

# Selection of sweet corn genotypes for resistance to Northern Leaf Blight using longitudinal analysis

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## Abstract

Northern Leaf Blight (NLB) of maize (*Zea mays*) is one of the most common maize diseases. The economic damage it causes is frequently linked to inadequate crop management and susceptible cultivars. To select sweet corn genotypes with a higher level of resistance to NLB, 18 single-cross hybrids, six populations, four interpopulation hybrids, and two controls were evaluated in two field trials. Genotypes were evaluated three times, and results were analyzed according to linear mixed models and repeated measures over time, using days to silk as a covariate. The entry effect was sliced into single-crosses, populations, controls and interclasses. Single-crosses were sliced in line, tester, and line by tester. Results indicate higher ear yield (EY) and greater severity in early genotypes, thereby validating the use of covariates. In all analyses, the selection accuracy was high, above 0.86. For NLB, population and simple hybrid means varied from one environment to another, while simple hybrids were always higher for yield. In the joint analysis of NLB, there was a significant difference between Entry and Entry x Location, Line x Location, Control x Location, and Interclass x Location interactions. For yield, only Entry, Single-Cross, Population, Interclass, Entry x Location, Single-Cross x Location, and Interclass x Location effects were significant. The P8HS population, and the interpopulation and simple hybrids from this population, showed less severity in the joint analysis. As a result, the breeding program should focus on lines derived from the P8HS population to select genotypes more resistant to NLB and with high EY.

## Abbreviations

BLUE – Best Linear Unbiased Estimation  
 BLUP – Best Linear Unbiased Prediction  
 C8HS – SH2-8HS-CIMMYT sweet corn population  
 CGZ – Campos dos Goytacazes-RJ/Brazil  
 CSH – SH2-CIMMYT8 sweet corn population  
 CV – coefficient of variation  
 DTS – days to silk  
 EY – ear yield  
 GxE – genotype x environment  
 ITA – Itaocara-RJ/Brazil, municipalitie

MSe – mean square error  
 MSg – mean square genotypes  
 NLB – northern leaf blight  
 P8HS – SH2-8HS-Piranão sweet corn population  
 PSH – SH2-Piranão sweet corn population  
 RRS – reciprocal recurrent selection  
 SC – single-crosses  
 SE – standard error  
 sh2 - shrunken-2 gene

## Introduction

Northern leaf blight of maize (NLB), caused by the fungus *Exserohilum turcicum* (Pass) K.J. Leonard & Suggs, is one of the most prevalent disease of maize. In its most common manifestation, this leaf disease leads to elliptical and elongated leaf lesions, grayish green in color, with lengths ranging from 2.5 to 15 centimeters (Kimati et al. 2005). It has high genetic variability (Bor-

hardt et al. 1998; Ferguson and Carson, 2004, 2007), although little variation in pathogenicity (De Rossi 2015).

The infectious phase starts in the oldest leaves after silking. The infections prior to this are linked to genotypes that are more sensitive and subject to yield loss (Pataky 1992).

In the United States and Canada, the estimated yield

**Table 1 - Correlation between traits evaluated in 30 sweet corn genotypes and four repetitions (120 observations) in two locations, Itaocara and Campos dos Goytacazes, North of Rio de Janeiro State, Brazil.**

	CGZ_NLB	CGZ_DTS	CGZ_EY	ITA_NLB	ITA_DTS
CGZ_DTS	-0.42**				
CGZ_EY	0.19*	-0.35**			
ITA_NLB	0.09	0.06	-0.2		
ITA_DTS	-0.48**	0.54**	-0.35**	-0.01	
ITA_EY	0.36**	-0.43**	0.55**	-0.10	-0.52**

CGZ – Campos dos Goytacazes; ITA – Itaocara; NLB – Northern Leaf Blight; DTS – Days to Silk; EY – Ear Yield; \* $p < 0.05$ ; \*\* $p < 0.01$ .

losses rose from 1.9 million tons in 2012 to 14 million tons in 2015, causing an estimated economic loss of \$1.9 billion in 2015 (Mueller et al. 2016). The primary means of controlling *E. turcicum* is by using resistant cultivars, with no consensus on which is the most important gene action, additive or non-additive (Carson 1995; Vivek et al. 2010).

