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Genetic variability affecting *Exserohilum turcicum* resistance in popcorn lines grown under high and low phosphorus conditions

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ABSTRACT. Northern leaf blight (NLB), caused by *Exserohilum turcicum*, is one of the main foliar diseases that affect popcorn culture. Farmers use many control measures to minimize damage caused by this disease, among which, the use of cultivars with genetic resistance is the most effective and economical. The aim of this study was to investigate genetic variability influencing resistance to NLB in 25 popcorn maize lines grown under high and low phosphorus conditions in relation to foliar fungal disease caused by *E. turcicum*. We evaluated the disease incidence and severity, by analysis of variance and cluster test (Scott-Knott). There was sufficient genetic variability between strains for resistance traits. Genotypic variance was higher than environmental variance, and had more discriminatory power. We conclude that new progenies could be selected for the establishment of future populations. P-7, P-9, L-59, L-71, and L-76 progenies possess promising characteristics that simultaneously reduce the severity and the incidence of NLB in popcorn plants.

Key words: Northern Leaf Blight; *Zea mays*; Disease resistance; *Exserohilum turcicum*; Scott-Knott cluster test; Genetic variability

INTRODUCTION

In Brazilian agribusiness, corn is ranked as the second highest cereal produced nationally, corresponding to 39.36% of the total grains produced in Brazil for the harvest of 2014/2015 (CONAB, 2015). Although most of the planted areas are cultivated with common corn, special maize cultivation is gaining space among producers, because of the differentiated market price, such as sweet corn and popcorn (Oliveira et al., 2016).

In this regard, popcorn (*Zea mays* L.) has received a lot of attention, primarily due to the emergence of the microwave oven in the 1940s, and to the number of consumers preparing it at home as a snack, thus increasing its consumption. Subsequently, with the "natural" foods era, there has been a further increase in popcorn consumption, which is considered to be a healthy energy food, and is rich in fibers, which promote good intestinal functioning. In addition, popcorn contains polyphenols, antioxidants, complex B vitamins, carbohydrates, manganese, and magnesium, and its routine ingestion can bring numerous health benefits (Paraginski et al., 2016).

However, popcorn breeding is disadvantaged owing to the narrow genetic base of the available germplasm, which leads to lower performance of agronomic traits of interest, including greater susceptibility to pests and diseases (Hallauer and Carena, 2009; Ribeiro et al., 2016; Vieira et al., 2016). Thus, assessment of genotypes in relation to their levels of disease resistance becomes even more important (Vieira et al., 2016).

Among the main foliar diseases that affect popcorn culture, northern leaf blight (NLB) is caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs (syn. *Helminthosporium turcicum* Pass.) stands out due to its relevance (Scapim et al., 2010; Wang et al., 2014; Hurni et al., 2015), wherein favorable climatic conditions and susceptible hosts cause damage that may vary between 27 and 90% of the total grain production (Pataky, 1992; Ward and Nowell, 1998; Ferguson and Carson, 2007, Wang et al., 2010; 2012; Ding et al., 2015; Ribeiro et al., 2016).

Many control measures aiming to minimize the damage caused by NLB disease have been used, including fungicide spraying, use of resistant varieties, crop rotation, use of adequate density and spacing, use of balanced fertilization, and elimination of crop residues (Payak and Sharma, 1985; Reuveni and Reuveni, 1998). Among these measures, the use of cultivars with genetic resistance is the most effective and economical method of control (Casela et al., 2006; Ferguson and Carson, 2007; Vieira et al., 2009; Ayiga-Aluba et al., 2015; Ding et al., 2015; Chen et al., 2016; Ribeiro et al., 2016).

In that regard, the evaluation of lines and hybrids has become necessary for the advancement of generations in breeding programs, where only the promising genotypes advance to subsequent stages. Thus, foliar disease resistance is an important criterion to be evaluated in relation to the generation of genotypes, and to measure its value (Gaytán-Bautista et al., 2009; Vieira et al., 2016).

In this context, gene banks are important for the assembly of genetic variability, which is needed to support plant-breeding programs aimed at obtaining superior genotypes (Quintal et al., 2012; Alves et al., 2014; Steffenson, 2016). Universidade Estadual do Norte Fluminense (UENF) possesses a popcorn gene bank with access to samples from different eco-geographical regions of Brazil and from other countries, which have been obtained through concessions and exchanges. These can be investigated for disease resistance, in order to identify sources of resistance to popcorn foliar diseases, for high productivity losses caused by leaf pathogens, and for the economic importance of culture.

