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Genetic resistance of new popcorn hybrids to foliar diseases

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ABSTRACT – *The level of genetic resistance to foliar diseases in 54 new simple popcorn hybrids was evaluated in field conditions. Two trials in a randomized complete block design were conducted in the 2006/07 growing season. The disease intensity in genotypes was compared by the area under the disease progress curve, expressing the severity. Significant differences ($P \leq 0.01$) of resistance to southern rust (*Puccinia polysora*) northern leaf blight (*Exserohilum turcicum*) and *Phaeosphaeria* leaf spot (complex *Phaeosphaeria maydis* / *Pantoea ananas*) were observed. The cluster analysis detected different resistance levels to the series of leaf diseases. In 12 of the new hybrids the resistance level to the set of diseases was similar as in the commercial hybrid IAC 112, considered the best reference for leaf disease resistance among commercial popcorn genotypes.*

Key words: *Zea mays*, pathogens, multivariate analysis, canonical variables, cluster analysis.

INTRODUCTION

Corn cultivation is affected by several leaf diseases, which, individually or together, can cause significant damage to yield and grain quality. The damage depends on the level of genetic resistance of the genotype, climatic conditions during the growth cycle and the production system (Reis et al. 2004).

The variability in the reaction of genotypes to leaf diseases is less reported for popcorn than for common corn. Some studies show different reactions to northern leaf blight, caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs (Fantin et al. 1991, Fernandes and Balmer 2002, Miranda et al. 2002) and to *Phaeosphaeria* leaf spot, caused by *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro (Fantin et al. 1991).

Resistant cultivars have been as the main control measure of corn leaf diseases (Reis et al. 2004, Casela and Guimarães 2005). The discrimination of resistance is more reliable when the genotypes are evaluated based on the area under the disease progress curve (Von Pinho et al. 2000) since several measurements of disease severity throughout the production cycle are included.

Techniques of multivariate analysis are appropriate when each experimental unit provides a number of variables that need to be correlated and considered simultaneously (Liberato et al. 1993). In plant pathology, multivariate techniques can be used mainly to determine the variability in isolates and identify genetic resistance to diseases. However, studies in the area of plant pathology based on this type of data analysis are rare.

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This study aimed to verify the level of genetic resistance to common rust (*Puccinia sorghi* Schwein) and southern rust (*P. polysora* Underw), northern leaf blight (*E. turcicum*), Phaeosphaeria leaf spot [complex *Phaeosphaeria maydis* / *Pantoea ananas* (Serrano) Mergaert et al.], to gray leaf spot (*Cercospora zea-maydis* Tehon & Daniels), and to the whole complex of these leaf diseases, considering them together in evaluations of 54 new simple popcorn hybrids, developed by the breeding program of special maize types of the Universidade Estadual de Maringá (UEM).

MATERIAL AND METHODS

Two tests were conducted in the 2006/07 growing season with new popcorn hybrids, in Maringá, PR (23° 21' S; 52° 04' W; 510 m asl). The experimental design was a randomized complete block, with four replications in trial 1 and three replications in experiment 2.

Sowing was performed in the second half of September 2006 (trial 1) and the first fortnight of December 2006, (trial 2). Each trial consisted of 30 treatments, with 27 simple popcorn hybrids, developed by a breeding program of special maize types of the UEM, derived from lines in the fifth and seventh inbreeding generation, and three commercial genotypes.

The lines were derived from local popcorn populations that had been collected on farms in the region or from commercial lines of unknown pedigree. The lines were selfed, using paper bags to collect the pollen of flowering plants. To produce the hybrids, the lines were sown pairwise, rows spaced 0.9 m and plants 0.4 m apart, in February 2006. The crosses were performed by hand.

The experimental plots consisted of three 5.0 m long rows, of which the center row was considered in evaluations. Rows were spaced 0.9 m apart, with a sowing density of five seeds per meter. The plots were fertilized with 50 kg ha⁻¹ K₂O, 20 kg ha⁻¹ N and 50 kg ha⁻¹ P₂O₅ at sowing and 100 kg ha⁻¹ sidedressing, when the plants had reached development stage V₄. The recommended cultural treatments were performed according to crop requirements.

The disease intensity was quantified individually based on the severity, evaluating the natural infestation by pathogens. In both trials, three evaluations were performed in the ear leaf and the first leaves

below and above approximately every 14 days, after flowering. Five plants per plot were sampled and identified in the first evaluation of each trial.