A study conducted with a sweet corn genotype concluded that *E. turcicum* is a "low sugar fungus" and that the development of *E. turcicum* can even be suppressed with the application of sucrose to the leaves (Levy and Cohen, 1984). However, no reference was made to the higher NLB resistance of sweet corn compared to common corn. The reason for this is that the increased sugar in sweet corn is related to the grain not the leaves, due to the failure to convert sucrose into starch (Tracy 2001).

In an attempt to select productive sweet corn genotypes resistant to foliar diseases, the Sweet Corn Breeding Program of the Universidade Estadual do Norte Fluminense (UENF) uses as genetic basis reciprocal populations of common corn, improved via reciprocal recurrent selection of full-sib (RRS) and the insertion of the shrunken-mutant gene ( $sh_2$ ) via backcrossing (Pereira et al. 2019). The genotypes carrying the  $sh_2$  gene are known as "super sweet", and are widely cultivated in Brazil mainly from the most commercialized cultivar Tropical Plus, from the company Syngenta. One of the advantages of the  $sh_2$  type is the longer post-harvest quality (Lertrat and Pulam, 2007), in addition to a longer harvest period without loss of quality (Tracy 2001).

The estimative of the level of resistance of a genotype to a given disease in comparison to other genotypes is performed by incidence (percentage of infected plants) (Spolti et al. 2015) or by severity (percentage of infected plant tissue), where the most commonly used methods are those that apply descriptive, rating, or diagrammatic scales (Cooke et al. 2006).

This study aimed to estimate, for selection purposes, the severity level of *E. turcicum* on single-cross hybrids and super sweet corn populations using repeated measures analysis over time (Probst et al. 2016; Bowers and Locke, 2004), including days to silk as covariate and modeling the error throughout the time, aiming to increase the results reliability. As a secondary objective, it aimed to verify the influence of the  $sh_2$  mutant gene on NLB severity in near-isogenic maize genotypes, hence the feasibility of selecting common corn genotypes for NLB before backcrossing.

**Table 2 - Statistic-genetic parameters estimated in 30 sweet corn genotypes in two locations, Itaocara and Campos dos Goytacazes, North of Rio de Janeiro State, Brazil.**

Parameters		Northern Leaf Blight			Ear Yield (t ha <sup>-1</sup> )		
		CGZ	ITA	Joint	CGZ	ITA	Joint
Entry	Mean	1.790	1.624	1.707	10.28	11.25	10.76
	Vg	0.030	1.41E-08	1.6E-07	4.159	9.817	5.2883
	rgg	0.94	0.86	0.93	0.95	0.95	0.97
Single-Crosses	Mean	1.868	1.584	1.687	10.93	12.743	11.89
	Vg	0.048	0.005	2.0E-08	6.086	16.121	9.876
	rgg	0.96	0.94	0.93	0.96	0.97	0.98
Populations	Mean	1.632	1.802	1.695	9.07	8.772	8.82
	Vg	5.0E-07	3.2E-08	1.4 E-08	5.0457	22.956	12.542
	rgg	0.86	0.86	0.93	0.96	0.98	0.98
UENF 506 11 (mean)		1.76	1.98	1.86	9.62	10.01	9.84
Tropical Plus (mean)		2.46	1.51	2.01	11.24	10.31	10.83
CV (%)		8.24	3.94	6.27	14.264	20.055	17.609

Vg – genetic variance; rgg – selective accuracy; CV – Coefficient of Variation; CGZ – Campos dos Goytacazes; ITA – Itaocara; Joint – Joint analysis