Therefore, the aim of this study was to investigate genetic variability for NLB resistance in 25 popcorn maize lines grown under high and low phosphorus conditions in relation to foliar fungal disease caused by *E. turcicum* and to select for promising genotypes with genetic resistance that may be used in future popcorn breeding programs, in an attempt to generate new NLB resistance cultivars for farmers.

MATERIAL AND METHODS

Two experiments were conducted at Colégio Agrícola Estadual Antônio Sarlo, located in Campos dos Goytacazes, RJ, with the following geographical coordinates: 21º42'48"S, 41º20'38"O, and 14 m in altitude. The climate that characterizes the Campos dos Goytacazes municipality is classified as tropical (Aw), with hot summers and mild winters, with rainfall that tends to be concentrated in the summer months.

Before the experiment was performed, soil chemistry was analyzed to characterize phosphorus availability (high and low) in soil environments using samples collected at depths of 0-10 and 10-20 cm in rows and interspersed between lines forming a sample from 10 sub-samples as shown in Table 1. The results in Table 1 showed that the soil was characterized as low phosphorus level.

Table 1. Soil chemistry and particle size analysis of samples from Campos dos Goytacazes.

Location	pH	P	K	Ca	Mg	Al	Na	C	OM	CEC	BS	V	Clay
	H_2O	mg/dm^3	mmolc/dm^3					g/dm^3		mmolc/dm^3		%	g/dm^3
Campos	6.1	8	3.7	16.6	11.7	0	1.5	11.8	20.34	57.2	36.2	63	305

We evaluated 25 S_7 popcorn lines from UENF gene bank in two environments contrasting in phosphorus availability: one with low phosphorus availability and the other with optimal phosphorus availability. Five of these lines were from the 'Viçosa' population, three were from the 'Beija-Flor' population, seven from the 'BRS-Ângela' population, and 10 populations were part of the 'UENF' program, as described in Table 2.

The experiment was a randomized block design with four replicates. Sowing was performed in conventional tillage. Each plot consisted of a 5-m line, spaced 0.2 m between plants, and 0.9 m between rows, totaling 25 plants per plot.

Table 2. S_7 popcorn lines, their respective genealogies, and climate adaptation.

Line	Population origin	Climate adaptation
L53	Beija-flor: UFV	Temperate/ Tropical
L54	Beija-flor: UFV	Temperate/ Tropical
L59	Beija-flor: UFV	Temperate/ Tropical
L61	BRS Angela: EMBRAPA	Tropical
L63	BRS Angela: EMBRAPA	Tropical
L65	BRS Angela: EMBRAPA	Tropical
L66	BRS Angela: EMBRAPA	Tropical
L69	BRS Angela: EMBRAPA	Tropical
L70	BRS Angela: EMBRAPA	Tropical
L71	BRS Angela: EMBRAPA	Tropical
L75	Viçosa: UFV	Temperate/ Tropical
L76	Viçosa: UFV	Temperate/ Tropical
L77	Viçosa: UFV	Temperate/ Tropical
L80	Viçosa: UFV	Temperate/ Tropical
L88	Viçosa: UFV	Temperate / Tropical
P1	Triple Hybrid Zélia	Temperate/ Tropical
P2	Compound CMS-42	Temperate/ Tropical
P3	Compound CMS-42	Temperate/ Tropical
P4	South American Races	Temperate/ Tropical
P5	Triple Hybrid Zaeli	Temperate/ Tropical
P6	Triple Hybrid Zaeli	Temperate/ Tropical
P7	Triple Hybrid Zaeli	Temperate/ Tropical
P8	IAC-112 Hybrid	Temperate/ Tropical
P9	IAC-112 Hybrid	Temperate/ Tropical
P10	IAC-112 Hybrid	Temperate/ Tropical

Fertilization for optimal phosphorus availability consisted of 30 kg/ha N, 70 kg/ha P_2O_5, and 40 kg/ha K_2O. In the environment with low phosphorus availability, fertilization consisted of 30 kg/ha N, 0 kg/ha P_2O_5, and 40 kg/ha K_2O. Topdressing was performed in both environments at a dose of 100 kg/ha N, when the plants reached the V6 stage. The supplementation of primary macronutrients was obtained based on the fertilization recommendation for popcorn culture in both environments, considering the amount of nutrients in the soil at the 0 to 20 cm layer provided by the chemical analysis. The low phosphorus environment received no supplementation. Other cultural practices were performed in accordance with the recommendations for culture in the region. Experiments received supplemental irrigation where necessary to prevent water stress.

