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Potential of popcorn germplasm as a source of resistance to ear rot

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ABSTRACT: Because of its multi-purpose nature, popcorn has sparked the interest of the World Trade Organization as regards fungal contamination by mycotoxins. However, no investigations have been conducted on popcorn for resistance of genotypes to ear rot. The aim of this study was to evaluate the potential of popcorn genotypes as to resistance to ear rot and rotten kernels, as an initial step for the implementation of a breeding program with the popcorn crop in Northern Rio de Janeiro State, Brazil. Thirty-seven accessions from different ecogeographic regions of Latin America were evaluated in 2 cultivation periods, in a randomized block design with 4 replications. We evaluated the incidence of rotten ears, incidence of rotten ears caused by *Fusarium* spp., severity of ears with *Fusarium* spp. rot, and

incidence of rotten kernels. The results were subjected to analysis of variance, and means were compared by the Scott-Knott clustering test ($p < 0.05$). A significant effect was observed for all evaluated variables, characterizing them as efficient in the discrimination of genotypic variability for reaction to fungal injuries in popcorn. The gene pool of the tropical and temperate Germplasm Collection evaluated here has the potential to generate superior segregants and provide hybrid combinations with alleles of resistance to diseases affecting ears and stored kernels. Based on the different variables and times, the experiment was conducted, and genotypes L65, L80, and IAC 125 showed the highest levels of resistance.

Key words: *Zea mays*, *Fusarium* spp., genetic resistance.

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INTRODUCTION

Mycotoxins are products of the secondary metabolism of fungi that can affect one's health when ingested, inhaled, or in simple contact with the skin. Mycotoxins can appear in food primarily by fungal infection of crops from agricultural productions, being directly absorbed by men, or indirectly, when contaminated diets are supplied to animals in whom mycotoxins can accumulate in different organs or tissues and enter the human diet through consumption of meat, milk, and eggs (Marin et al. 2013). In the Italian market, for instance, the presence of mycotoxins was detected in baby foods in at least 31% of cereal-based nutrients, and 27% of contaminated samples showed simultaneous occurrence of different mycotoxins. The mycotoxin levels found reveal a situation that puts at risk the health of vulnerable populations, as is the case of the infant group (Juan et al. 2014). A study in Northern Nigeria investigated the occurrence of mycotoxins in urine samples of 120 farmers of the region, most of whom depended on corn, sorghum, peanut, and millet growing. Of the studied samples, 50.8% showed contamination by mycotoxins (Ezekiel et al. 2014).

In general, questions about food safety are complex, requiring more than 1 control measure to effectively overcome the risk posed to health, which includes actions involving the different steps of the food production chain (Pitt et al. 2013). In the agricultural production scenario, the occurrence of damaged, namely rotten kernels, is a consequence of ear rots in corn, which is always related to the presence of mycotoxins, caused by fungi of the genus *Fusarium* spp. in batches of grains stored either for human consumption or to be used as a component in the formulation of animal diets (Juliatti et al. 2007). Besides the hazard of contamination by mycotoxins, the effects of fungal development markedly reduce grain yield and the quality of the end product (Mendes et al. 2012). Moreover, fungi of the genus *Fusarium* spp. have caused serious damage to cereal production, resulting in reductions of 10 to 40% (Bottalico and Perrone 2002; Linkmeyer et al. 2016). Nevertheless, the quantification of economic losses in popcorn production remains unknown.

Only in the last decade, studies about the control of diseases in popcorn have become a concern to breeders

(Arnhold 2008; Vieira et al. 2011; Sanches et al. 2011; Vieira et al. 2012; Noor et al. 2015; Vieira et al. 2016). To complicate matters, no research has been developed on resistance to ear rot in popcorn. In this regard, the evaluation of germplasm banks is of great importance during pre-breeding, mainly to support breeding programs in the identification of sources of resistance genes (Sanches et al. 2011; Resh et al. 2015; Vivas et al. 2015).