**Table 3 - Likelihood Ratio Test (random effects) and Ward test (fixed effects) for the main effects and unfolded effects of two trials performed in two locations, evaluating Northern Leaf Blight and Ear Yield in 27 sweet corn and 3 field corn genotypes.**

Source	DF	Deviance/ Ward	
		NLB	EY
Days to Silk (covariate)	1	17.7***	-
Block(Loc) (F)	6	29.347**	6.347
Loc (F)	1	2033.19***	16.240***
Measures (F)	2	591.61***	-
Measures x Entry (F)	58	175.077***	-
Measures x Loc (F)	2	21.059**	-
Measures x Entry x Loc (F)	58	48.282	-
Entry (F)	29	71.087**	414.202***
Single-crosses (R)	17	6.897e-06	13.036***
Line (R)	5	0.36492	0.025
Tester (R)	2	-1.373e-05	3.109
Line x Tester (R)	10	-1.690e-06	0.533
Populations (R)	9	-8.398e-07	9.480**
Control (F)	1	18.100	0.792
Interclasses (F)	2	5.1775	68.688***
Entry x Loc (F)	29	21.059**	83.029***
Single-crosses x Loc (R)	17	-3.826e-06	3.975*
Line x Loc (R)	5	4.01639*	0.105
Tester x Loc (R)	2	-8.782e-06	0.416
Line x Tester x Loc (R)	10	-4.375e-07	1.494
Populations x Loc (R)	9	-1.973e-06	1.429
Control x Loc (R)	1	6.342*	0.891
Interclasses x Loc (F)	2	12.357**	9.568**
Residual error variance		0.0361	3.599
Measurement error		0.0473	-

(R) – Random effect; (F) – Fixed Effect; DF- Degree of Freedom; Loc – Location; \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\*

