

Popcorn germplasm resistance to fungal diseases caused by *Exserohilum turcicum* and *Bipolaris maydis*

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ABSTRACT: Compared with the common corn, popcorn shows greater susceptibility to pests and diseases occurrence, being the use of resistant cultivars the most efficient strategy. In this regard, Germplasm Banks deserve special attention because they contain accessions that can be used as sources of resistance in breeding programs. The State University of Northern Rio de Janeiro (UENF) maintains a popcorn Germplasm Bank with accessions from tropical and temperate countries, including Diversity Centers for the species. In this study, we investigated the performance of 37 popcorn accessions landraces, hybrids, and inbreed lines from the Germplasm Collection of UENF's Popcorn Breeding Program with respect to the fungal leaf diseases northern leaf blight (NLB) and southern leaf blight (SLB),

caused by *Exserohilum turcicum* and *Bipolaris maydis*, aiming to select promising accessions for genetic resistance to be used in future crosses to generate new resistant cultivars for farmers. The dendrogram scattering by the UPGMA multivariate technique was efficient in discriminating resistant accessions. Satisfactory results were obtained for resistance to NLB in accessions L71, L75, L76, P7, and PARA-172, which can thus be considered remarkable sources of resistance. For resistance to SLB, the superior accessions that can be indicated as sources of resistance are ARZM-05083, ARZM-07049, and PARA-172.

Key words: *Zea mays*, northern leaf blight, genetic resistance, plant breeding.

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INTRODUCTION

Popcorn (*Zea mays* L.) is a type of corn whose main characteristics are its hard and small kernels and the ability to expand due to the pressure formed inside the grains upon heating. When compared with cultivars of common corn, popcorn plants usually display greater susceptibility of pests and diseases occurrence (Arnhold 2008; Hallauer and Carena 2009; Ribeiro et al. 2016). Among the leaf diseases affecting popcorn, the common blotch (northern leaf blight, NLB), caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs (syn. *Helminthosporium turcicum* Pass.), is characterized as one of the major (Harlapur et al. 2008; Hurni et al. 2015; Ishfaq et al. 2014; Sabato et al. 2013; Wang et al. 2014). Under favorable climatic settings and in susceptible hosts, the pathogen may cause losses in grain yield that range from 27 to 90% (Ferguson and Carson 2007; Muiru et al. 2010; Wang et al. 2010; 2012).

Another disease, the southern leaf blight (SLB), caused by the fungus *Bipolaris maydis* (Nisik.) Shoemaker (synonym of *Helminthosporium maydis* Nisik. and Myiake), occurs in corn with different intensities, depending on the degree of resistance, the crop management system, and the climatic conditions during its growth (Ali et al. 2011; Altaf et al. 2016; Hussain et al. 2016). Southern leaf blight is one of the most important diseases affecting corn and represents a great threat to corn growing all around the globe (Altaf et al. 2016; Kump et al. 2011; Noor et al. 2015). Under certain conditions, this fungus dramatically reduces the stand and yield of crops, with losses that can be as high as 70% (Ali et al. 2011; Hussain et al. 2016). The factors determining the magnitude of losses are the growth stage of the crop, the susceptible varieties, and the planting time (Ali et al. 2011).

Several control measures are implemented to minimize the damage caused by spot blotches (NLB and SLB), e.g., the spraying of fungicides and the planting of resistant varieties. Furthermore, some practices can be adopted, such as crop rotation, the use of the adequate planting density and spacing, and the use of balanced fertilization and elimination of crop residues (Bergamin Filho and Amorim 2011). The use of cultivars with genetic-resistance potential against the main popcorn diseases is the most viable and economic control measure (Ayiga-Aluba et al. 2015; Ferguson and Carson 2007; Ishfaq

et al. 2014; Vieira et al. 2009b). Associated with this method, the knowledge of the type of control as well as of the heritability of the traits involved in the expression of resistance are highly important in the development of resistant cultivars. This is because these data provide guidance for more efficient selection in subsequent works of breeding programs (Vieira et al. 2009a).

