

Indirect selection for resistance to ear rot and leaf diseases in maize lines using biplots

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ABSTRACT. Leaf disease and ear rot have caused reductions in maize yield in Brazil and other producer countries. Therefore, the aims of this study were to analyze the association between husked ear yield and the severity of maize white spot, gray leaf spot, helminthosporium, and ear rot caused by *Fusarium verticillioides* and *Diplodia maydis* using biplots in a mixed-model approach. The responses of 238 lines introduced to Brazil and four controls were evaluated using an incomplete block design with three replicates in two locations: Lavras and Uberlândia, Minas Gerais, Brazil. Two experiments were conducted in each location, one with *F. verticillioides* and the other with *D. maydis*. The mixed models elucidated the relationship between yield, leaf disease, and ear disease. Significant genotype x environment and genotype x pathogen interactions were observed. In conclusion, husked ear yield is more associated with ear rot than with the leaf diseases evaluated,

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justifying the indirect selection for resistance to kernel rot in maize-*F*. *verticillioides* and maize-*D*. *maydis* pathosystems by yield evaluation.

Key words: Indirect selection; Grain yield; Leaf disease; Ear rot

INTRODUCTION

Recently, a greater incidence and severity of leaf diseases and rotten grain has been observed in maize in several regions of Brazil. This has caused great concern, because these diseases constitute one of the main limiting factors for continuing to obtain gains in breeding programs. Among the maize leaf diseases that occur in Brazil, gray leaf spot, maize white spot, and helminthosporium are the most important, and are caused by *Cercospora zeae-maydis*, *Pantoea ananatis*, and *Exserohilum turcicum*, respectively, which, under conditions of early infection in susceptible hybrids, may cause significant reductions in crop yield potential (Do Amaral et al., 2005; Brito et al., 2011).

Ear rot in maize is caused by the fungi *Fusarium verticillioides* and *Diplodia maydis*, which are considered the diseases that have the greatest economic impact on this cereal crop throughout the world, because of their wide distribution and low tolerance of infected grains in the commercialization (Van Egmond et al., 2007; Reid et al., 2009). With increasing mean global temperatures, it is expected that damage from these diseases will increase, particularly in regions with mild climates (Mesterházy et al., 2012). *F. verticillioides* is responsible for the production of mycotoxins of the fumonisin group, which, upon being ingested, cause serious damage to swine, poultry, and humans (Lanubile et al., 2010). *D. maydis* is the causative agent of Diplodia ear rot and synthesizes the toxin diplodiatoxin, which is harmful to poultry and cattle (Odriozola et al., 2005).

The growing of hybrids with low levels of resistance to diseases, inadequate irrigation management, planting maize as a second crop, adoption of the no-till system, and an absence of crop rotation are factors that have contributed to the multiplication and preservation of the inocula of pathogens, consequently increasing the economic losses arising from these pathogens in regard to grain yield and quality.

The most efficient method of disease control is the use of resistant genotypes (Hefny et al., 2012; Mesterházy et al., 2012), and indirect selection for pathogen resistance is a common strategy in plant breeding. Indirect selection is based on the use of related agronomic traits, and is particularly recommended for gaining traits that are difficult to evaluate (Rossouw et al., 2002). Tembo et al. (2012) reported the success of this strategy in the selection of maize lines resistant to *Stenocarpella maydis* and *Fusarium graminearum* when selection is practiced on the former, because this fungus colonizes plant tissues faster than the latter.

When conducting a breeding program, the genotype x environment interaction makes it difficult to select superior genotypes with the desired level of disease resistance, because disease development is highly affected by environmental conditions, which may lead to incorrect estimates of genotype resistance, and, consequently, to a lack of success in selection (Mukanga et al., 2010). Descriptive and/or quantitative techniques can be used to minimize the impact of the genotype x environment interaction. Among these methodologies, the additive main effects and multiplicative interaction (AMMI) and the genotype and genotypes by environments interaction (GGE) are visual, descriptive approaches to genotype x environment interactions, and allow better analysis of the information contained in the data (Crossa et al.,

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1990). The main advantage of the GGE biplot compared to the AMMI is the fact that the GGE biplot explains an intermediate proportion of the sum of squares of the genotype and the genotype x environment interaction (Yan et al., 2000, 2007).