## Material and methods

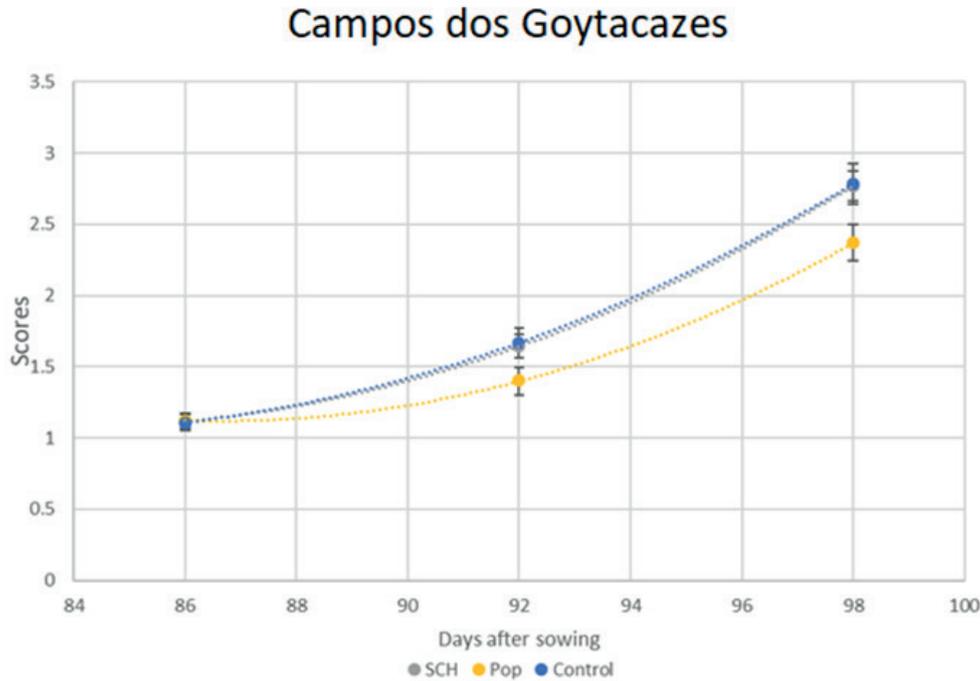
### Genotypes

Thirty genotypes divided into three groups were evaluated: 18 single-crosses (SC); ten broad-genetic based genotypes (six populations and four interpopulation hybrids); and two controls (one sweet corn single-cross hybrid and one field corn interpopulation hybrid). Among populations, two are of field corn that belong to different heterotic groups, the Piranão8 population in the dent group and the CIMMYT8 population in the flint group, in the eighth cycle of reciprocal recurrent selection (RRS). One of the genotypes in the control groups is an interpopulation hybrid cultivar of field corn named UENF 506-11, which originated from the F1 cross between Piranão11 and CIMMYT11 populations already in the 11<sup>th</sup> RRS, and a super sweet single-

cross hybrid cultivar ( $sh_2$ ) from the Syngenta company, Tropical Plus.

The super sweet populations originated from recurrent populations of Piranão8 and CIMMYT8, which were backcrossed with two populations carrying the shrunken-2 ( $sh_2$ ) mutant gene (SH2 and SH28HS populations), resulting in the generation of the following four super sweet corn populations: SH2-CIMMYT8 (CSH), SH2-8HS-CIMMYT (C8HS), SH2-Piranão (PSH), and SH2-8HS-Piranão (P8HS). Intersubpopulation hybrids were derived by crossing super sweet corn populations of distinct heterotic groups (CSH x PSH; CSH x P8HS; C8HS x PSH; C8HS x P8HS).

The 18 SC were obtained from crosses between three homozygotic testers deriving from the CSH population (LCHS113; LCHS116; LCHS119) and six inbred



**Fig. 1 - Disease progress curve of three groups of sweet corn genotypes: Single-Cross Hybrids (SCH); Populations (Pop) and Control, evaluated in Campos dos Goytacazes, North of Rio de Janeiro State, Brazil.**

lines, three originating from the P8HS population and three from the PSH population (LP8HS125; LP8HS129; LP8HS130; LPSH134; LPSH139; LPSH140).

#### Experimental design

According to the severity scale of *Exserohilum turcicum* developed by AGROCERES (1996), in which severity scores are obtained based on the whole plant, and with scores ranging from 1 to 9, the 30 genotypes were evaluated in the 2016/2017 crop season, in two experiments in a randomized complete block design and four replicates. The experimental units consisted of a row 3 m long, 0.90m between rows, and 0.30 m between plants. Evaluations were initiated 9 to 23 days after silking (female flowering), depending on the genotype, and performed at an average interval of 7 days on three occasions.

The trials were sown on August 3, 2017 in Itaocara (latitude 21°40'09" S and longitude 42°04'34" W, 60 m altitude) and August 22, 2017 in Campos dos Goytacazes (latitude 21°24'48" S and longitude 41°44'48" W, 14 m altitude), both located in the northern part of Rio de Janeiro State. The climate classification – according to Köppen classification – is Aw (tropical climate with summer rains) and, as the environments are close to the coast, at approximately -21° latitude, the climate is controlled by equatorial and tropical air masses influenced by the tropical marine mass.

The evaluations were initiated on October 25, 2017 and November 16, 2017 in Itaocara and Campos dos Goytacazes, respectively.