In this scenario, germplasm banks take on an indispensable importance in regard to the congregation of genetic variability, which is necessary especially to give basis to breeding programs of plants to obtain superior genotypes (Newton et al. 2011; Quintal et al. 2012; Vivas et al. 2015). UENF has a popcorn Germplasm Bank with accessions from different eco-geographic regions of Brazil and abroad, obtained from grants and exchanges, which have the potential to be investigated for resistance to diseases. This investigation should start with the identification of sources of resistance to leaf diseases in popcorn, as a result of the high losses caused by leaf pathogens and of the economic importance of the crop.

That said, in this study we investigated the performance of 37 popcorn genotypes with respect to fungal leaf diseases caused by *E. turcicum* and *B. maydis* and to select promising genotypes with genetic resistance to be used in future popcorn breeding programs, aiming to generate new resistant cultivars for farmers.

MATERIAL AND METHODS

Genetic material

Thirty-seven popcorn accessions from the Germplasm Bank were used (Table 1). Nine of them were crossbreeds originating from CIMMYT (ARZM-05083, ARZM-0749, ARZM-13050, BOYA-462, BOZM-260, CHZM-13134, PARA-172, URUG-298-*Amarelo*, and URUG-298-*Roxo*); one commercial hybrid (IAC-125); and 27 inbred lines – six of the ‘*Beija-Flor*’ population (L51, L52, L53, L54, L55, and L59), six of the BRS-Angela population (L61, L63, L65, L66, L70, and L71); five of the ‘*Viçosa*’ population (L75, L76, L77, L80, and L88), three of the Zaeli hybrid (P5, P6, and P7), three of the IAC-112 hybrid (P8, P9, and P10), two of the CMS-42 compound (P2 and P3), one of the Zélia hybrid (P1), and one of South-American breeds (P4).

Table 1. Description of popcorn genotypes from the Germplasm Bank of the State University of Northern Rio de Janeiro.

Genotypes	Type	Origin	Year of Obtainment	Climate adaptation	Institution of Development
L51	Line	<i>Beija-flor</i> : UFV	2009	Temperate/ Tropical	UENF
L52	Line	<i>Beija-flor</i> : UFV	2009	Temperate / Tropical	UENF
L53	Line	<i>Beija-flor</i> : UFV	2009	Temperate / Tropical	UENF
L54	Line	<i>Beija-flor</i> : UFV	2009	Temperate / Tropical	UENF
L55	Line	<i>Beija-flor</i> : UFV	2009	Temperate / Tropical	UENF
L59	Line	<i>Beija-flor</i> : UFV	2009	Temperate / Tropical	UENF
L61	Line	BRS Angela: EMBRAPA	2009	Tropical	UENF
L63	Line	BRS Angela: EMBRAPA	2009	Tropical	UENF
L65	Line	BRS Angela: EMBRAPA	2009	Tropical	UENF
L66	Line	BRS Angela: EMBRAPA	2009	Tropical	UENF
L70	Line	BRS Angela: EMBRAPA	2009	Tropical	UENF
L71	Line	BRS Angela: EMBRAPA	2009	Tropical	UENF
L75	Line	Viçosa: UFV	2009	Temperate / Tropical	UENF
L76	Line	Viçosa: UFV	2009	Temperate / Tropical	UENF
L77	Line	Viçosa: UFV	2009	Temperate / Tropical	UENF
L80	Line	Viçosa: UFV	2009	Temperate / Tropical	UENF
L88	Line	Viçosa: UFV	2009	Temperate / Tropical	UENF
P1	Line	Hybrid Triple Zélia	2006	Temperate / Tropical	UEM
P2	Line	Compound CMS-42	2006	Temperate / Tropical	UEM
P3	Line	Compound CMS-42	2006	Temperate / Tropical	UEM
P4	Line	Races South-Americans	2006	Temperate / Tropical	UEM
P5	Line	Hybrid Triple Zaeli	2006	Temperate / Tropical	UEM
P6	Line	Hybrid Triple Zaeli	2006	Temperate / Tropical	UEM
P7	Line	Hybrid Triple Zaeli	2006	Temperate / Tropical	UEM
P8	Line	Simple hybrid IAC-112	2006	Temperate / Tropical	UEM
P9	Line	Simple hybrid IAC-112	2006	Temperate / Tropical	UEM
P10	Line	Simple hybrid IAC-112	2006	Temperate / Tropical	UEM
ARZM-05083	Race	Argentina	2008	Temperate	CIMMYT
ARZM-07049	Race	Argentina	2008	Temperate	CIMMYT
ARZM-13050	Race	Argentina	2008	Temperate	CIMMYT
BOYA- 462	Race	Bolivia	2008	Tropical	CIMMYT
BOZM-260	Race	Bolivia	2008	Tropical	CIMMYT
CHZM-13134	Race	Chile	2008	Temperate	CIMMYT
PARA-172	Race	Paraguay	2008	Temperate	CIMMYT
URUG-298- <i>Amarelo</i>	Race	Uruguay	2008	Temperate	CIMMYT
URUG-298- <i>Roxo</i>	Race	Uruguay	2008	Temperate	CIMMYT
IAC-125	Hybrid Top cross	Campinas: IAC	2013	Temperate / Tropical	IAC