Analyses of the genotype x environment interaction in traits related to diseases have been conducted in several pathosystems (Sharma and Duveiller, 2004; Twizeyimana et al., 2008; Reid et al., 2009; Maroya et al., 2012). Yan et al. (2000) suggested that the genotype interaction patterns of various traits could be investigated using biplot analysis. However, the use of GGE biplot analysis is still rare in mixed-model frameworks, particularly in disease studies. Balestre et al. (2009) suggested that genotype and interaction effects should be random terms in a GGE biplot analysis, because, in general, their best linear unbiased predictions (BLUPs) are more accurate than when using fixed models. This is because the estimates are adjusted for heritability. Therefore, the aims of this study were to analyze the association between husked ear yield and the severity of maize white spot, gray leaf spot, helminthosporium, and ear rot caused by *F. verticillioides* and *D. maydis* using biplots in a mixed-model approach.

MATERIAL AND METHODS

F. verticillioides and *D. maydis* inocula were replicated, and the isolates of *F. verticillioides* were grown in a complete medium over a period of seven days before inoculation. The *D. maydis* isolates were grown in a complete medium and stored in glass tubes for 30 days. A conidial suspension of both fungi was adjusted to 10⁶ conidia/mL, by counting in a Neubauer chamber on the day of inoculation. Inoculations were conducted 15 days after 100% of the plants in the plot had developed style-stigmas. A pipette was used to inoculate each ear with 1 mL suspension. The methodology of Clements et al. (2003), with some modifications, was used in this step of the study.

The study was carried out during the 2012/2013 crop season in two municipalities in the state of Minas Gerais, Brazil: Lavras [910 m above mean sea level (amsl), 21°14'S 45°00'W] and Uberlândia (863 m amsl, 18°55'S 48°16'W), where the climate is classified as temperate highland tropical and tropical wet and dry or savanna, respectively, according to the Köppen climate classification. The responses of 238 lines introduced to Brazil and four controls were evaluated, and two separate experiments were conducted in each location. In the first experiment, *F. verticillioides* was used as the inoculum; *D. maydis* was used in the second. The lines were randomly distributed in each experiment in incomplete blocks, with common controls interspersed among them. Each block included 10 treatments (8 + 2) and three replicates. The experimental plots consisted of one 3-m row with a spacing of 0.7 m. The design was not balanced due to the loss of some plots.

The severities of gray leaf spot (CERC), maize white spot (PHA), and helminthosporium (HELM) in the maize lines were evaluated using the diagrammatic scale proposed by Agroceres (1996). The scores range from 1 to 9, where 1 = 0% disease, 2 = 0.5%, 3 = 10%, 4 = 30%, 5 = 50%, 6 = 70%, 7 = 80%, 8 = 90%, and 9 = 100% of the leaf area with lesions. A single evaluation was carried out 85 days after plant emergence. In all of the experiments, the yield in husked ear weight (PROD) was evaluated, obtained in kg/plot, and then converted to kg/ha. The data for this trait were then standardized for 13% moisture (Brasil, 2009).

Three traits were evaluated regarding ear rot, and included only the symptoms related to the inoculated fungus, and consisted of the mean score for the incidence of rotten kernels (SCO), the proportion of ears with symptoms of ear rot (PROP), and the percentage of rotten

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kernels (ARD). For evaluation of the first trait, a diagrammatic scale of scores method was adopted, which ranged from 1 to 7 (1 was least severe): 1 = 0%, 2 = 1-3%, 3 = 4-10%, 4 = 11-25%, 5 = 26-50%, 6 = 51-75%, and 7 = greater than 75% (Reid et al., 2002). For the second trait, the proportion of ears with symptoms of rotting compared to the total number of ears of the plot was recorded. Evaluation of the third trait was based on the procedure proposed by Ordinance No. 11, 12/04/96 (Brasil, 1996), which is the percentage of grains with symptoms of discoloring on more than one quarter of their total surface area in a sample of 230 g grain per plot. Based on these traits and the experimental design described above, statistical analyses were conducted using the following mixed linear model:

$$y = X\beta + Za + \Omega b + \Delta \alpha + W\delta + \Psi \omega + e$$
 (Equation 1)

where y is an observation of the plot for each trait, X is a matrix of fixed effects that includes inoculum, location, replication within fungi within location, and a fungi x environment interaction, Z is a matrix of the random effect of line, Ω is a matrix of the random effect of the block within the replication, fungi, and location, Δ is a matrix of the random effect of a line x fungus interaction, W is a matrix of the random effect of a line x environment interaction, Ψ is a matrix of the random effect of a line x fungus x environment interaction, and ε is a vector of experimental error.