Corn has a great genetic importance worldwide held in genetic reservoirs, such that there is no shortage of favorable alleles in germplasm banks capable of contributing, among other aspects, with resistance to diseases, tolerance to abiotic stresses, greater productivity, and better nutritional quality (Prasanna 2012). The institution of development of this study has a popcorn germplasm bank with accessions from different eco-geographic regions that have been made available for research focused on resistance to diseases. This collection contains germplasm from Brazil and abroad — Latin America, Mexico, and USA — obtained from grants and exchanges.

Given the impacts of mycotoxins, along with the scarcity of investigations on resistance to ear rot in popcorn, the present study was developed to evaluate the performance of genotypes from the Germplasm Collection concerning ear rot by quantifying the incidence and severity of symptoms and the percentage of rotten kernels resulting from susceptibility to infection by *Fusarium* spp.

MATERIAL AND METHODS

Genotypes

Thirty-seven accessions of popcorn from the Germplasm Bank were used to evaluate their reaction to *Fusarium* ear rot (Table 1).

Implementation of the experiment

The experiment was implemented in Campos dos Goytacazes, Northern Rio de Janeiro State, Brazil, in the first harvest (October 2013 to March 2014), when the average temperature was 26 °C, and in the second harvest (May to September 2014), with an average temperature of 22 °C.

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Table 1. Description of popcorn genotypes from the Germplasm Bank.

Genotype	Type	Originating variety	Climatic adaptation	Institution of development
L 88	Line S7	Viçosa: UFV	Temperate/Tropical	UENF
L 70	Line S7	Ângela: Embrapa	Tropical	UENF
L 65	Line S7	Ângela: Embrapa	Tropical	UENF
L 80	Line S7	Viçosa: UFV	Temperate/Tropical	UENF
L51	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 77	Line S7	Viçosa: UFV	Temperate/Tropical	UENF
L 76	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 75	Line S7	Viçosa: UFV	Temperate/Tropical	UENF
L 66	Line S7	Ângela: Embrapa	Tropical	UENF
L 53	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 52	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 55	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 54	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 61	Line S7	Ângela: Embrapa	Tropical	UENF
L63	Line S7	Ângela: Embrapa	Tropical	UENF
L 59	Line S7	Beija-flor: UFV	Temperate/Tropical	UENF
L 71	Line S7	Ângela: Embrapa	Tropical	UENF
P1	Line	Hybrid Zélia	Temperate/Tropical	UEM
P2	Line	Compound CMS-42	Temperate/Tropical	UEM
P3	Line	Compound CMS-42	Temperate/Tropical	UEM
P4	Line	South-American varieties	Temperate/Tropical	UEM
P5	Line	Hybrid Zaeli	Temperate/Tropical	UEM
P6	Line	Hybrid Zaeli	Temperate/Tropical	UEM
P7	Line	Hybrid Zaeli	Temperate/Tropical	UEM
P8	Line	Hybrid IAC112	Temperate/Tropical	UEM
P9	Line	Hybrid IAC112	Temperate/Tropical	UEM
P10	Line	Hybrid IAC112	Temperate/Tropical	UEM
BOYA 462	Variety	Unknown	Temperate/Tropical	CIMMYT
URUG 298 Roxo	Variety	Unknown	Temperate/Tropical	CIMMYT
URUG 298 Amarelo	Variety	Unknown	Temperate/Tropical	CIMMYT
BOZM 260	Variety	Unknown	Temperate/Tropical	CIMMYT
ARZM 07049	Variety	Unknown	Temperate/Tropical	CIMMYT
CHZM 13134	Variety	Unknown	Temperate/Tropical	CIMMYT
ARZM 13050	Variety	Unknown	Temperate/Tropical	CIMMYT
ARZM 05083	Variety	Unknown	Temperate/Tropical	CIMMYT
PARA 172	Variety	Unknown	Temperate/Tropical	CIMMYT
IAC 125	Triple hybrid	Unknown	Temperate/Tropical	IAC

UENF = Northern Fluminense State University "Darcy Ribeiro"; UEM = Maringá State University; CIMMYT = International Maize and Wheat Improvement Center; IAC = Agronomic Institute of Campinas.