To estimate the severity of common and southern rust, a diagrammatic scale (Peterson et al. 1948) was used, modified by Cobb. The scale of Smith (1989) was used for gray leaf spot. The scales used for northern leaf blight and Phaeosphaeria leaf spot had been proposed by Canteri et al. (1999), with severity classes of 0, 2, 4, 7, 13, 23, 37%, and 0, 2, 5, 10, 15, 20 and 35% respectively.

Data of disease severity were used to calculate the area under the disease progress curve (AUDPC), as proposed by Campbell and Madden (1990). Bartlett's test was applied to check the homogeneity of variances. To verify the data and error distribution the method of Kolmogorov-Smirnov was used. The Mauchly test of sphericity was applied to verify the equality of covariance matrices of disease intensity and zero-order correlations. To meet the assumptions of normality and homogeneity of variances, the AUDPC was transformed to $(x + 1) / 2$.

The multivariate analysis of variance (MANOVA) was applied to AUDPC in both trials. By MANOVA, the variances associated with the disease were analyzed jointly by means of the matrices A, H, B and E, representing the total sum of squares and products, respectively, of the treatments, blocks and errors. The matrix dimensions of 4 x 4 were due to the four diseases examined. The univariate analysis of variance was applied to determine which diseases caused the source of variation in MANOVA. When the F values were significant at 5% probability, the mean test of Scott-Knott (Scott and Knott 1974) was applied, at 10% probability.

The canonical analysis was also applied to the AUDPC of the diseases evaluated, and four canonical variables were determined with their canonical coefficients in relation to each disease. By the canonical coefficients and the transformed AUDPC values of each disease for each plot, canonical scores were calculated, in a procedure proposed by Cruz and Regazzi 1994, representing the resistance of the complex of evaluated leaf diseases. By this procedure, the multidimensional space of the four diseases was reduced to a two-dimensional space of the grades obtained from the first two canonical variables.

The canonical scores of the first two canonical variables were used to obtain the matrix of genetic dissimilarity of genotypes, estimated based on Mahalanobis' generalized distance.

Considering the matrix of genetic dissimilarity, cluster analysis was performed to discriminate the genotype resistance to the disease complex. The modified Tocher optimization method was used as clustering algorithm, as suggested by Vasconcelos et al. (2007). As proposed, the calculation of the dissimilarity distance is based on the yet ungrouped plants, so that the grouped ones would not interfere with the formation of other groups.

Statistical analyses were performed using the Genes software (Cruz 1998) and the STAT module of the SAS version 8.2 for Windows (SAS Institute 2000).

RESULTS AND DISCUSSION

By the Mauchly test the condition of sphericity for the variance matrices between pairs of disease intensity was rejected at 1% probability. Significant correlation coefficients were observed between the error matrices of disease, confirming the association between them. The multivariate approach is therefore justified.

The Shapiro–Wilks test, applied in MANOVA to assess the validity of the treatment vectors, was significant ($P < 0.001$) in both trials. Consequently, the hypothesis of equality for the mean vectors was rejected, indicating that one or more genotypes differ in genetic resistance to the disease complex.

By the F test, applied individually to the AUDPC of the diseases underlying MANOVA, significant differences ($P \leq 0.01$) were observed between genotypes with regard to resistance to *Phaeosphaeria* leaf spot, northern leaf blight and southern rust.

Regarding *Phaeosphaeria* leaf spot, in trial 1, the AUDPC indices in 13 UEM hybrids (27, 22, 05, 01, 25, 26, 18, 17, 16, 20, 13, 19, and 23) and the commercial hybrids IAC 112 and Jade were lowest and therefore formed the group of most resistant genotypes, with similar resistance levels within the group (Table 1). Hybrid IAC 112 is moderately disease-resistant (Instituto Agronômico de Campinas – IAC, 2001), however, under the disease intensity of the trial, the performance of hybrid Jade, considered susceptible (Embrapa Maize and Sorghum 2008), was similar to 'IAC 112'. The

intermediate resistance group of the trial comprised 12 UEM hybrids and hybrid Zélia, considered disease-susceptible (Embrapa Maize and Sorghum 2008). The disease intensity of the hybrids UEM10 and UEM12 was significantly higher than of the other genotypes of the trial, characterizing them as the most susceptible. In trial 2, 17 UEM hybrids (41, 45, 33, 39, 44, 43, 28, 51, 30, 29, 54, 31, 49, 52, 32, 53, and 50) had similar resistance levels as 'Jade', 'BRS Angela', and 'IAC 112' (Table 2). The resistance of hybrid Jade and variety BRS Angela is considered, respectively, susceptible and moderate (IAC 2001, Embrapa Maize and Sorghum 2008). The other UEM hybrids of the trial constituted the group with highest disease susceptibility. Different resistance levels to *Phaeosphaeria* leaf spot is often reported for common maize genotypes (Santos et al. 2002, Souza and Duarte 2002, Pegoraro et al. 2002, Juliatti and Souza 2005, Lopes et al. 2007). The negative correlation between disease severity and grain yield (-0.45), reported by Pegoraro et al. (2001), reinforces the need of using genotypes with greater resistance.