Genotype performance in relation to leaf diseases was monitored by estimating the incidence and severity of symptoms, for which two estimation methods were adopted: i) assessment of the incidence of disease symptoms throughout the plant, expressed as a percentage, o; and ii) the quantification of severity of the symptoms along the leaf immediately below the first spike, expressed as a percentage. Foliar diseases manifest through natural infection with causal agents in the field. The evaluations were performed by taking six competitive plants per plot, and assessments were performed every 7 days from flowering to senescence.

The incidence of NLB symptoms on the plants was estimated with the aid of a diagrammatic scale, as adopted by Agroceres (1996). The scale has a range of 1 to 9, wherein 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 on the leaf, we used six competitive plants per plot, and the assessments were performed every 7 days from flowering to senescence. Therefore, we used the diagrammatic scale proposed by Vieira et al. (2014), containing the severity ranges in percentage corresponding to 0.5, 1.6, 5.0, 15.0, 37.0, 66.0, 87.0, and 96.0%, through which the percentage of the lesioned area was estimated.

Analysis of individual variance and estimates of genetic parameters for environments with high and low phosphorus were performed for the two variables. When the mean effect was significant, we performed an average cluster test (Scott-Knott) at 5% probability. In addition, the line graphic dispersion was carried out from the severity and incidence averages deviations of each line compared to the average of these variables in each environment where they were plotted in a scatter plot in which the x-axis represents the high phosphorus deviations, and the ordinate axis represents low phosphorus deviations. Thus, the lines were divided into four quadrants depending on their performance and resistance in contrasting environments for phosphorus availability. For a more comprehensive analysis of the results, a joint analysis of variance was performed for the two environments. All statistical procedures were performed in the GENES Program (Cruz, 2013).

RESULTS AND DISCUSSION

Analysis of variance (Table 3) showed that for the source of variation "treatments", was significant at a 1% level of probability by the F test, confirming that the genotypes showed different performances for the evaluated characteristics.

Table 3. Analysis of variance and estimated genetic parameters for *Exserohilum turcicum* incidence and severity in popcorn lines under high and low phosphorus levels.

SV	d.f.	*E. turcicum* incidence		*E. turcicum* severity	
		High P	Low P	High P	Low P
Blocks	3	55.57	119.93	103.11	60.19
Treatment	24	491.61**	672.13**	917.07**	1085.33**
Error	72	60.52	59.99	104.39	64.88
Average estimates and genetic parameters					
Genotypic variance		107.77	153.04	203.17	255.11
Environmental variance		15.13	15.00	26.10	16.22
Phenotypic variance		122.90	168.03	229.27	271.33
Heritability (%)		87.69	91.08	88.62	94.02
CVg/CVe		1.33	1.60	1.40	1.98
CVe (%)[a]		11.38	11.17	15.88	12.02
CVg(%)[b]		15.19	17.83	22.16	23.84
Means		68.35	69.37	64.33	67.01

**Significant at 0.01 probability by the F test. [a]Coefficients of experimental variation; [b]Coefficients of genetic variation.

Table 3 shows that the magnitude of genotypic variance increased from high to low phosphorus for both NLB incidence based on plant and for leaf, with values very close to those for phenotypic variance. This result reveals the highly heritable characteristics in this study, where most of the total variation was due to the genotype and not the environment. This result was confirmed by the low values for environmental variance obtained in this experiment.

These results show that high heritability values were observed, which ranged from 87.69 to 94.02%. These high heritability magnitudes indicate that much of the phenotypic variance observed in the studied lines is genetically controlled. These results confirm that the disease resistance of lines can be easily explored in future crosses through simple breeding methods. Similar results were observed by Chen et al. (2016), who evaluated the severity and size of the lesion caused by *E. turcicum* in 207 lines of maize, using pathogen inoculation.