A randomized block design with 4 replications was adopted in the 2 harvests. Experimental units consisted of simple 3.0 m rows containing 16 plants per row. The experimental field was prepared mechanically by harrowing followed by furrowing. Seeding was performed manually, with 0.2 m spacing between plants and 0.9 m between rows. Cultivation practices included seedling thinning 15 days after emergence, control of weeds by hoeing, periodic irrigation with maintenance of soil at field capacity, and topdressing performed 30 and 45 days after emergence using a 20-0-20 NPK formulation with 300 and 200 kg·ha⁻¹ urea, respectively.

The ears of each genotype were harvested by visually observing when the bracts were dry, at each cultivation period, to prevent early or late harvesting.

Evaluated traits

Incidence of rotten kernels (IRK): after the ears were threshed, two 100-kernel samples were counted and represented the portions of work. The incidence of rotten kernels was expressed in percentage, determined according to the criteria for classification of corn established by Ordinance no. 11 from the Brazilian Ministry of Agriculture, Livestock and Food Supply, of April 12, 1996. The method consists of the visual segregation of symptomatic kernels showing discoloration in over ¼ of their total surface.

Incidence of rotten ears (IRE): determined adopting the total number of ears obtained from the harvest of each experimental unit. Ears infected at more than 50% by the action of ear-rot fungi were counted, and the incidence of rotten ears was the result of the division between the number of rotten ears and the total number of ears harvested per plot on the field, with values expressed in percentage.

Severity of ear rot caused by *Fusarium* spp. (SRF): the diagrammatic scale for ear rot caused by *Fusarium* spp. proposed by the International Maize and Wheat Improvement Center (CIMMYT 1985) was adopted, with values of 0, 10, 20, 30, and 40%, according to the rot severity level.

Incidence of ears damaged by *Fusarium* spp. (IDF): after the characteristic symptom of the disease was detected, all symptomatic ears infected by *Fusarium* spp. were counted. The incidence was obtained in percentage, as the division between the number of symptomatic ears and the total number of ears harvested per plot on the field, with values expressed in percentage.

Statistical analysis

Data were subjected to analysis of variance, and, when a significant difference was detected, the Scott-Knott means-clustering algorithm was used ($p < 0.05$). All analyses were performed using the computer resources of Genes software (Cruz 2013).

RESULTS AND DISCUSSION

There was a significant effect of the sources of variation genotype and time for the group of evaluated traits (data not shown), revealing the existence of genotypes with contrasting responses, among the evaluated accessions. This is of fundamental importance for applied breeding, from the perspective of potentiation of variability for the generation of superior segregants, or even for studies of heritability. These results also suggest that the 2 planting times were distinct enough to cause differences between the evaluated traits. The source of variation genotype *versus* time interaction revealed a significant effect on IDF, SRF, and IRK, whereby we concluded that the genotypes have different performances, determining the use of estimates of means based on each cultivation time.

On the other hand, for the trait incidence of rotten ears (IRE), no significant genotype *versus* time interaction effect was detected; consequently, the clustering of genotypes was presented and discussed with respect to means between harvests, since the cultivation time does not change the performance of genotypes. For this trait, by clustering the means, 2 groups were formed (Figure 1). The group of resistant genotypes included 83.78% of the studied accessions; of those from the Northern Fluminense State University "Darcy Ribeiro" (UFENF), the following lines stood out: L53, L54, L59, L61, L63, L65, L66, L70, L71, L76, L77, and L80; and among those from the Maringá State University (UEM), lines P1, P2, P3, P4, P5, P6, P7, P9, and P10 were notable. For the accessions from the CIMMYT, varieties ARZM 05083, ARZM 07049, ARZM 13050, BOYA 462, BOZM 260, CHZM 13134, PARA 172, URUG 298 Amarelo, and URUG 298 Roxo manifested resistance to ear rot, as well as hybrid IAC 125, originating from the Agronomic Institute of Campinas (IAC; Figure 1).