Implementation of the experiment

Two experiments were implemented in the municipality of Campos dos Goytacazes, Rio de Janeiro, Brazil, during

a 1st season (October 2013 to March 2014), with an average temperature of 26 °C, and a 2nd season (May to September 2014), with an average temperature of 22 °C.

The experiment was implanted as a randomized-block design with four replications, in both growing seasons. The experimental units consisted of simple 3.0 m row containing 16 plants each. The experimental field was prepared mechanically by harrowing followed by furrowing. Seeding was performed manually, with plants spaced 0.20 m apart and rows by 0.90 m. Cultivation practices included thinning the seedlings 15 days after emergence, weeding out, periodic irrigation maintaining the soil under field capacity, and topdressing applied 30 and 45 after emergence, using a 20-0-20 NPK formulation with 300 kg·ha⁻¹ and 200 kg·ha⁻¹ urea, respectively. The ears of each genotype were collected after visual observation of the moment when the bracts were dry, in each growing season, to prevent early or late harvests. The useful plot of the experiment consisted of six competitive plants, totaling 0.90 m².

Evaluated traits

The performance of the genotypes concerning the leaf diseases was monitored by estimating the incidence and severity of the symptoms. To this end, two estimation methods were adopted:

- i. Measurement of incidence of the disease symptoms along the plant, expressed as a percentage;
- ii. Quantification of severity symptoms along the first leaf below the first ear, expressed as a percentage.

Leaf diseases were manifested by a natural infection of the etiological agents on the field. Evaluations were performed by taking six competitive plants per plot, this is, only plants with correct spacing and in the center of the plot were evaluated.

The incidence of NLB and SLB symptoms based on the plant was estimated by using a diagrammatic scale adopted by Agrocere (1996). The scale presents an interval of 1 through 9, in which 1 = 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% incidence. To evaluate the severity of NLB in the leaf, six competitive plants were used per plot, and the evaluations occurred every seven days from flowering to the senescence stage. Considering that only three evaluations were conducted, and that our goal was simply to compare genetic materials, we believe that the analysis based on the average of the evaluations better

reflects the potential of the evaluated genotypes. For this variable, the diagrammatic scale proposed by Lazaroto et al. (2012) was adopted, containing the severity intervals in percentage corresponding to 0.5%, 1.0%, 2.5%, 6.5%, 15.5%, 30.0%, and 54.0%, based on which the percentage of the leaf area compromised by the disease was estimated. The scale proposed by James (1971) was used to estimate the leaf severity of SLB, with severity intervals expressed by the following scale: 0%, 1%, 5%, 25%, and 50%.

Statistical analysis

The traits were statistically evaluated by analysis of variance, and when a significant difference was detected, Scott-Knott's mean clustering algorithm was employed at the 5% probability level. Phenotypic (rp), genotypic (rg) and environmental (re) correlation coefficients were estimated, and the significance level was evaluated by the *t* test. In the estimate of genetic diversity, Generalized Mahalanobis Distance was used as a dissimilarity measure, and subsequently the accessions were grouped based on the UPGMA method, using, for this, the traits incidence and severity of SLB and NLB. The adjustment between the matrix of distances and the dendrogram was estimated by the cophenetic correlation coefficient (CCC), developed by Sokal and Rohlf (1962). All analyses were performed using the computer resources of Genes software (Cruz 2013).

RESULTS AND DISCUSSION

Resistance to Northern Leaf Blight (NLB)

There was a significant genotype effect for incidence of plants with symptoms in both growing seasons. Two groups were formed in the first, and four in the second growing season. Genotypes L71, L75, L76, P7, and PARA-172 were allocated in the group with highest resistance in both evaluated periods (Table 2).