Those authors obtained heritability values ranging from 83 to 87%, consistent with the values achieved in the present study.

Table 3 shows that the experiment was conducted with good precision since the experimental variation coefficient ranged from 15.19 to 23.84%. Other authors obtained higher values for this parameter. Vieira et al. (2009) assessed *E. turcicum* resistance in 10 popcorn hybrids, and obtained 31.87% CV for incidence and 35.41% for disease severity, showing that the experiment generated an acceptable error for the study of this feature.

When we analyze this information with genotypic variation coefficients and heritability (Table 3), this character is observed to have low coefficients of genetic variation (CVg) and coefficients of experimental variation (CVe), and high heritability, indicating that it is little influenced by the environment, as reported by Granato et al. (2016) for nutritional stress and Ahmad et al. (2016) for water deficiency. The high heritability coefficients observed are due to the genetic variability for the characteristics in the population studied.

The relationship between CVg and CVe presented values above unity, indicating that the selection may be effective. Vencovsky and Barriga (1992) concluded that when this relationship is equal or greater than the unity, it may be possible to obtain a representative genetic gain in breeding.

Table 4 shows the average values for incidence in popcorn lines grown in soil containing high and low levels of phosphorus, based on the severity of NLB in the plant and leaves.

Table 4. Average incidence of symptoms based on plant and leaf severity of *Exserohilum turcicum*, evaluated in popcorn lines grown under high and low levels of phosphorus.

Lines	*E. turcicum* incidence				*E. turcicum* severity			
	High P		Low P		High P		Low P	
L-53	66.67	B*	64.72	C	77.05	B	65.95	C
L-54	88.06	A	94.45	A	90.43	A	96.45	A
L-55	88.89	A	93.89	A	89.53	A	97.03	A
L-59	62.50	C	52.78	D	48.95	D	47.72	D
L-61	71.67	B	69.17	C	59.93	C	63.75	C
L-63	86.11	A	91.67	A	85.78	A	87.93	B
L-65	81.94	A	81.12	B	90.05	A	87.08	B
L-66	58.89	C	64.45	C	55.15	C	59.85	C
L-69	71.67	B	72.78	C	59.98	C	68.50	C
L-70	81.11	A	83.06	B	65.95	C	82.93	B
L-71	55.56	C	58.89	D	53.08	D	47.85	D
L-76	66.04	B	47.50	D	44.63	D	33.70	E
L-77	84.17	A	81.11	B	83.43	A	81.03	B
L-80	58.33	C	63.06	C	56.65	C	69.68	C
L-88	60.00	C	64.17	C	52.85	D	65.45	C
P-1	70.56	B	83.34	B	70.98	B	83.68	B
P-2	71.39	B	74.45	B	74.00	B	77.58	B
P-3	60.83	C	62.50	C	52.68	D	55.73	C
P-4	65.83	B	69.17	C	66.60	C	65.73	C
P-5	56.95	C	60.28	C	48.60	D	57.15	C
P-6	68.33	B	64.72	C	73.05	B	65.08	C
P-7	56.95	C	53.61	D	42.20	D	46.20	D
P-8	66.11	B	63.34	C	59.18	C	59.52	C
P-9	50.28	C	56.67	D	45.70	D	46.90	D
P-10	60.00	C	63.34	C	61.93	C	62.78	C
Average	68.35		69.37		64.33		67.01	

*For the same variable, means followed by the same letter consist of a homogeneous group based on the Scott-Knott test at 5% probability.

Three groups were formed for the incidence of *E. turcicum* at high phosphorus. Group I possessed the highest average and contained six lines L-54, L-55, L-63, L-65, L-70, and L-77. Group II contained genotypes with intermediate means for disease incidence and included nine lines, which are as follows: L-53, L-61, L-69, L-76, P-1, P-2, P-4, P-6, and P-8. All other lines comprised Group III, which had lower averages and contained 10 cultivars. In the latter group, the lines P-5, P-7, P-9, and L-71 stand out with the lowest average incidence of NLB.