The means-clustering test for IDF performed for the conditions of the first harvest formed 4 groups (Table 2).

The group of genotypes resistant to the occurrence of the disease was composed of 18.92% of the studied accessions, namely L80, L65, L70, from UENF; varieties PARA 172, URUG 298, Amarelo, and URUG 298 Roxo, from CIMMYT; and, lastly, hybrid IAC 125 (Table 2). There were no resistant genotypes in this harvest originating from UEM. For the second harvest, the means-clustering test formed 3 groups. The group of most resistant genotypes corresponded to

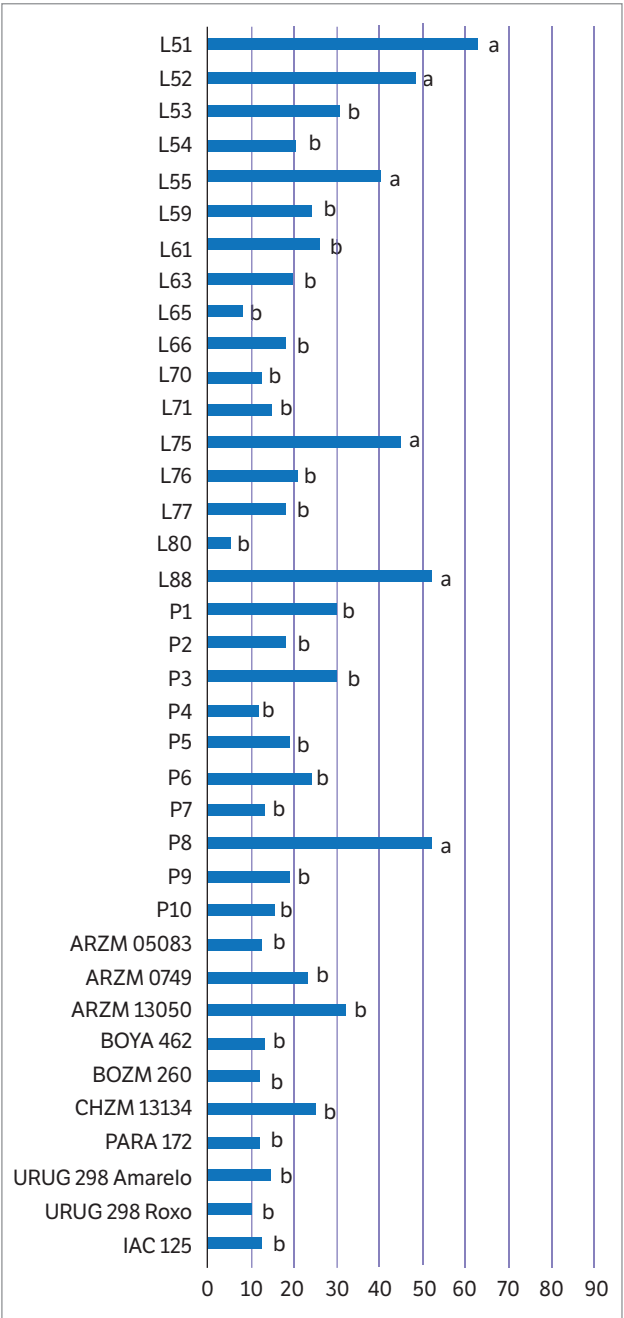


Figure 1. Incidence of rotten ears estimated in 37 popcorn genotypes evaluated in the first (2013/2014) and second (2014) harvests.

Table 2. Incidence of ears damaged by *Fusarium* spp. and severity of ear rot caused by *Fusarium* spp. in 37 popcorn genotypes evaluated in the first (2013/2014) and second (2014) crops.