For leaf severity, a significant effect of genotype was only observed in the second evaluation period. The means clustering tests also revealed the presence of more than one group in the second harvest (Table 2). Thirty-one genotypes were included in the group with the lowest means, with noteworthy values found for L71, L75, L76, P7, and PARA-172. In addition to these,

Table 2. Average of three assessments in percentage of incidence and severity of the fungal leaf diseases northern leaf blight (NLB), caused by *E. turcicum*, in the first and second popcorn growing seasons.

Genotypes	Incidence of NLB		Severity of NLB	
	Season 1	Season 2	Season 1	Season 2
L51	31.98a	37.26b	7.96a	13.84a
L52	40.02a	43.63b	4.67a	24.85b
L53	33.38a	46.96c	3.68a	23.87b
L54	42.52b	56.93c	6.31a	21.12b
L55	46.15b	72.38d	5.70a	31.56b
L59	34.72a	46.55c	2.82a	14.33a
L61	26.70a	31.70b	5.25a	1.57a
L63	46.99b	52.37c	8.22a	10.58a
L65	62.52b	71.11d	10.43a	27.30b
L66	45.00b	24.45a	4.04a	2.68a
L70	58.10b	49.05c	6.81a	6.05a
L71	33.06a	19.46a	3.26a	6.11a
L75	33.09a	21.54a	3.45a	2.84a
L76	34.17a	23.49a	4.67a	3.39a
L77	38.89a	34.18b	8.81a	9.18a
L80	37.22a	42.24b	5.32a	21.52b
L88	31.27a	35.51b	3.90a	13.64a
P1	40.00a	42.17b	8.45a	6.38a
P2	41.58a	40.72b	4.45a	8.53a
P3	28.63a	37.26b	2.70a	6.99a
P4	55.86b	47.93c	4.97a	8.78a
P5	36.68a	34.20b	4.84a	6.52a
P6	57.28b	39.05b	5.40a	9.49a
P7	23.63a	21.97a	2.15a	3.71a
P8	28.33a	36.95b	1.97a	7.46a
P9	31.14a	36.41b	4.15a	8.27a
P10	33.61a	38.39b	3.48a	11.35a
ARZM-05083	48.33b	25.87a	3.34a	1.08a
ARZM-07049	55.00b	26.29a	9.11a	2.55a
ARZM-13050	53.66b	37.30b	7.03a	5.07a
BOYA-462	49.46b	39.51b	4.27a	5.68a
BOZM-260	46.11b	29.38a	4.68a	2.41a
CHZM-13134	47.22b	41.96b	4.88a	13.92a
PARA-172	22.79a	23.34a	1.84a	0.93a
URUG-298- <i>Amarelo</i>	44.47b	29.19a	3.15a	1.47a
URUG-298- <i>Roxo</i>	56.96b	33.36b	7.15a	1.88a
IAC-125	33.64a	38.64b	3.36a	6.63a

Means followed by the same letters represent a statistically homogenous group according to Scott-Knott's algorithm. Season 1 = growing season from October 2013 to March 2014 and Season 2 = growing season from May to September 2014.

L61, L66, P3, P8, ARZM-05083, BOYA-462, BOZM-260, URUG-298-*Amarelo*, URUG-298-*Roxo*, and IAC-125 were highlighted for showing values lower than 5% of their leaf area affected, considering the average between the first and second harvests (Table 2). P3, P7, and P8 are lines from the State University of Maringá (UEM); P3 was extracted from the CMS-42 compound of Embrapa Maize & Sorghum; P7, from the ‘Zaeli’ triple hybrid; and P8 from the IAC-112 hybrid (Silva et al. 2011). The resistance of line P3 is possibly related to the fact that it originates from the genetically bred population CMS-42 (Pacheco et al. 1998). The line P7 may have its expression of resistance because its genealogy – Zaeli – holds alleles favorable to NLB, as stated by Vieira et al. (2009b). The P8 line probably showed good performance because it originated from hybrid IAC-112, which has recognized resistance to NLB (Vieira et al. 2009b).