The analyses were conducted using a mixed-model approach by restricted maximum likelihood. After the analyses, the BLUPs regarding the lines (a) and the interaction (δ) were clustered in a double-input table, obtaining a G + GE matrix. This matrix was broken down into vectors and singular values using principal components analysis, thereby obtaining a GGE biplot. To investigate interactions between the traits, the principal components technique was used in the BLUP matrix for PROD, PROP, SCO, ARD, CERC, PHA, and HELM. Genetic correlations between these traits were obtained by biplot analysis using the cosines of the angles between the variables in the biplot (Yang et al., 2009). In addition, a Pearson correlation of the BLUPs was conducted for each trait. A Mantel test was also conducted, and included 5000 simulations. The GGE biplot analysis was conducted using the program SAS v. 8.0 with Interactive Matrix Language and SAS GRAPH (SAS Institute, 2000). The phenotypic and genetic variances, heritabilities, confidence intervals, and selective accuracy of the genetic variances and heritabilities of PROP, SCO, ARD, CERC, PHA, and HELM were estimated (Hicks, 1973; Knapp et al., 1985; Resende and Duarte, 2007).

RESULTS

The formation of three distinct groups was observed, which corresponded to husked ear yield, leaf disease, and traits related to the incidence of rotten kernels (Figure 1). This biplot accounted for 81.4% of the total variation for these five traits, showing that the first two principal components explained a significant part of the variation. PROP, SCO, and ARD were clustered together (Figure 1), and a significant negative correlation was observed between PROD and these traits, which was confirmed by the correlation between the BLUPs and by the cosines of the angles between these traits (Table 1 and Figure 1). The leaf diseases were also clustered together (Figure 1), and the correlations between PROD and the leaf diseases were negative and nonsignificant (Figure 1 and Table 1).

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Figure 1. Biplots generated from 242 lines in two environments (Lavras and Uberlândia, Minas Gerais, Brazil) and inoculated with two fungi (*Diplodia maydis* and *Fusarium verticillioides*). SCO = Mean score for the incidence of rotten kernels; ARD = percentage of rotten kernels; PROP = number of ears containing rotten kernels; CERC = gray leaf spot; PHA = maize white spot; HELM = helminthosporium.

Table 1. Estimates of biplot and Pearson's (in parentheses) correlations between the best linear unbiased
predictions (BLUPs) for the mean score for the incidence of rotten kernels (SCO), percentage of rotten kernels
(ARD), number of ears containing rotten kernels (PROP), gray leaf spot (CERC), maize white spot (PHA),
and helminthosporium (HELM).

	PROD	CERC	HELM	PHA	PROP	SCO	ARD
PROD CERC	1.00	-0.25 (-0.36 ^{ns}) 1.00	-0.35 (-0.34 ^{ns}) 0.99 (0.97**)	-0.49 (-0.56 ^{ns}) 0.96 (0.93**)	-0.97 (-0.99**) 0.08 (0.15 ^{ns})	-0.93 (0.98**) -0.05 (0.03 ^{ns})	-0.84 (-0.86**) -0.28 (-0.06 ^{ns})
HELM PHA			1.00	0.98 (0.91**) 1.00	0.13 (0.08**) 0.31 (0.38 ^{ns})	-0.03 (0.05 ^{ns}) 0.15 (0.02 ^{ns})	-0.26 (-0.12 ^{ns}) -0.08 (-0.12 ^{ns})
PROP SCO					1.00	0.99 (0.99**) 1.00	0.92 (0.85**) 0.97 (0.94**)
ARD							1.00

**Significant by the Mantel test (5000 permutations obtained at level of 1%); ns = not significant.

Low estimates of heritability were obtained for the leaf diseases, suggesting that phenotypic evaluation was not an effective genotypic indicator (Table 2). Experimental precision in the evaluation of these traits was low, as indicated by the accuracy values (34.61 to 38.03%; Table 2) (Resende and Duarte, 2007). In addition, a significant genotype x environment interaction was observed, which was shown by the cosine of the angle formed between the straight lines corresponding to the locations evaluated (Figure 2). Although the estimates of heritability were low, there was a significant correlation between these traits which shows that similar results for the selection of superior lines in regard to these diseases would be obtained during the selection process. In the combined analysis of the traits that were related to the incidence of rotten kernels, PROP had the greatest heritability

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(64.52%), indicating that the phenotypic evaluation of this trait was a reliable indicator of the genetic value of the lines (Table 2). The experimental precision for this trait was high, according to the accuracy estimate of 80.32% (Resende and Duarte, 2007). SCO and ARD exhibited low levels of heritability (4.49 and 26.50%, respectively), as a result of the low accuracy estimates obtained for them (Table 2). Nevertheless, these traits were significantly correlated with PROP (0.99 and 0.92, respectively; Figure 1 and Table 1). For the traits that were related to the SCO, a weaker genotype x environment interaction was found for PROP than for the other traits (Figure 2).