In trial 1, the AUDPC indices for northern leaf blight of 19 UEM hybrids (27, 22, 01, 25, 26, 18, 17, 16, 13, 19, 21, 24, 07, 15, 11, 08, 14, 12, and 10) and the commercial hybrids IAC 112 and Zélia were similar (Table 1). Seven other UEM hybrids together with Jade formed a group of intermediate resistance while hybrid UEM05 was more susceptible than all other genotypes. The absence of significant differences between 'IAC 112' and 'Zelia', which are considered, respectively, as disease-resistant and susceptible (IAC, 2001; Embrapa Maize and Sorghum 2008), is possibly linked to the disease intensity observed in the trial. In trial 2, the disease intensity of the UEM genotypes 46, 39, 40, 29, 30, 51, 28, 43, 50, 49, 31, 52, and 32, and of IAC 112 did not differ statistically, indicating similar resistance levels (Table 2). The AUDPC indices of the other UEM hybrids and the cultivars BRS Angela and Jade were higher than of the above genotypes.

Different resistance levels to southern rust were observed among the genotypes evaluated in trial 2 (Table 2). The UEM hybrids 34, 36, 47, 35, 48, 41, 45, 42, 33, 39 and 37 were statistically as resistant as the commercial genotypes and more resistant than the other UEM hybrids. Variability of reaction to southern rust was reported in common corn hybrids (Von Pinho et al. 2001) and lines (Silva et al. 2001).

Table 1. Mean values of the area under the disease progression curve (AUDPC) of *Phaeosphaeria* leaf spot, northern leaf blight, common rust, and gray leaf spot for trial 1

Genotype	<i>Phaeosphaeria</i> leaf spot	Northern leaf blight	Common rust	Gray leaf spot
UEM27	0.00a ¹	0.00a	0.93	0.00
UEM22	0.00a	2.80a	5.13	0.48
UEM05	1.40a	43.17 c	3.03	0.00
IAC 112	1.65a	1.28a	2.57	0.00
UEM01	1.75a	4.20a	13.42	0.37
UEM25	3.26a	0.00a	1.63	0.00
Jade	4.57a	20.30 b	3.85	0.58
UEM26	5.71a	3.03a	5.13	0.23
UEM18	9.10a	1.63a	3.73	0.68
UEM17	10.20a	5.60a	1.40	0.25
UEM16	11.08a	1.98a	5.25	0.82
UEM20	11.20a	16.10 b	2.57	1.40
UEM13	14.00a	2.80a	2.92	1.51
UEM19	14.46a	4.20a	3.85	0.12
UEM23	15.19a	15.17 b	2.57	0.70
UEM21	16.45 b	0.47a	5.13	0.82
UEM06	19.02 b	13.18 b	2.22	0.35
UEM24	19.36 b	0.00a	1.87	0.93
UEM09	19.40 b	9.57 b	3.50	1.63
UEM02	19.72 b	9.45 b	4.32	1.63
UEM07	25.20 b	3.85a	4.32	0.23
UEM15	25.43 b	3.73a	2.80	0.70
UEM11	27.65 b	5.02a	2.68	0.58
UEM08	25.67 b	0.00a	5.60	0.00
UEM04	27.06 b	12.25 b	9.45	1.17
UEM14	29.17 b	1.75a	8.28	0.47
UEM03	35.12 b	15.17 b	6.88	0.70
Zélia	36.30 b	5.02a	6.18	0.00
UEM12	59.73 c	3.03a	1.40	0.46
UEM10	79.80 c	3.50a	1.98	0.72
P>F ²	<0.001	0.014	0.172	0.126
CVe ³	34.60	56.57	30.47	21.07

¹Means followed by the same letter in a column do not differ significantly by the Scott-Knott test, at 10% probability. ²Minimum probability of significance by the F test, for the genotype effect. ³Coefficient of experimental variation, in percentage

Under the conditions of disease intensity and assumed significance level α , no significant differences were detected for common rust (Table 1) and gray leaf spot (Tables 1 and 2).

In both trials, the error estimates were expressed by the coefficient of experimental variation (CVe). On average, the CVe was similar to results of Fantin et al. (1991). When the disease occurs at low severity or at certain points in the trial, the replications of the

treatments contributed to an uncommon magnitude of error, for example, for yield. As in the study cited above, it was observed that even under low disease intensity, different levels of genetic resistance were detected.