Genotype	IDF		SRF	
	1 st harvest	2 nd harvest	1 st harvest	2 nd harvest
L51	100.00a*	50.11a	71.00a	79.54a
L52	90.48a	35.72b	89.33a	59.17a
L53	87.20a	19.97c	91.67a	43.75b
L54	65.21b	17.86c	88.58a	59.17a
L55	95.83a	11.31c	100.00a	43.75b
L59	89.59a	12.73c	93.23a	58.33a
L61	70.00b	9.82c	80.00a	70.00a
L63	37.34c	18.02c	67.50a	28.75b
L65	20.96d	9.90c	47.50b	26.25b
L66	46.49c	8.85c	67.69a	33.33b
L70	25.64d	9.98c	75.63a	59.75a
L71	37.91c	3.57c	48.54b	25.00b
L75	83.33a	49.11a	100.00a	72.92a
L76	59.66b	13.19c	60.83b	56.88a
L77	43.33c	14.92c	49.59b	24.38b
L80	11.82d	3.57c	40.00b	25.00b
L88	97.50a	67.31a	69.03a	63.61a
P1	70.83b	18.13c	97.29a	23.50b
P2	69.36b	25.54c	72.71a	64.96a
P3	42.55c	14.26c	76.15a	48.33b
P4	52.58c	14.38c	67.29a	69.58a
P5	64.76b	24.04c	66.74a	64.21a
P6	51.94c	20.14c	37.68b	55.79a
P7	57.55b	14.14c	75.00a	62.71a
P8	35.60c	25.99c	54.44b	65.94a
P9	44.16c	14.77c	57.06b	82.29a
P10	60.57b	24.13c	71.97a	78.33a
ARZM 05083	42.64c	6.89c	67.50a	41.25b
ARZM 07049	46.29c	23.82c	63.67a	84.32a
ARZM 13050	73.76b	31.95b	75.58a	49.50b
BOYA 462	40.63c	15.57c	17.32b	78.75a
BOZM 260	36.26c	4.85c	72.17a	66.25a
CHZM 13134	41.39c	24.59c	88.33a	72.92a
PARA 172	19.71d	13.46c	43.75b	71.46a
URUG 298 Amarelo	31.41d	17.64c	43.39b	57.08a
URUG 298 Roxo	20.06d	4.55c	86.17a	13.13b
IAC 125	31.92d	16.01c	53.19b	44.92b

*Means followed by common letters represent a statistically homogeneous group by the Scott-Knott algorithm. IDF = Incidence of ears damaged by *Fusarium* spp.; SRF = Severity of ear rot caused by *Fusarium* spp.

86.48% of the evaluated accessions: L53, L54, L55, L59, L61, L63, L65, L66, L70, L71, L76, L77, and L80. For this second harvest, all accessions from UEM showed resistance to incidence of *Fusarium* spp. As for the accessions originating from CIMMYT, varieties ARZM 05083, ARZM 07049, BOYA 462, BOZM 260, CHZM 13134, PARA 172, URUG 298 Amarelo, and URUG 298 Roxo stood out. Triple hybrid IAC 125 also manifested resistance.

Concerning SRF, for the first harvest, 2 statistically distinct groups were formed regarding severity of *Fusarium* ear rot. The group of resistant genotypes included 32.43% of the studied accessions, and variations between resistant and susceptible accessions reached 82.68% between genotypes BOYA 462 and L55, respectively (Table 2). For the second harvest, the means test generated statistically distinct groups, among which the group of most resistant genotypes comprised 37.84% of the studied accessions that responded positively to severity of *Fusarium* spp. The most significant variation between resistant and susceptible genotypes reached the magnitude of 71.19% (Table 2).

For a reliable indication of promising genotypes in terms of resistance to ear rots, especially those caused by *Fusarium* spp, it is necessary to study the set of factors influencing the occurrence and severity of post-harvest diseases. Thus, considering the 3 variables simultaneously and the cultivation times, it was found that lines L65, L80 and triple hybrid IAC 125 possess favorable alleles for traits that provide resistance to ear rots, such as those caused by fungi of the genus *Fusarium* spp. Pacheco et al. (2005), evaluating cycles of selection of BRS Angela, observed that, up to selection cycle VI, variety BRS Angela showed higher levels of ear health when compared with controls IAC 112 and Zélia. This fact may indicate congregation of favorable alleles for resistance to ear diseases for lines extracted from the genetically improved cultivar BRS Angela, as is the case of line L65. Inferring about the potential of popcorn cultivars, Miranda et al. (2003) evaluated variety Viçosa for agronomic traits, and, for percentage of diseased ears, this cultivar showed an average resistance of 20% in relation to the others investigated in the range of 10% to 30%. Regarding triple commercial hybrid IAC 125, the estimates for ear health are possibly linked to the good performance *per se* of the genotype.

