Gene effects from *Bipolaris maydis* incidence and severity on popcorn

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ABSTRACT: Popcorn suffers severe economic losses resulting from harmful effects caused by several diseases such as southern corn leaf blight (SCLB), which is one of the main leaf diseases able to cause considerable damages to corn crops. The realization conducting genetic studies in order to identify genotypes presenting genetic resistance to this disease it is essential. Accordingly, the aim of the current study is to estimate gene effects and combining abilities in popcorn, with emphasis on the resistance to *Bipolaris maydis*. Full diallel crosses were performed, including reciprocal crosses between eight lines (L76, L88, L70, L77, L55, L61, P1 and P8), in order to produce 56 hybrids (F₁'s and reciprocal hybrids). The experiment followed a randomized block design, with four replicates; in two different cultivation periods (first and second periods), in Campos dos Goytacazes, RJ. Southern corn leaf blight incidence and severity, caused by *B. maydis*, were assessed in each cultivation period. No reciprocal effect was observed for the evaluated characteristics, since the diallel analysis did not find significant effect from this variation source. With respect to SCLB incidence and severity in the two cultivation periods, the mean square of the effects showed that the dominance components were superior to the additivity ones. Hybrids that stood out in the two cultivation periods to the assessed features were: L88 x L61, L88 x L70, L88 x L76, L61 x L77, L61 x P1, L70 x P1, P1 x L76, L77 x L76, and P8 x L77.

Key words: combining ability; genetic resistance; southern corn leaf blight; *Zea mays* L.

Efeitos gênicos da incidência e severidade de *Bipolaris maydis* em milho-pipoca

RESUMO: O milho-pipoca sofre severas perdas econômicas devido aos efeitos danosos da incidência de doenças. A helmintosporiose é uma das principais doenças foliares e representa um considerável potencial de dano à cultura; tornando-se evidente a necessidade de estudos genéticos visando à identificação de genótipos que apresentem resistência genética para essa doença. O estudo teve por objetivo estimar os efeitos gênicos e as capacidades combinatórias em milho-pipoca, com enfoque para a resistência a *Bipolaris maydis*. Foram realizados cruzamentos dialêlicos completos, incluindo os recíprocos entre oito linhagens (L76, L88, L70, L77, L55, L61, P1 e P8), para a obtenção de 56 híbridos (F₁'s e recíprocos). Instalou-se experimento em delineamento em blocos ao acaso com quatro repetições, em duas épocas de cultivo, em Campos dos Goytacazes, RJ. Avaliaram-se a incidência e a severidade causada por *B. maydis* para cada época. Observou-se a ausência de efeito recíproco para as características avaliadas, uma vez que não houve efeito significativo desta fonte de variação na análise dialêlica. Para a incidência e severidade de helmintosporiose, nas duas épocas de cultivo, o quadrado médio dos efeitos revelou que os componentes de dominância foram superiores aos de aditividade. Os híbridos que se destacaram, nas duas épocas de cultivo, com base no conjunto de características avaliadas, foram: L88 x L61, L88 x L70, L88 x L76, L61 x L77, L61 x P1, L70 x P1, P1 x L76, L77 x L76 e P8 x L77.

Palavras-chave: capacidade combinatória; resistência genética; helmintosporiose; *Zea mays* L.
Introduction

Popcorn is quite consumed worldwide, and although it is intended to human consumption, it is also considered a high-profitability crop, since its final product is widely accepted by the consumers. Thus, interest in grain production has increased in several regions, a fact that turned Brazil into the second largest popcorn producer (Paraginski et al., 2016). However, popcorn cultivation remains modest, mainly due to limitations in finding genotypes able to provide quality, good yield and resistance to diseases. Consequently, it is necessary importing grains, mainly from Argentina and the United States of America (Rinaldi et al., 2007).

Although the limited number of national popcorn cultivars and hybrids is the main barrier for the crop expansion, there has been improvement in both its extension and production capacity. However, the crop lacks genotypes holding other favorable agronomic features, such as the resistance to diseases. Overall, popcorn is less resistant to the main leaf diseases affecting corn crops (Hallauer & Carena, 2009; Ribeiro et al., 2016). This fact, aside from contributing to lower yields, increases the production risks. Hence, developing genetically resistant genotypes is the most viable alternative to control the main leaf diseases (Ayiga-Aluba et al., 2015).