In both trials, the first two canonical variables explained about 80% of the variation (Table 3). Therefore, according to criteria of Cruz and Regazzi (1994), it is possible to conclude that a sufficient part of the variation of genotypes in terms of resistance to the disease complex could be concentrated in the grades of the first two canonical variables.

Table 2. Mean values of the area under the disease progression curve (AUDPC) of southern rust, *Phaeosphaeria* leaf spot, northern leaf blight, and gray leaf spot, in trial 2

Genotype	Southern rust	<i>Phaeosphaeria</i> leaf spot	Northern leaf blight	Gray leaf spot
BRS Angela	43.03 a ¹	0.00 a	10.66 b	1.94
Jade	60.30 a	1.60 a	20.33 b	0.93
UEM34	83.05 a	34.17 b	7.99 b	1.20
UEM46	83.52 a	14.02 b	0.00 a	1.43
UEM47	85.46 a	13.55 b	12.77 b	3.51
UEM35	85.98 a	42.49 b	30.17 b	4.32
UEM48	88.70 a	22.37 b	15.60 b	1.68
UEM41	93.81 a	3.96 a	22.01 b	7.99
UEM45	99.14 a	11.76 a	10.83 b	4.53
UEM42	100.30 a	25.03 b	11.02 b	3.57
UEM33	111.03 a	8.42 a	12.64 b	2.67
UEM39	114.07 a	3.11 a	4.53 a	3.49
UEM37	119.80 a	16.97 b	6.98 b	4.96
IAC 112	120.21 a	6.92 a	1.38 a	0.96
UEM40	123.96 b	37.78 b	3.07 a	5.41
UEM38	126.08 b	17.88 b	17.87 b	8.61
UEM44	132.98 b	7.10 a	16.44 b	1.41
UEM36	137.88 b	20.88 b	8.31 b	4.82
UEM43	139.01 b	11.75 a	5.27 a	0.96
UEM28	144.69 b	0.00 a	0.64 a	0.00
UEM51	145.09 b	7.87 a	4.91 a	3.36
UEM30	147.33 b	5.93 a	0.42 a	7.01
UEM29	149.95 b	6.67 a	0.42 a	2.00
UEM54	151.16 b	1.60 a	7.49 b	5.54
UEM31	151.93 b	8.67 a	0.00 a	1.33
UEM49	163.81 b	7.17 a	1.38 a	2.96
UEM52	174.70 b	11.20 a	3.59 a	11.56
UEM32	179.81 b	10.53 a	1.27 a	3.38
UEM53	191.35 b	2.84 a	18.37 b	2.50
UEM50	213.47 b	3.09 a	6.39 a	4.83
P>F ²	<0.001	<0.001	0.001	0.212
CVe ³	16.00	45.60	45.97	39.30

¹Means followed by the same letter in a column do not differ significantly by the Scott-Knott test, at 10% probability. ²Minimum probability of significance by the F test, for the genotype effect. ³Coefficient of experimental variation, in percentage

Table 3. Summary of the canonical analysis of disease severity

Canonical variables	Canonical coefficients for:				Percentage of explained variance	Accumulated percentage of variance explained
	Rust ¹	Northern leaf blight	Phaeosphaeria leaf spot	Gray leaf spot		
Trial 1						
VC1	0.03887	-0.04344	0.07126	0.50349	57.38	57.38
VC2	0.13657	0.05871	0.01799	-1.11030	18.26	75.64
VC3	0.12363	0.06996	0.00706	0.48377	15.42	91.06
VC4	0.14129	0.01301	-0.00284	0.72104	8.94	100.00
Trial 2						
VC1	-0.01073	0.06268	0.09075	0.02593	58.33	58.33
VC2	0.00746	-0.05220	0.04240	0.18525	23.31	81.64
VC3	0.00662	0.07460	-0.02412	0.12161	14.56	96.20
VC4	0.00902	0.02440	0.01506	-0.10118	3.80	100.00

¹Common and Southern rust in the trials 1 and 2, respectively

The cluster analysis discriminated different groups and subgroups of resistance to the disease complex in both trials. The groups for the genotypes tested in trial 1 are shown in Table 4, and for the genotypes of trial 2 in Table 5.