For the incidence of *E. turcicum* in the low P environment, four distinct groups were formed. Group I with lines L-54, L-55, and L-63, which possess the highest average. Group IV with lines showing the lowest average: L-59, L-71, L-76, P-7, and P-9. All other lines showed intermediate means for NLB incidence and were grouped into Groups II and III (Table 4).

Regarding the *E. turcicum* severity, four groups were formed with high P levels. Group I was formed by L-54, L-55, L-63, L-65, and L-77 and comprised the genotypes with a higher average. In relation to the lowest averages, we can highlight the L-59, L-71, L-76, L-88, P-3, P-5, P-7, and P-9 lines, included in group IV. The other lines composed the groups II and III, with intermediate means of *E. turcicum* severity under high P conditions.

Five groups were formed based on the values obtained for *E. turcicum* severity under low P conditions. Group V, which had the lowest average, was composed only by the L-76 line. Group IV grouped the G-59, L-71, L-76, P-7, and P-9 lines, which showed low means for disease severity. With the higher means, the L-54 and L-55 lines formed Group I. The other genotypes were in Groups II and III, representing intermediate values for *E. turcicum* severity in low P.

In general, the lines that showed higher susceptibility were L-54 and L-55, which always grouped in Group I, i.e., the group with the highest average NLB incidence and severity under high and low P conditions. Other lines highlighted for their susceptibility were L-63, L-65, and L-77. Moreover, the lines L-59, L-71, L-76, P-7, and P-9 had a good level of resistance because they possess the lowest average for disease incidence and severity in high and low P environments.

Overall, we verified that the high P condition resulted in lower NLB incidence (based on the plant) leaf severity average. This is because plants with a better nutritional balance are more efficient at tolerating, or even at resisting attack from pests and diseases, which is known as the Trofobiose Theory (Chaboussou, 1987) and it consists mainly of the principles of organic and biodynamic agricultural approaches.

Many studies have demonstrated the effects of mineral nutrition on growth and productivity, with emphasis on the role of nutrients in plant metabolism (Huber and Arny, 1985; Perrenoud, 1990; Marschner, 1995). However, mineral nutrition can also have a secondary effect on the resistance of plants to pests and diseases (Marschner, 1995). According to Silveira and Higashi (2003), nutritional deficiencies and imbalances lead to morphological and biochemical changes in plants, and can make certain genetic materials more susceptible to pathogen infections. Therefore, the efficient use of fertilizers combined with the resistance of genetic material can reduce the level of disease severity and incidence.

Figure 1 shows the distribution of 25 popcorn lines in different quadrants of a Cartesian plane, considering the deviation in the average incidence and severity of each line, in relation to the average of these variables in the environment containing high and low levels of phosphorus, which forms four groups. It is noted that the genotypes with high susceptibility, L-54, L-55, L-63, L-65, L-70, and L-77, formed Group I, which was distinct in the upper right quadrant as the group with the highest average for disease incidence and severity. Close to these genotypes is Group II, composed of the P-1 and P-2 lines, which also exhibited high mean values for NLB occurrence.

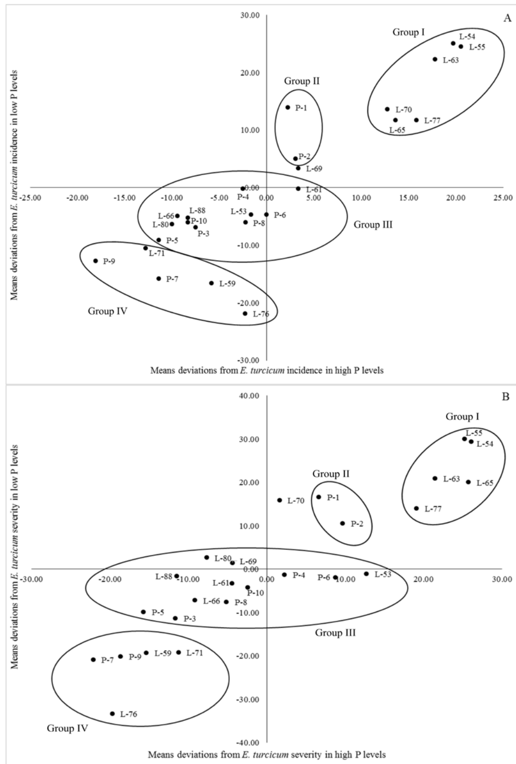

Figure 1. Distribution of 25 popcorn lines in different quadrants of a Cartesian plane: northern leaf blight (NLB) (*Exserohilum turcicum*) incidence (**A**) based on plant and (**B**) leaf severity.