Southern Corn Leaf Blight (SCLB) caused by the fungus Bipolaris maydis (Nisik.) Shoemaker [synonym Helminthosporium maydis Nisik and Myiaka], stands out among the main leaf diseases affecting popcorn crops. This fungus may drastically reduce crop stand and yield depending on the resistance level of some cultivars, the crop management system and the climate conditions during cultivation; yield losses may reach 70% (Altaf et al., 2016). According to Negeri et al. (2011), the use of quantitative resistance to SCLB may help mitigating the disease in pathogen-vulnerable regions, besides avoiding the use of expensive, highly harmful to the environment, fungicides. The case gains relevance in Brazil due to lack of records concerning specific fungicides able to control this pathogen.

Accordingly, several techniques have been suggested in order to help selecting parents to produce hybrids able to present favorable agronomic features. Among the aforementioned techniques, the diallel cross has been widely used to give many genetic information it may provide to breeders (Oliboni et al., 2013). The methodology by Griffing (1956) allows estimating genetic parameters, such as combining abilities, in order to support the indication of breeding strategies and the recommendation of promising genotypes.

Studies about popcorn genetic resistance to leaf diseases are scarce; most of these assessed such resistance based on intervarietal diallels. Among them, it is worth mentioning Rangel et al. (2007) and Vieira et al. (2009). On the other hand, Pinto et al. (2007), Pajic et al. (2008), Silva et al. (2010), Viana et al. (2011), Vieira et al. (2011), Moterle et al. (2012) and Cabral et al. (2013) stand out when it comes to studies providing information about the diallel cross between popcorn lines.

However, there is limited information about the genetic resistance of the herein addressed crop to B. maydis. Thereby, the aim of the study was to investigate the existence of reciprocal effect, in order to estimate the combining ability of popcorn parents and hybrids according to the method by Griffing (1956), as well as to indicate the best combinations able to enhance plant resistance to southern corn leaf blight.

Materials and Methods

Eight popcorn (S$_7$) lines (P1, P8, L55, L61, L70, L76, L77 and L88) produced by the Popcorn Reproduction Program from the State University of Northern Rio de Janeiro, were previously identified as of interest for the accomplishment of this research in the study developed by Kurosawa et al (2017), and when submitted to complete diallel cross; it was possible to make 56 hybrid combinations, including reciprocal hybrids. Evaluations were conducted at the Antônio Sarlo State Agricultural College, located in Campos dos Goytacazes - RJ, Brazil. Eight parents, along with 56 hybrids and 6 controls (IAC 125, Angela BRS, UENF 14, UFV M2-Barão de Viçosa, L70 x L54 hybrid and P8 x L54 hybrid) were evaluated in two growth periods: the first one from October 2014 to January 2015, with variable temperature between 24 °C and 25 °C and the relative air humidity ranging from 70 to 76%; and the second one from May to August 2015, with temperature between 21 °C and 23 °C and air humidity from 79% to 85%.

The experiment followed a randomized block design, with four replicates. Each plot consisted of a 5 m-long row holding 25 plants spaced 0.90 m between rows and 0.20 m between plants. Sowing comprised 3 grains per pit, placed 0.05 m down in the soil; thinning was performed 30 days after the sowing and left one plant per pit. The plots were fertilized at sowing using 60 kg ha$^{-1}$ of K$_2$O, 30 kg ha$^{-1}$ of N, and 60 kg ha$^{-1}$ of P$_2$O$_5$, in addition to 100 kg ha$^{-1}$ of N as topdressing, 30 days after the sowing.

