenic with predominance of the additive effects. Dominance was observed in a few small-effect loci and high heritability values.

Key words: Cercospora zeae-maydis, genetic resistance, Zea mays.

INTRODUCTION

Gray leaf spot, whose etiologic agent is the fungus Cercospora zeae-maydis is currently one of the main leaf diseases of maize in Brazil, both in terms of losses it has caused to susceptible hybrids as well as its broad distribution in all maize-producing regions of the country (Brito et al. 2008). As of 2000, this endemic disease, appearing in the form of small and sparse spots on leaves, began to assume epidemic proportions in various regions of the country (Juliatti et al. 2004).

In Brazil, maize breeders agree that one of the main causes of inconstancy in the use of commercial hybrids is the high severity of diseases such as gray leaf spot (GLS), with the appearance of variations in the pathogen population, brought about mainly by the planting of susceptible hybrids and by changes in the production system. These facts evidence the need of breeding hybrids with genetic resistance to GLS (Vieira et al. 2009). Fortunately, the effectiveness of this strategy has already been demonstrated in the U.S. and in Africa, where GLS-losses had been reported prior to those in Brazil (Menkir and Ayodele 2005, Derera et al. 2008).

In a study by Pozar et al. (2009), QTLs related to resistance to C. zeae-maydis in Brazil were mapped and characterized by means of microsatellite markers (SSR - Simple sequence repeats) and single nucleotide markers (SNP - Single nucleotide polymorphism). According to the same author, the QTL combination has an impact on the effectiveness of selection procedures monitored by markers in breeding programs for the development of commercial hybrids.
Although QTL mapping allows a more rapid development of GLS-resistant hybrids by molecular marker-assisted selection, the chance for the emergence of variations in the pathogen population is great, resulting in QTL x environment interaction, reinforcing the importance of field selection.

The course of any breeding program is facilitated if information on the genetic control of the target trait is available (Lüders et al. 2008), which allows breeders to choose the most adequate working strategy. Nevertheless, it is important to highlight that the studies reported in the literature used germplasm adapted to the distinct edaphoclimatic conditions of Brazil. Although these studies have generated valuable information, they are generally only applicable to the source of specific germplasm and for the range of environments tested (Falconer and Mackay 1996). In addition, there is little information resulting from the study of inheritance of GLS-resistance in maize in Brazil using divergent breeding lines and their hybrid generations. The purpose of this study was to analyze the nature and magnitude of the genetic effects of resistance to gray leaf spot (GLS) based on assessments of lines with divergence for GLS-resistance and hybrid generations.

MATERIAL AND METHODS

The lines GNS31, GNS30 and GNS84 from the genetic breeding program of the company Geneseeds - Recursos Genéticos Ltda. were assessed and the generations F1, F2, BC11 and BC21, derived from the crosses GNS30 x GNS31 and GNS84 x GNS31. The lines GNS30 and GNS31 were obtained from the same population resulting from lines derived from the germplasm Cateto and Caribbean. Both have flint kernels, short plant height and a semi-late cycle. GNS31 is susceptible and GNS30 resistant to GLS. Line GNS84 was obtained from selfed varieties of Tuxpeno germplasm; it has medium dent kernels, medium plant height, an early cycle and GLS-resistance. Seeds of the parent lines and the F1, F2, BC11 and BC21 generations were obtained in the 2007/2008 growing season, by selfing and crosses performed in the field.

The experiments were initiated on two sowing dates about one month apart (11/19/2008 and 12/15/2008), in an experimental area of the Universidade Federal de Lavras (lat 21° 14’ S, long 45° 00’ W and alt 918 m asl), MG, Brazil. According to Köppen’s classification, the local climate is Cwa (subtropical, with rainy summers and dry winters). The soil of the experimental area was classified as Dystrophic Red Latossol, with clayey texture and 9% slope.

Two experiments were conducted for each sowing date and cross, in a randomized block design with three replications. Each replication consisted of 11 plots, one per parent line and F1 generation, two per BC11, two per BC21 and four per F2 generation. In this way, it was possible to use plots of always the same size, namely four 5-m rows, spaced 0.8 m apart. Thus, the representativity of the segregating populations was increased, to around 80 plants per plot.