Group IV was formed by lines with the highest resistance levels, L-59, L-71, L-76, P-7, and P-9. Although in general, the lines behave better in environments with ideal P conditions, it is clear that genetic resistance is crucial in response to the pathogen. It is clear that the resistant genotypes performed better in both evaluations.

The other genotypes were placed in Group III, which included those that expressed intermediate averages, and are considered to have intermediate resistance.

Figure 1 shows that the distribution of lines was wide in the Cartesian plane. This is due to the genetic variability between genotypes for *E. turcicum* resistance. Plant breeding is only possible if there is genetic variability. Therefore, with respect to NLB resistance, it can be inferred that there is genetic variability in popcorn lines grown under conditions of high and low phosphorus.

We also highlight in Table 3, that the ratio between the largest and smallest mean square residue was equal to 1.74. As the value obtained was less than 7, this confirms that there is homogeneity in residual variance (Pimentel-Gomes, 1990; Cruz, 2005), which enabled the joint analysis of variance to be determined (Table 5). The joint analysis of variance indicated that the lines differed significantly ($P < 0.01$) in terms of *E. turcicum* incidence and severity, confirming the existence of genetic variability for disease resistance (Table 5).

Table 5. Joint analysis of variance and estimates of genetic parameters for *Exserohilum turcicum* incidence and severity in popcorn lines grown under high and low phosphorus levels.

Source of variation	d.f.	*E. turcicum* incidence	*E. turcicum* severity
Blocks/environment	6	87.750	81.648
Blocks	3	92.134	155.902
Blocks x environment	3	83.367	7.394
Treatment	24	18.073**	22.410**
Environments	1	0.586^{ns}	4.383^{ns}
Treatments x environments	24	1.241^{ns}	1.250^{ns}
Error	144	60.254	84.633
Average		68.86	65.67
CV(%)		11.27	14.01
Genotypic quadratic component		128.59	226.50
Quadratic component G x E		3.63	5.29
Residual Variance		60.25	84.63
Genotypic determination coefficient (%)		94.47	95.54
Intraclass correlation		68.09	72.80
Genetic variation coefficient (CVg%)		16.47	22.92
CVg/CVe ratio		1.46	1.64

^{ns,}**Not significant and significant by the F test at 0.01 probability, respectively.

The lack of significance of the G x E interaction indicates that the evaluated lines did not have a distinct behavior in both environment. The coefficient of genotypic determination is a parameter related to heritability; however, it allows inferences about genotypes (fixed effects) and not of the population (random effect) in terms of heritability (Vasconcelos et al., 2012). The coefficient of genotypic determination was 94.47% for *E. turcicum* incidence, and 95.54% for *E. turcicum* severity (Table 5). This indicates that approximately 95% of the observed variation has a genetic origin.

The coefficient of genetic variation was 16.47% for *E. turcicum* incidence, and 22.92% for *E. turcicum* severity (Table 5). This coefficient expressed, as a percentage, the genetic fraction of the experiment average. These data show the ratio between the genotypic variation coefficient and the environmental variance was greater than unity for the characters studied (Table 5). The ratio of CVg and CVe shows the proportion of the total variance that is explained by genotype (Vasconcelos et al., 2012). These results suggest that NLB resistance can be selected for and can lead to genetic gain for this feature.

The intraclass correlation coefficient ranged from 68.09 to 72.80, for *E. turcicum* incidence and severity, respectively. These results confirm good experimental precision, which was confirmed by the CVs obtained. Values of repeatability between 40% and 75%, as noted, are considered satisfactory (Table 5) (Cruz et al., 2004).

In the present study, we show that there was variability for all characteristics studied. We observed that the genotype variances were higher than the environmental variances, and thus possess more discriminatory power. Therefore, it is possible to select for new progenies for the establishment of future populations. The P-7, P-9, L-59, L-71, and L-76 progenies are promising due to their characteristics that simultaneously reduce *E. turcicum* severity and incidence in popcorn plants.

Conflicts of interest

The authors declare no conflict of interest.

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