Treatments reaction to leaf diseases was monitored through the manifestation of symptoms by natural field infection. Assessed features were B. maydis incidence (BMI) and severity (BMS) on the plant. The BMI assessment that considers the rate of damaged leaves in the whole plant by using all leaves was performed employing the Agroceres (1996) rating scale, with grades ranging from 1 to 9, where grade 1 indicates 0% incidence; 2 - 0.5% incidence; 3 - 10% incidence; 4 - 30% incidence; 5 - 50% incidence; 6 - 70% incidence; 7 - 80% incidence; 8 - 90% incidence and 9 - 100% leaf incidence; James’s (1971) diagrammatic scale, with percentage values of 1, 5, 25 and 50 of the injured leaf area, was employed to measure BMS considering only the upper ear leaf in the plant. The 5 firsts and lasts plants were excluded from the assessment; only the 15 central plants were considered as useful plot. The first plant in each useful plot was marked and assessed; the subsequent 2 were omitted; with this procedure being repeated until the last plant. Thereby, 5 plants were assessed in each plot.

Data concerning each cultivation period were individually subjected to analysis of variance. The means of the treatments...
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Results and Discussion

The analysis of variance applied to the first cultivation period did not show significant variance (p < 0.01) in genotype variation sources GCA and SCA, except for BMI (Table 1). These results indicate that all hybrid combinations were similar to the mean of the parents; therefore, the chances of finding a superior hybrid combination for the herein assessed set of hybrids in this cultivation period were limited. Possibly, the effect of the pathogen on the leaves at the first cultivation period may have been small or absent, which results in the impossibility of discriminating the genotypes.

The second cultivation period showed significant differences (p < 0.01) in genotype variation sources, GCA and SCA, in both herein assessed features (Table 1), and it indicated the existence of additive and non-additive effects controlling these features. Significant effect on combining abilities revealed variability resulting from both effects. With these results, it was possible to conclude that the evaluation performed in this environment with significant presence of the pathogen on plants, favored significant variability among the genotypes in the evaluated characteristics; this variability resulted from the combining ability effects. Incidence and severity of fungal diseases are aggravated, depending on climatic conditions, especially through the temperature and air humidity present in the region. Weather conditions in Campos dos Goytacazes are not well defined, according to the imposed sowing season. Infestation and proliferation of southern corn leaf blight causing pathogens may vary due to existing climate instability. Environmental influence on GCA and SCA estimates for disease resistance and agronomic characteristics have been reported by Parodas & Hayes (1971) and Pixley & Bjarnason (1993). This fact is intensified in tropical regions, where interaction is of particular interest, as there is a significant variation between locations, even when they are nearby. Variations in latitude, length of day, humidity and temperature are greater in these regions, changing what is expected to be found in disease assessments when differences in growing seasons.

Results of the analyses indicate the existence of non-additive effects on the herein assessed features. Non-additive effects are very important to popcorn breeding because they directly affect the hybrid varieties exploitation. On the other hand, if one was interested in developing corn lines, these results would indicate that the selection based on the herein assessed features would be more efficient in advanced generations when the dominance effects are less pronounced.

Table 1. Analysis of variance applied to the general (GCA) and specific combining abilities (SCA) and mean squares of the GCA and SCA effects on Bipolaris maydis incidence (BMI) and severity (BMS), assessed in 8 parents and 56 popcorn hybrids intercrossed with the reciprocal hybrids in full diallel, in the first harvest (from October 2014 to January 2015) and the second harvest (from May to August 2015), in Campos dos Goytacazes, RJ.

<table>
<thead>
<tr>
<th>VS</th>
<th>DF</th>
<th>First Harvest</th>
<th>Second Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BMI</td>
<td>BMS</td>
</tr>
<tr>
<td>Genotype</td>
<td>63</td>
<td>24438.1439**</td>
<td>6.8788**</td>
</tr>
<tr>
<td>GCA</td>
<td>7</td>
<td>101714.4614*</td>
<td>10.1684**</td>
</tr>
<tr>
<td>SCA</td>
<td>28</td>
<td>25385.1855**</td>
<td>8.5268**</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>28</td>
<td>4172.0229**</td>
<td>4.4083**</td>
</tr>
<tr>
<td>Residual</td>
<td>189</td>
<td>4632.2361</td>
<td>6.5320</td>
</tr>
</tbody>
</table>

Mean squares of effects

<table>
<thead>
<tr>
<th>VS</th>
<th>First Harvest</th>
<th>Second Harvest</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>BMI</td>
<td>BMS</td>
</tr>
<tr>
<td>GCA</td>
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<td>0.0568</td>
</tr>
<tr>
<td>SCA</td>
<td>5188.2373</td>
<td>4.4987</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>-57.5266</td>
<td>-0.2655</td>
</tr>
</tbody>
</table>

VS = variation source, DF = degrees of freedom, GCA = general combining ability, SCA = specific combining ability, ** = significant at 1% probability level, *= non-significant at 1% probability level.
in the control of the resistance expressed by this feature; thus, the epidemic affected genotypes presenting a certain cytoplasm type.