For both experiments on both sowing dates, 400 kg ha-1 of N-P2O5-K2O fertilizer (as 8-28-16+0.5% zinc) was applied at sowing. When the plants had 4-5 leaves, 300 kg ha-1 urea (as 30-00-20) was applied as top dressing. A second application of N- fertilizer (100 kg ha-1 urea) was top-dressed when the plants had 8-9 leaves. Crop treatments and pest control were duly applied according to the crop requirements.

The onset of the disease occurred by natural infection. To assess disease severity, 20 plants from each plot were labeled for data collection at the time of flowering and evaluated 95 days after plant emergence. For this purpose, the disease-affected leaf area was visually assessed, based on a graded scale of 1 - 9, proposed by Pinho et al. (2001), where: 1 = 0% (highly resistant); 2 = 1% (resistant); 3 = 1% and < 10% (resistant); 4 = > 10% and ≤ 20% (moderately resistant); 5 = > 20% and ≤ 30% (moderately susceptible); 6 = > 30% and ≤ 40% (moderately susceptible); 7 = > 40% and ≤ 60% (susceptible); 8 = > 60% and ≤ 80% (susceptible); 9 = > 80% (highly susceptible).

The experimental data for both sowing dates were analyzed in parallel, for the two crosses. For this purpose, the phenotypic variance was estimated within the plots and the mean values per plot subjected to analysis of variance, according to the following statistical model, considering all effects as fixed, except for the effect of experimental error: 

\[ Y_{ij} = m + t_i + b_j + e_{ij} \]

where: 

- \( Y_{ij} \) value observed in block \( j \) of generation \( i \);
- \( m \) general mean;
- \( t_i \) effect of generation \( i \);
- \( b_j \) effect of block \( j \);
- \( e_{ij} \) effect of experimental error.

Estimates of the genetic mean components were obtained using the weighted least square method (Kearsey and Pooni 1996). An additive-dominance model without epistasis was considered, with estimation of the following parameters: \( m \): mean of the homozygotic genotypes; \( a \): sum of the effects of the homozygotic genotypes in relation to the midpoint (additive effects) and \( d \): sum of the effects of the heterozygotic genotypes in relation to the midpoint (dominance effects). The average dominance degree \((d/a)\) was calculated from these estimates.

The genetic variance components were estimated considering that the origin of the phenotypic variance of the parent lines and the F1 generation is only environmental, \( \sigma^2_E \); that is, \( \sigma^2_{F1} = \sigma^2_E \). and that the F2 variance
contains, in addition to the environmental variance, additive genetic variance ($\sigma^2_A$) and dominance genetic variance ($\sigma^2_D$), that is, $\sigma^2_F_2 = \sigma^2_A + \sigma^2_D + \sigma^2_E$ and the sum of the phenotypic variances of the two backcrosses contains: $\sigma^2_{BC11} + \sigma^2_{BC21} = 1\sigma^2_A + 2\sigma^2_D + 1\sigma^2_E$. The phenotypic variance components, $\sigma^2_A$, $\sigma^2_D$, and $\sigma^2_E$, were estimated using the procedure proposed by Mather and Jinks (1984).

All analyses were performed using software SAS (SAS Institute Inc 1993), Proc GLM for analyses of variances between generations and the Proc IML to estimate the mean and variance genetic components. The model fitting was verified based on estimates of the determination coefficients, involving the observed and expected values.

The narrow-sense heritability ($h^2_r$) and number of genes ($K$) involved in the trait control were estimated by the expressions proposed by Ramalho et al (1993).

**RESULTS AND DISCUSSION**

From the results of analyses of variance (Table 1), significant differences ($p \leq 0.01$) were observed between the different generations, i.e., between the parent lines, F1 hybrids and segregating generations (F2, BC11 and BC21) for the two crosses. The variation between the parent lines and the other generations involved (Table 2) was high. The existence of variation is an essential condition for the study of genetic control of the trait.

With the exception of the means of the parents and of the F1 and BC21 generations of the cross GNS31 x GNS84, the means of the generations of the two crosses increased significantly in the second sowing (Table 2). This is probably due to the fact that the inoculum concentration in the experiments of the second sowing was higher. In addition, the environmental conditions of the second sowing were more favorable for the establishment of the pathogen.