Among genotypes developed by UENF that stood out — L61, L66, L71, L75, and L76 —, lines L61, L66, and L71 had good performance, likely because they originated from cultivar BRS Angela, developed by Embrapa Maize & Sorghum, after six cycles of intrapopulation recurrent selection (Pacheco et al. 2005). According to Bleicher and Balmer (1993), recurrent selection is efficient to increase the level of resistance to NLB in susceptible corn cultivars, confirming the assertion of Parteniani and Miranda Filho (1978). Based on this premise, Kasozi et al. (2015) observed that two S_1 recurrent selection cycles were effective in improving the ‘Longe5’ population for yield and resistance to the damage caused by the maize weevil and to the main corn diseases, among them NLB. These authors achieved a 29% increase in grain yield and 15% reduction of the NLB disease from C_0 to C_2 , demonstrating the potential for improvement of these traits through recurrent selection. In an evaluation of the history of the NLB disease in seven cycles of recurrent selection, Ribeiro et al. (2016) concluded that selection was effective in maintaining the equilibrium of the disease in the improved population of popcorn UENF-14 and that the source of resistance was not lost with the advance of the selection cycles. These authors also stated that it is possible to combine genes for resistance to NLB with the other agronomic traits of interest.

The superiority of lines L75 and L76 can be attributed to the fact that they originated from cultivar Viçosa, which was evaluated under natural conditions of NLB

infestation by Miranda et al. (2003) and was considered an expressive source of resistance. Nevertheless, Arnhold et al. (2008) evaluated S_1 progenies from Viçosa population for resistance to diseases and expression of agronomic features, and found superior resistance to NLB compared with the control IAC-112.

The breeds ARZM-05083, BOYA-462, BOZM-260, URUG-298-*Amarelo*, URUG-298-*Roxo*, and PARA-172 originate from CIMMYT, and genealogies or any expression of resistance to diseases for them are unknown. These accessions expressed similar resistance to that of commercial hybrid IAC-125, which is recognized for having moderate resistance to NLB (Cruz et al. 2014). Of these, PARA-172 stood out for showing an even superior resistance to that of hybrid IAC-125, which makes its use in breeding programs for introgression of resistance to NLB strategic.

Considering the performance of the genotypes in both growing seasons, lines L71, L75, L76, P7 and accession PARA-172 are considered superior.

Resistance to Southern Leaf Blight (SLB)

There was a significant effect of genotype in all evaluations performed, with three groups formed in the first harvest and four in the second, for incidence in the plant. Genotypes ARZM-05083, ARZM-07049 and PARA-172 had the lowest estimates for incidence of SLB in both evaluation periods (Table 3). For severity of SLB in the leaf, there was a significant genotype effect only in the second growing season, with three and four groups formed for the first and second periods, respectively (Table 3). The genotypes that expressed the highest levels of resistance to SLB, in both growing seasons, were L61, L66, L70, L76, P2, P3, P4, P7, ARZM-05083, ARZM-07049, BOZM-260, PARA-172, URUG-298-*Amarelo*, and IAC-125 (Table 3).

Lines L61, L66, and L70 are from recurrent selection (Pacheco et al. 2005), whereby it is concluded that the resistance shown was due to the combination of favorable alleles stemming from the implementation of recurrent selection. Of the accessions from UEM, the resistance of lines P2 and P3 is attributed to the fact that they are from the improved population originating from compound CMS-42 (Pacheco et al. 1998). The resistance observed in hybrid top cross IAC-125 was expected to occur, as described in the table provided by EMBRAPA about the behavior of corn cultivars available in the Brazilian market in the 2013/14 crop regarding the main diseases (Cruz et al. 2014).

Table 3. Average of three assessments in percentage of incidence and severity of the fungal leaf diseases southern leaf blight (SLB), caused by *B. maydis*, in the first and second popcorn growing seasons.