SCA is more important than GCA in some corn culture diseases. Nihei & Ferreira (2012) used corn lines to investigate several leaf diseases, in a study based on diallel analysis, and found that the dominant effects were more important to the resistance against E. turcicum. Such result differs from the responses found by Paterniani et al. (2000), Carson (2006) and Vivek et al. (2010). It may happen when crossing using non-related lines, since it increases the possibility of not finding similarity between the loci controlling the feature; consequently, it increases the possibility of finding a genetic complementation in hybrids expressing gene dominance effects, thus decreasing the GCA effect. This study recorded higher non-additive effects, meaning that, in this case, complementation between hybrids in the loci showed some dominance degree. Therefore, breeding procedures based on hybridizations are the best alternative to explore the gene-complementation effect, since it may generate superior genetic gains (Freitas Junior et al., 2006).

With respect to the estimates concerning the $\hat{g}_i$ effects on the studied features, the current study recorded values whose sign ranged from positive to negative depending on the used parent (Table 2). It is noteworthy that the selection lies on individuals showing high $\hat{g}_i$ estimates and positive-sign value; however, negative-sign $\hat{g}_i$ values may be more convenient depending on the feature, as in the case of disease resistance-related features.

In the first harvest, it was not possible to indicate potential parents for the IBM and SBM characteristics, since they had no significant effect for CGC in the analysis of variance (Table 1). On the other hand, in the second harvest, parents who expressed the highest negative estimates of overall combining ability for the characteristics related to B. maydis (IBM) and B. maydis (SBM) were: L61, L70 and L76 (Table 2). L61 parent had the highest negative estimate for the characteristics, IBM (-91.2664) and SBM (-4.9361). Since this current study addresses disease resistance, it is possible stating that the genotype presenting negative GCA is potentially superior to be used in breeding programs (Cruz et al., 2012). Genotypes L70 and L61 recorded negative BMI and BMS estimates for the second harvest, thus constituting a powerful resistance source to be used in breeding programs.

Genotypes L61, L70 and L76 are relevant parents in the production of superior segregant genotypes in advanced generations, since these lines are more prone to hold alleles resistant to southern corn leaf blight. On that account, they are recommended to be used in crosses aimed at reducing southern corn leaf blight levels in crops. Hence, using these parents in hybridizations increases the possibility of finding hybrid combinations showing favorable $s_{ij}$ estimates for all features.

The $s_{ij}$ estimates were positive for both features in the two cultivation periods, except for BMI in the first cultivation period, when parents L88, P8 and L55 were used (Table 3). Thus, negative dominance deviations were recorded; consequently, there was unidirectional dominance. Therefore, Table 3. $s_{ij}$ estimate and $s_{ij}$ effects, assessed in a full diallel cross using 8 parents, the reciprocal crosses included, in the first harvest (from October 2014 to January 2015) and the second harvest (from May to August 2015), Campos dos Goytacazes, RJ.
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**Conclusions**

It was not possible to highlight favorable parents to reduce the incidence and severity of southern corn leaf blight in the first harvest.

Genetic control for the characteristics related to resistance to southern corn leaf blight, in the second harvest were influenced by the additive and non-additive effects, and the non-additive effects were higher for this set of parents.

Assessments for reciprocal effect showed that there is no difference when a genotype is used as donor or recipient of pollen, since there is presence of nuclear genes effect for the characteristics related to resistance to *B. maydis*.

Hybrids that stood out in the two harvest on the resistance to southern corn leaf blight, and are thus recommended for cultivation, were L88 x L61, L88 x L70, L88 x L76, L61 x L77, L61 x P1, L70 x P1, P1 x L76 and L77 x L76.

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