Regardless of the sowing date, high disease severity was observed for the susceptible line GNS31, common to both crosses, with scores ranging from 8.85 to 8.95. For cross GNS30 (P1) x GNS31 (P2), the mean disease severity of the GLS-resistant line GNS30 was 1.1, while the performance of the F1 hybrid was intermediate to the parent lines, in both sowings. In the cross GNS84 (P1) x GNS31 (P2), line GNS84 had a disease severity of 1.1 and the F1 hybrid a performance similar to that of the resistant parent, with score 3.20 and 2.15 for the first and second sowing, respectively. Differences of this magnitude were also reported elsewhere (Coates and White 1998, Menkir and Ayodele 2005). The prerequisite of divergent parents, one of the basic conditions for the study of genetic control, was also met.

In principle, these results suggest that the additive effects are predominant in the control of resistance to C. zeae-maydis, since in the segregating generations there are non-effective alleles. The means of the F1 and F2 generations, near the midpoint of the parent lines, also indicate predominance of the additive effects in the trait control.

The study of mean components showed that the additive-dominance model was adequate to explain the variation observed. For both crosses of the first sowing, the correlations between the estimated and the expected means were close to unity, as well as the coefficient of determination of the model ($R^2$). The estimates of the mean components (Table 2) showed that the additive ($a$) and dominance ($d$) effects were significantly different from zero ($p \leq 0.01$) for the two crosses for both sowings. The negative estimates of $d$ indicate dominance in the direction of reducing the disease severity, which is a favorable condition for breeding programs for resistant hybrids, with the exception of the positive value obtained for cross GNS31 x GNS30 in the second sowing, due to the increase in disease severity observed in the segregating generations.

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**Table 1.** Summary of analysis of variance for disease severity (scores) of Cercospora zeae-maydis obtained in experiments for assessment of parent lines and F1, F2, BC11 and BC21 generations of two crosses and two sowings dates (1: 11/19/2008 and 2: 12/15/2008)

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>Sowing 1</th>
<th>Sowing 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GNS84 x GNS31</td>
<td>GNS30 x GNS31</td>
</tr>
<tr>
<td>Blocks</td>
<td>2</td>
<td>3.4137</td>
<td>2.7103</td>
</tr>
<tr>
<td>Generations</td>
<td>5</td>
<td>36.7071**</td>
<td>38.7212**</td>
</tr>
<tr>
<td>Error</td>
<td>25</td>
<td>0.6987</td>
<td>1.1581</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>4.38</td>
<td>4.74</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>19.06</td>
<td>22.71</td>
</tr>
</tbody>
</table>

** Significant at 1 % probability by the F test.
The average dominance degree \( (d/a) \) obtained from these estimates was 0.06 and 0.66 for GNS31 x GNS30, and 0.47 and 0.44 for GNS31 x GNS84, for the first and second sowing respectively, suggesting partial dominance. The results recorded here are in agreement with most reports in the literature regarding predominance of additive effects and significant dominance effects (Thompson et al. 1987, Elwinger et al. 1990, Ulrich et al. 1990, Donahue et al. 1991, Gevers et al. 1994, Coates and White 1998, Verma 2001, Menkir and Ayodele 2005, Derera et al. 2008).

Phenotypic and genetic variance components (Table 3) showed that the additive-dominance model explained nearly all variation, which can be verified by the \( R^2 \) estimates. Corroborating the study of mean components, the variance components showed greater importance of the additive effects in the inheritance of GLS-resistance. The estimates of additive variance \( (\hat{\sigma}_A^2) \) were, for the most part, significantly different from zero. Additive variance may be defined as a linear relationship between the genotypic values of the plants of a population and the number of favorable alleles they have (Cruz et al. 2004). Thus, the prevalence of additive effects in the genetic control of GLS-resistance indicates that superior genotypes, with greater concentration of favorable alleles and therefore a condition favorable to genetic breeding, can easily be identified.

However, the dominance variance proved to be very small for the two crosses, for both sowings, with non-significant values, contrary to the results obtained by the mean components on the importance of the dominance effects on the resistance control for these crosses. Nevertheless, it must be highlighted that in the estimates of genetic components of variance, using the least square method, dominance variance corresponds to the deviations of the linear regression equation used in obtaining additive variance (Bernardo 2002). Therefore, the estimates of dominance variance may be underestimated since this method tends to minimize the deviations in relation to the regression line. Significant dominant effects were also described by Elwinger et al (1990), Gevers et al. (1994), Coates and White (1998), Verma (2001) and Menkir and Ayodele (2005), indicating that the genetic trait control may vary between different resistance sources. This must be considered in breeding programs, where the resistance sources should be characterized to ensure an effective selection process.