Genotypes	Incidence of SLB		Severity of SLB	
	Season 1	Season 2	Season 1	Season 2
L51	81.48c	66.41c	4.97c	45.43c
L52	86.96c	76.50d	4.82c	55.29d
L53	75.61b	68.29c	3.24b	55.05d
L54	69.75a	69.36c	2.35a	56.13d
L55	64.33a	68.37c	1.86a	46.09c
L59	69.50a	67.34c	2.55a	45.33c
L61	71.15a	66.46c	1.84a	33.87a
L63	66.74a	67.16c	3.01b	31.06a
L65	65.88a	62.16b	2.25a	38.62b
L66	64.20a	65.89c	2.40a	34.24a
L70	61.18a	62.68b	1.88a	30.73a
L71	66.45a	66.80c	3.21b	32.89a
L75	76.15b	67.33c	2.82a	38.66b
L76	73.38b	66.89c	2.70a	33.29a
L77	71.98b	67.49c	2.46a	45.47c
L80	72.22b	73.74d	2.18a	56.13d
L88	68.92a	64.09b	2.60a	40.92b
P1	72.41b	69.15c	3.16b	43.20c
P2	64.46a	65.90c	1.70a	31.12a
P3	67.83a	65.07b	2.20a	34.38a
P4	66.68a	61.66b	1.82a	29.57a
P5	74.48b	67.93c	2.41a	42.04c
P6	67.28a	64.56b	1.94a	39.42b
P7	68.09a	62.49b	2.16a	32.22a
P8	69.49a	61.66b	2.61a	37.43b
P9	68.95a	63.56b	1.97a	39.20b
P10	67.53a	65.12b	1.96a	40.80b
ARZM-05083	68.13a	57.91a	2.25a	32.51a
ARZM-07049	65.61a	57.00a	2.04a	30.10a
ARZM-13050	68.65a	68.90c	2.15a	37.72b
BOYA-462	69.75a	63.22b	3.16b	32.07a
BOZM-260	68.10a	62.97b	1.94a	34.69a
CHZM-13134	65.23a	72.72d	3.75b	48.59c
PARA-172	65.06a	59.29a	1.97a	26.92a
URUG-298-Amarelo	64.78a	61.53b	1.65a	31.00a
URUG-298-Roxo	66.68a	63.42b	1.77a	36.32b
IAC-125	66.40a	67.47c	2.19a	34.93a

Means followed by the same letters represent a statistically homogenous group according to Scott-Knott's algorithm. Season 1 = growing season from October 2013 to March 2014 and Season 2 = growing season from May to September 2014.

The breeds from CIMMYT — ARZM-05083, ARZM-07049, BOZM-260, PARA-172 and URUG-298-*Amarelo* — have shown to be materials of interest for breeding aiming at resistance to diseases. These cases are genotypes from temperate countries, like Argentina (ARZM-05083, ARZM-07049), Paraguay (PARA-172), and Uruguay (URU-298-*Amarelo*) and tropical country like Bolivia (BOZM-260). These findings indicate these genotypes can be recommended in crosses with plants that have desirable agronomic traits, given the expansion of the genetic basis of popcorn accessions and the development of new cultivars. In addition, the selection could be applied within some accession, in order to achieve improved lines for explore hybrids, for instance Silva et al. (2015) evaluated the genetic divergence among and within accessions of popcorn and found that accession BOZM-260 showed the highest proportion of heterozygous plants among the evaluated accessions and also exhibited allelic variation in all microsatellite loci analyzed. These authors also found that ARZM-05083 was genetically distant from the other genotypes assessed, which corroborates the strategy of use of popcorn germplasm from temperate climates to broaden the genetic base of breeding programs.

Considering the accessions performance in both growing seasons, for both incidence and severity, three accession can be indicated as the highest sources of resistance to SLB: breeds ARZM-05083, ARZM-07049, and PARA-172, all originating from CIMMYT. Special emphasis should be placed on genotype PARA-172, which expressed the highest degree of resistance for both NLB and SLB (Table 3), thus showing to be an excellent alternative in crosses aiming at resistance to popcorn leaf diseases or for the extraction of lines for the generation of superior hybrids. With this goal, UENF's Popcorn Breeding Program has acted intensely in the development of lines from superior germplasms; nine S_6 descendants of PARA-172 are already under growth in the field for the generation of individuals from the next inbred generation.