Regarding each fungus separately, a greater genotype x environment interaction was seen for all of the traits related to rotten kernels when inoculated with *D. maydis* than with *F. verticillioides* (Figure 3). When considering the biplots for PROP and the genotype x pathogen interaction in Lavras and Uberlândia, the behavior of these lines was not coincident (Figure 4). The biplots in Figures 2, 3, and 4 explained around 70.0% of the variation seen in the responses of the lines to the different factors, which demonstrates that the first two principal components explained most of the variation.

Table 2. Estimates of heritability and genetic variance of 242 maize lines related t	to the mean score for the
incidence of rotten kernels (SCO), percentage of rotten kernels (ARD), number of ears	containing rotten kernels
(PROP), gray leaf spot (CERC), maize white spot (PHA), helminthosporium (HE	LM), and yield (PROD).

Parameter	PROP	РНА	SCO	ARD	CERC	HELM
Genetic variance	0.03	0.33	0.05	18.21	0.16	0.20
(LL-UL)*	(0.02 - 0.04)	(0.10-4.70)	(0.00-0.00)	(9.07-53.48)	(0.04-5.88)	(0.05 - 8.01)
Heritability (%)	64.52	14.47	4.49	26.50	12.15	11.98
(LL-UL)*	(52.79-73.14)	(-10.51-33.79)	(-23.77-26.19)	(-3.54-46.61)	(-15.04-32.65)	(-1.02-20.26)
Accuracy (%)	80.33	38.03	21.18	51.48	34.85	34.61



*Lower limit (LL) and upper limit (UL) of the confidence interval, obtained at the level of 5% confidence.

Figure 2. Biplots generated from 242 lines in two environments [Lavras (LAV) and Uberlândia (UBE), Minas Gerais, Brazil] and inoculated with two fungi (*Diplodia maydis* and *Fusarium verticillioides*). A) Mean score for the incidence of rotten kernels (SCO); B) percentage of rotten kernels (ARD); C) number of ears containing rotten kernels (PROP); D) gray leaf spot (CERC); E) maize white spot (PHA); F) helminthosporium (HELM).

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Figure 3. Biplots generated from 242 lines in two environments [Lavras (LAV) and Uberlândia (UBE), Minas Gerais, Brazil] for *Diplodia maydis* and *Fusarium verticillioides* separately. **A)** Percentage of rotten kernels (ARD) infected by *D. maydis*; **B)** percentage of rotten kernels (ARD) infected by *F. verticillioides*; **C)** number of ears containing rotten kernels (PROP) infected by *D. maydis*; **D)** number of ears containing rotten kernels (PROP) infected by *F. verticillioides*; **E)** mean score for the incidence of rotten kernels (SCO) infected by *D. maydis*; **F)** mean score for the incidence of rotten kernels (SCO) infected by *D. maydis*; **F)**



Figure 4. Biplot generated from 242 lines in relation to the number of ears containing rotten kernels (PROP) and inoculated with two fungi, *Diplodia maydis* (DI) and *Fusarium verticillioides* (FU) in Lavras (A) and Uberlândia (B), Minas Gerais, Brazil.

DISCUSSION

The results indicate that the use of mixed models and multivariate techniques allow the relationships between yield, leaf diseases, and ear rot to be elucidated (Figure 1).

The significant negative correlation between PROD and traits related to rotten kernels (Figure 1 and Table 1) is a result of rotten kernels drastically reducing maize grain weight, as reported by Vigier et al. (2001), who observed losses of up to 48% in grain yield due to infection by *F. graminearum*. Therefore, practicing indirect selection for resistance to *F. verticillioides* and *D. maydis* by the evaluation of PROD is effective.

The correlations between the severity of the leaf diseases and yield were not as signifi-

cant as found by Carson (2005), Katwal et al. (2013), or Yeshitila (2003) for CERC, PHA, or HELM, respectively. However, the lines that were more resistant to these diseases had satisfactory yields (Figure 1), which demonstrates that productive performance is partly explained by variations in the severity of these pathogens.