Heritability estimates for the first sowing were greater and show that the environmental effect was more pronounced for the second sowing (Table 3). This fact may explain the unexpected result of the generation means in the second sowing and mainly shows that studies involving horizontal genetic resistance to pathogens may be affected by late sowing, in which the pathogen pressure may be greater.

Additive genetic variation was high, indicating a great possibility of success with selection. Heritability estimates with a higher genetic than environmental variation have been reported elsewhere (Coates and White 1998).

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**Table 2. Means of disease severity (scores) of *Cercospora zeae-maydis* of generations assessed and estimates of mean components for mean severity of gray leaf spot obtained in two crosses and in two sowings dates (1: 11/19/2008; 2: 12/15/2008)**

<table>
<thead>
<tr>
<th>Generations</th>
<th>Sowing 1</th>
<th>Sowing 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GNS31 x GNS84</td>
<td>GNS31 x GNS30</td>
</tr>
<tr>
<td><strong>P</strong>₁</td>
<td>8.93</td>
<td>8.85</td>
</tr>
<tr>
<td><strong>P</strong>₂</td>
<td>1.08</td>
<td>1.12</td>
</tr>
<tr>
<td><strong>F</strong>₁</td>
<td>3.20</td>
<td>4.87</td>
</tr>
<tr>
<td><strong>F</strong>₂</td>
<td>5.10</td>
<td>4.24</td>
</tr>
<tr>
<td><strong>BC</strong>₁₁</td>
<td>6.59</td>
<td>7.20</td>
</tr>
<tr>
<td><strong>BC</strong>₂₁</td>
<td>1.43</td>
<td>1.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean components</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>m</strong></td>
</tr>
<tr>
<td><strong>a</strong></td>
</tr>
<tr>
<td><strong>d</strong></td>
</tr>
<tr>
<td><strong>R²</strong></td>
</tr>
</tbody>
</table>

1 Standard error of the estimate
** Significant at 1 % probability of error
With the identification of predominance of the additive effects and high heritability estimates, it becomes clear that the chances of obtaining GLS-resistant lines by selection are great. According to Kearsey and Pooni (1996), the results found related to dominance ($d$ - small and significant; $\hat{\sigma}_D^2$ - insignificant) indicate a low dominance level in resistance inheritance. In other words, there are few small-effect loci with dominance on the trait control. This shows that the most recommended breeding strategy would be to obtain hybrids from resistant lines, taking advantage of the additive effects of the large-effect loci of the parents and the dominance that will be expressed in divergent loci of smaller effect.

In relation to the number of genes involved in the resistance control of this pathogen, considering the absence of dominance, the presence of 11.4 genes, on average, was observed when the cross GNS84 x GNS31 was considered and 10.2 genes, on average, for the cross GNS30 x GNS31, considering both sowing dates. These results show that the genetic control is polygenic. Studies performed by Bubeck et al. (1993) showed that various quantitative trait loci (QTLs) with additive gene action are associated with resistance, indicating that it is horizontal or polygenic.

The high heritability and the great importance of the additive gene effect in the trait control are favorable for breeding for characteristics controlled by several genes.

ACKNOWLEDGEMENTS

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Controle genético da resistência à cercosporiose do milho em germoplasma tropical

**Resumo** - O objetivo deste trabalho foi estudar a natureza e a magnitude dos efeitos gênicos da resistência à Cercosporiose. Foi utilizado o delineamento experimental de blocos ao acaso, com três repetições. A avaliação da resistência foi realizada utilizando escala diagramática com notas. Os dados obtidos foram analisados por experimento, utilizando-se médias de parcela. Foi considerado um modelo aditivo-dominante sem epistasia, com a estimação dos componentes de média e de variância. O controle genético da resistência à Cercosporiose é poligênico, com predominância dos efeitos aditivos, com dominância em poucos locos de pequeno efeito e alta herdabilidade, condição essa favorável à seleção.

**Palavras-chave:** Cercospora zeae-maydis, resistência genética, Zea mays.
REFERENCES