Cluster analysis

There was a good fit in the graphic representation of the clustering distances, since the cophenetic correlation coefficients (CCC) were 0.87 for the first and 0.78 for the second growing season. For the first harvest, the genotypes were gathered in three larger groups (Figure 1a),

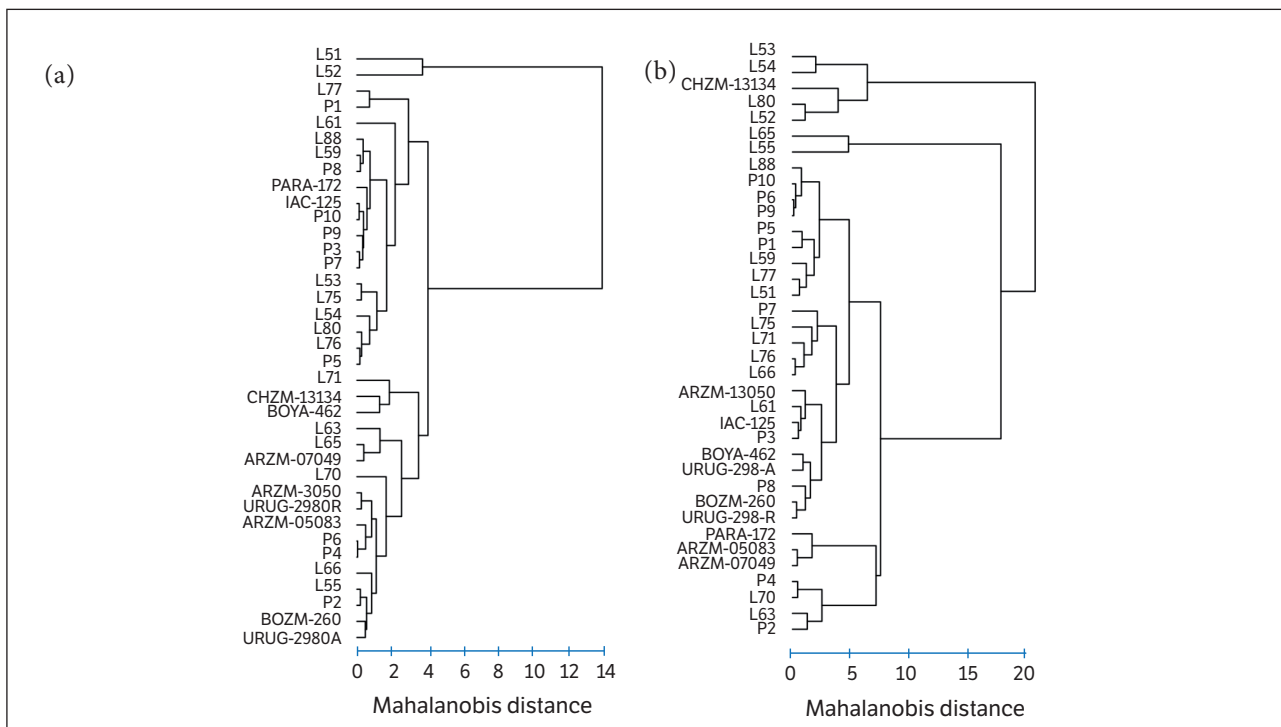


Figure 1. Dendrograms of 37 genotypes of popcorn by the UPGMA method using Generalized Mahalanobis Distance as a measure of genetic dissimilarity, for both growing seasons. (a) Growing season from October 2013 to March 2014 and (b) Growing season from May to September 2014.

one of which was formed by lines L51 and L52, that were the most susceptible to the two diseases evaluated here (Tables 2 and 3). The second group included 18 of the 35 remaining genotypes. A subgroup was formed in this group, consisting of L59, P8, L88, IAC-125, P10, P3, P7, P9, and PARA-172, which were the genotypes most resistant to both diseases (Tables 2 and 3). The third group contained 17 accessions, and except for P2 and L71, which showed resistance to NLB and SLB in the first harvest, the others were moderately resistant to the action of the fungus *Bipolaris maydis*.

Four groups were formed for the second harvest (Figure 1b); the first included the genotypes of highest susceptibility – L53, L54, L80, L52, and CHZN-13134, whilst the second group allocated lines L55 and L65, which were susceptible to NLB in the second harvest and moderately resistant to NLB and SLB in the second harvest. It is thus unquestionable that these reactions facilitated the agglutination of these genotypes in the same group for both growing seasons. The third group contained 23 accessions, which were moderately resistant to NLB and SLB, except for the subgroup formed by lines P5, P1, L77, L51, and L59, which were all moderately resistant to NLB and susceptible to SLB. In the second group, a subgroup was formed containing accessions PARA-172, ARZM-05083, and ARZM-07049, which stood out for having consistently the highest levels of resistance to SLB in the second growing season, especially PARA-172, which revealed the highest estimates for severity to both diseases in the second growing season (Table 3). In this way, these are populations with high potential to be used as sources of resistance. Moreover, the consistent variability present

between these populations — confirmed by the high genetic diversity estimates as estimated by ISSR markers (Amaral Júnior et al. 2011) — make them even more interesting for the production of superior segregants.