For the trait IRK, 3 groups were formed in the first harvest, whereas there was no statistical difference between the genotypes for the second cultivation period (Table 3).

Table 3. Incidence of rotten kernels in 37 popcorn genotypes evaluated in the first (2013/2014) and second (2014) harvests.

Genotype	Incidence of rotten kernels	
	1 st harvest	2 nd harvest
L51	69.500a*	13.375a
L52	50.833a	6.000a
L53	44.375b	3.000a
L54	23.125c	2.500a
L55	65.655a	3.750a
L59	41.750b	3.000a
L61	19.250c	0.500a
L63	12.750c	1.375a
L65	10.750c	1.250a
L66	24.375c	1.750a
L70	15.750c	0.375a
L71	29.375c	0.625a
L75	56.168a	8.125a
L76	40.500b	1.500a
L77	14.875c	1.125a
L80	23.125c	0.375a
L88	57.500a	3.625a
P1	37.875b	4.500a
P2	24.000c	2.875a
P3	19.125c	0.000a
P4	8.625c	1.250a
P5	24.875c	1.375a
P6	10.375c	1.250a
P7	23.125c	2.125a
P8	8.750c	3.500a
P9	10.375c	0.250a
P10	18.875c	2.875a
ARZM 05083	9.875c	0.750a
ARZM 07049	12.625c	2.875a
ARZM 13050	27.375c	2.375a
BOYA 462	36.500b	0.125a
BOZM 260	12.875c	0.000a
CHZM 13134	35.250b	0.750a
PARA 172	6.500c	0.250a
URUG 298 Amarelo	9.750c	2.875a
URUG 298 Roxo	21.375c	0.500a
IAC 125	12.250c	2.750a

*Means followed by common letters represent a statistically homogeneous group by the Scott-Knott algorithm.

That being said, the factor cultivation time significantly changed the rates of contamination of in rotten kernels, making the season effect remarkable. The first harvest, in general, favored the occurrence of rotten kernels for all studied genotypes and, therefore, all accessions evaluated showed values above the maximum tolerated limits for commercial classification, according to Normative Instruction no. 61 of the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA) of December 22, 2011. The second harvest significantly favored the health quality of the kernels, at a magnitude of 54.05% of the genotypes evaluated when compared with the first harvest. However, the values fitting the maximum commercial tolerance limits established by MAPA in 2011 do not necessarily apply to all genotypes that showed significant reductions of rotten kernels between the first and second harvests. For instance, lines L51, L52, and L75 showed significant reductions in the rotten-kernel incidence between periods; however, their lowest incidence values did not meet the minimum tolerance requirements established. However, at the second harvest, 54.05% of genotypes fitted type 01, with variations of 0% to 1.75% of rotten kernels;

21.62% fitted type 02, with variations of 2.12% to 2.87% of rotten kernels; and 16.21% of genotypes fitted group 03, with variations of 3% to 4.5% of rotten kernels (Table 3). It is known that the rotten kernels are a reflection of ear rots, caused mainly by fungi, still on the field. Initially, it was found that genotypes L65, L80, and IAC 125, which best stood out against ear rots, were also present in the group of genotypes most resistant to the incidence of kernel rot, and are thus promising to be used in breeding programs.

CONCLUSION

The adopted variables were efficient in detecting the existing variability for severity of *Fusarium* spp., allowing a reliable discrimination of superior accessions.

Genotypes L65, L80, and IAC 125 displayed the highest levels of resistance to incidence and severity of rot caused by *Fusarium* spp. and can thus be considered of interest for the introgression of resistance genes in popcorn breeding programs.

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