In trial 1, 24 UEM hybrids and the commercial genotypes IAC 112, Zélia and Jade constituted group I. After a second canonical and cluster analysis, group I was divided into 4 resistance subgroups. The genotypes UEM25 and UEM27 were grouped with the commercial genotypes IAC 112 and Jade, indicating a similar resistance level to the disease complex. Based on information from the IAC (2001) and Embrapa Maize and Sorghum (2008), hybrid IAC 112 is the commercial popcorn genotype with greatest resistance to the diseases evaluated. The hybrids UEM14, UEM08, UEM03, UEM04, and Zélia had a similar resistance level, although 'Zélia' is considered rather susceptible to

diseases (Embrapa Maize and Sorghum 2008). The other hybrids of the trial constituted groups and subgroups, which indicated a resistance level to the disease complex that differed from the already grouped UEM hybrids and the commercial genotypes.

In trial 2, 24 UEM hybrids were grouped with commercial genotypes (group I). The subgrouping of group I divided these genotypes into 5 groups. The genotypes UEM29, UEM31, UEM49, UEM51, UEM32, UEM39, UEM43, UEM54, UEM30, UEM28, and IAC 112 constituted a single subgroup, indicating similar resistance levels. The resistance of the genotypes UEM41 and Jade was moderate, since they were grouped with variety BRS Angela, which is considered moderately resistant to the evaluated diseases (Embrapa Maize and Sorghum 2008). In this trial, 16 UEM hybrids differed from the other genotypes in terms of resistance, also indicating different resistance levels.

Table 4. Genotype grouping for resistance to the disease complex by the modified Tocher method, based on Mahalanobis' generalized distance (Trial 1)

Group ¹	Subgroup ²	Genotypes
I	a	UEM25, IAC 112, UEM27 and Jade
	b	UEM02, UEM09, UEM13, UEM24, UEM15, UEM21, UEM16, UEM11, UEM18, UEM07, UEM19, UEM17, UEM06, UEM23, UEM20, UEM26, UEM22
	c	UEM14, Zélia, UEM08, UEM03 and UEM04
	d	UEM01
II		UEM05
III		UEM10 and UEM12

¹Cluster analysis for all genotypes tested in the trial. ²Cluster analysis only for the genotypes in group I

Table 5. Genotype grouping for resistance to the disease complex by the modified Tocher method, based on Mahalanobis' generalized distance (Trial 2)

Group ¹	Subgroup ²	Genotypes
I	a	UEM29, UEM31, UEM49, UEM51, UEM32, UEM39, IAC 112, UEM43, UEM54, UEM30 and UEM28
	b	BRS Angela, Jade and UEM41
	c	UEM36, UEM37, UEM45, UEM47, UEM33, UEM42, UEM46, UEM48, UEM38 and UEM44
	d	UEM50 and UEM53
	e	UEM52
II		UEM34 and UEM40
III		UEM35

¹Cluster analysis for all genotypes tested in the trial. ²Cluster analysis only for the genotypes in group 2

CONCLUSIONS

The levels of genetic resistance to *Phaeosphaeria* leaf spot, northern leaf blight and southern rust differ among the new popcorn hybrids.

The resistance to the disease complex in the UEM hybrids 25, 27, 28, 29, 30, 31, 32, 39, 43, 49, 51, and 54 is similar to the resistance level of the commercial hybrid IAC 112. The resistance of hybrid UEM41 is similar to variety BRS Angela and hybrid Jade. The resistance of the hybrids UEM03, UEM04, UEM08 and UEM14 is

similar to hybrid Zélia. The resistance level of 37 UEM hybrids differs from the UEM hybrids and commercial genotypes.

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Resistência genética de novos híbridos de milho-pipoca a doenças foliares

RESUMO – O nível de resistência genética a doenças foliares de 54 novos híbridos simples de milho-pipoca foi avaliado em condições de campo. Dois ensaios em blocos completos casualizados foram conduzidos na safra 2006/07. A intensidade das doenças nos genótipos foi comparada pela área abaixo da curva de progresso da doença, calculada com base na severidade. Verificam-se diferenças significativas ($P \leq 0,01$) de resistência em relação à ferrugem polissora (*Puccinia polysora*), helmintosporiose (*Exserohilum turcicum*) e mancha branca (*Phaeosphaeria maydis*/*Pantoea ananas*). A análise de cluster discriminou diferentes níveis de resistência ao complexo de doenças foliares. Dentre os novos híbridos, doze apresentaram nível de resistência semelhante ao híbrido comercial IAC 112 quanto ao complexo de doenças foliares avaliado, considerado o melhor referencial de resistência a doenças foliares dentre os genótipos comerciais de milho-pipoca.

Palavras-chave: *Zea mays*, Patógenos, Análise multivariada, Variáveis canônicas, Análise de cluster.

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