The efficiency of the UPGMA clustering method is thus proven in the discrimination of reaction to NLB and SLB. As such, it is an interesting multivariate statistical alternative in the indication of genotypes for breeding programs aimed at the introgression of genes providing resistance to NLB and SLB in segregating populations, or even to obtain superior hybrids.

Estimates of correlations

Elevated estimates for phenotypic and genotypes correlations were detected for incidence and severity of NLB in both growing seasons, similarly to what occurred for incidence and severity of SLB (Table 4). The magnitude of genetic correlations were superior to the phenotypic correlations, evidencing a strong correspondence of selection based on the phenotype with the genetic effects between incidence and severity. Thus, only one of the two traits would already be sufficient for the selection of resistant genotypes and, in this aspect, because of the greater practicality, it is recommended to use the incidence traits as the main variable in experiments conducted in the field, under natural inoculation conditions. Under controlled inoculation conditions, however, it is suggested to use severity as a criterion of selection. This is because in experiments undertaken in greenhouses, the distribution of diseases is dependent upon the inoculation being

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Table 4. Estimates of correlations among incidence of NLB in the plant (ETP), severity of NLB in the leaf (ETF), incidence of SLB in the plant (BMP), and severity of SLB in the leaf (BMF) for two popcorn growing seasons.

Trait	Correlation	Season 1			Season 2		
		ETF	BMP	BMF	ETF	BMP	BMF
ETP	Phenotypic	0.6046**	-0.3458*	-0.1879	0.8064**	0.2806	0.4252**
	Genotypic	0.6539**	-0.6649**	-0.1631	0.8713**	0.2781	0.4644**
	Environmental	0.6017**	0.0698	-0.2235	0.5684**	0.2912	0.2326
ETF	Phenotypic		0.0389	0.1082		0.5263**	0.749**
	Genotypic		-0.0533	0.2901		0.6109**	0.8344**
	Environmental		0.1175	-0.0269		0.1903	0.3208*
BMP	Phenotypic			0.7251**			0.7485**
	Genotypic			0.9821**			0.8285**
	Environmental			0.2195			0.2765

** , * = Significant at the 1 and 5% probability levels , respectively, according to the *t* test. Season 1 = growing season from October 2013 to March 2014 and Season 2 = growing season from May to September 2014.

performed; on the other hand, for experiments in the field, the disease is typically distributed more evenly. Furthermore, incidence is a more practical variable to be evaluated.

In turn, were observed genetic, phenotypic and environmental correlation estimates varying of low to high magnitude for incidence and severity of NLB and SLB, depending on the pair of traits assessed and the season in which this evaluation took place (Table 4). Therefore, there is no direct relationship between traits that provide resistance to these diseases. These results are probably because the mechanisms of resistance to these diseases have distinct origins. According to Chang and Peterson (1995), *Bipolaris maydis* is controlled by two recessive genes in corn, in which the dominant gene provides susceptibility to SLB. On the other hand, for *E. turcicum*, the genetic control of NLB can be achieved by quantitative resistance (by using several genes of small effects) or even by qualitative resistance (from few genes of high effect in reducing the intensity of the disease) (Vieira et al. 2009b). Ogliari et al. (2005) emphasize

that these two types of mechanisms of resistance to *E. turcicum* may work separately or together, suggesting the existence of different mechanisms providing resistance to NLB and SLB, which will require greater accuracy in the development of breeding programs when aiming at the combination of these diseases in progenies under selection.

CONCLUSION

A good source of resistance was found to NLB through accessions L71, L75, L76, P7 and PARA-172, which can thus be considered remarkable sources of resistance. For resistance to SLB, the superior accessions that can be indicated as sources of resistance are ARZM-05083, ARZM-07049, and PARA-172. Thus, with respect to leaf fungal diseases caused by *E. turcicum* and *B. maydis*, promising genotypes with genetic resistance may be used in future crosses in popcorn breeding programs, in order to generate new resistant cultivars for farmers.

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