The results of the present study may be due to a disease severity that was insufficient to cause expressive losses in yield. This is unfavorable when discriminating between genotypes with respect to different levels of resistance to disease, and is frequently observed when experiments are conducted under conditions of the natural incidence of the pathogens (Santos et al., 2002).

The significant genotype x environment interaction seen for these diseases (Figure 2) may be explained by the climatic differences between the environments, because in Lavras, the climate is classified as highland tropical, and in Uberlândia as tropical wet and dry.

Differences between the locations may have contributed to a greater variability among the populations of the pathogens in the experimental areas, which generated inconsistent responses by the genotypes in these environments (Brito et al., 2011). Therefore, the genotype x environment interaction increased the phenotypic standard deviation, and consequently reduced accuracy and heritability (Matheson and Raymond, 1986) (Table 2).

Regarding the traits that were related to the SCO, PROP required less visual effort in the identification of infected ears and had a weak genotype x environment interaction, explaining the greater accuracy observed for this trait (Table 2). Consequently, PROP exhibited high heritability, which corroborates the results obtained by Robertson et al. (2006), who recorded the proportion of rotting ears caused by *F. verticillioides*, where the estimates of heritability were 0.47 and 0.80 considering two different populations.

Scoring using a diagrammatic scale and the separation of rotten kernels to obtain the SCO and the ARD, respectively, are highly subjective methodologies and are strongly influenced by the evaluator, since they depend on a visual inspection of the kernels. This contributed to the low accuracy and consequently, to the low heritability estimates (Table 2) (Ramalho et al., 2012).

However, when analyzing the correlations between SCO and ARD with PROP, a high degree of association was found between these traits (Figure 1 and Table 1), indicating their importance in the selection of resistant lines. Because of the precision and heritability of the PROP evaluation method, it was appropriate for identifying genotypes resistant to ear rot, as was found by Rossouw et al. (2002).

Considering the need for evaluating genotype yield potential in breeding programs, in which hundreds, and even thousands, of genotypes are evaluated each crop season, and bearing in mind the viability of the indirect selection of PROD with consequent gains in resistance to *D. maydis* and *F. verticillioides*, PROD would be the most viable trait for incorporation in a maize breeding program, due to its great practicality. The indirect selection of PROD to increase resistance against rotten kernels is recommended by Martin et al. (2012), because they found it effective against *F. graminearum*.

D. maydis exhibited a stronger genotype x environment interaction than *F. verticillioides* (Figure 3). This was attributed to the preferential conditions for the establishment of each fungus. *F. verticillioides* exhibits great homeostasis, which explains its broad geographical range in maize cultivation areas (Mesterházy et al., 2012), whereas *D. maydis* is restricted to high altitude and moist conditions, which characterizes Lavras.

The genotype x pathogen interaction seen for the PROP trait (Figure 4) indicates that the mechanisms of resistance to *D. maydis* and *F. verticillioides* are not the same, which, in

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this case, implies the ineffectiveness of indirect selection, making it necessary to carry out experiments for the individual evaluation of these fungi to identify lines that are resistant to each of them, as was conducted in this study and by Tembo et al. (2012). Therefore, knowledge of the degree of association between the mechanisms of resistance to pathogens is fundamental for experimental planning, decision-making with respect to the possibility of conducting indirect selection, and optimization of the selection process.

The GGE biplot method has been frequently used in the selection of maize genotypes with resistance to pathogens of economic importance (Egesi et al., 2007; Reid et al., 2009; Badu-Apraku and Akinwale, 2011; Sibiya et al., 2012), and it is relevant not only in studies on the stability and adaptability of genotypes in different locations (Yan et al., 2000) but also on the effects of different pathogens or isolates (Sharma and Duveiller, 2004; Twizeyimana et al., 2008; Reid et al., 2009). Because the fungal complexes that cause leaf diseases and ear rot include many species and isolates, analysis of the genotype x pathogen interaction is crucial when the identification of genotypes with multiple resistances is desired.

In this study, the GGE biplot method explained most of the variation in the data, and allowed an analysis of the responses of the different lines in relation to the environments and the pathogens studied (Yan et al., 2007; Yang et al., 2009). The use of mixed models made it feasible to analyze our data, despite the design being unbalanced (Resende and Duarte, 2007).

In conclusion, husked ear yield is more closely associated with ear rot than with the leaf diseases studied, justifying the indirect selection for resistance to kernel rot in maize-*F. verticillioides* and maize-*D. maydis* pathosystems by yield evaluation.

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