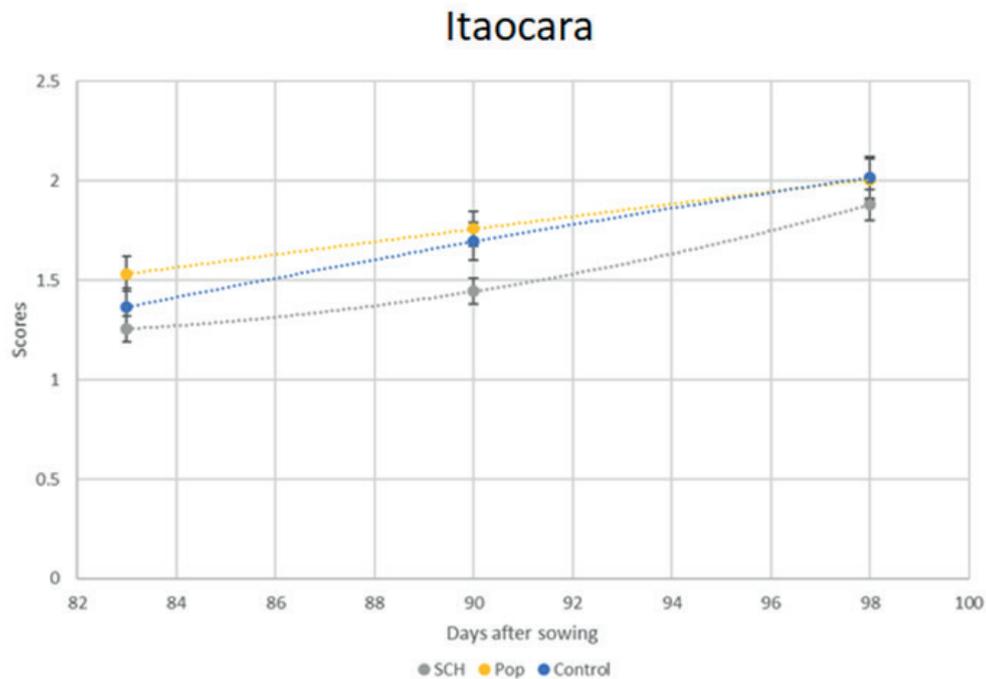
#### Statistical Analyses

Three observations of *E. turcicum* severity were analyzed using restricted maximum likelihood analysis and repeated measures over time. A repeated-measures analysis is defined by the variation between experimental units with respect to the number and range of observations and by time-dependent covariates (Davis 2002). Therefore,  $y_i = (y_{i1}, y_{i2}, y_{i3})$  is considered to be the response vector of the measures and the generalized linear model is:

$$y = Xb + e$$

where  $y$  = observation vector;  $b$  = vector of fixed block effects, locations, measurements and genotype classes;  $e$  = vector of random residuals modeled using the autoregressive spatial structure; and  $X$  is the incidence matrices of fixed effects.

Entry effect and location by entry effect were sliced in several effects considering the different kinds of entries. In this way, the 29 degrees of freedom (DF) for entry were sliced in 17 DF to compare single-crosses, 9 DF for populations, 1DF to compare controls and 2 DF to compare interclasses. Single-cross effects were a



**Fig. 2 - Disease progress curve of three groups of sweet corn genotypes: Single-Cross Hybrids (SCH); Populations (Pop) and Control, evaluated in Itaocara, North of Rio de Janeiro State, Brazil**

random effect and it was also sliced in line, tester, and line by tester effects, all of them considered as random effects.

To consider the repeated measurements, a first-order autoregressive ( $\sigma_{ij} = \sigma^2\rho^{|i-j|}$ ) covariance structure was fitted, given the lowest value observed in Akaike model selection criteria when compared with unstructured and antedependence models. The first-order autoregressive covariance structure is also justified in the repeated-measures analyses in linear models, when the intervals between measures are the same and there is a correlation between measures because of permanent (plot) effects (Mrode 2014).

As disease onset in maize usually occurs after flowering, the covariate of days to silk (DTS) was added to improve the accuracy of the model and adjust the predicted/estimated values. The observed values of DTS were centered ( $S_{ijk} - S_{\mu}$ ) according to the recommendation of the ASREML statistical package (Gilmour *et al.* 2015). The significance of fixed effects was achieved by the Wald method, while the significance of random effects was determined by the likelihood ratio test (Gilmour *et al.* 2015).

Based on the BLUPs (Best Linear Unbiased Predictions) of the SC and Populations, and the BLUEs (Best Linear Unbiased Estimations) of the Controls, a second-

degree polynomial curve of the disease progress was generated, and, based on the BLUE of the Entry, a bar graph was made with the genotype means per environment and across environments.

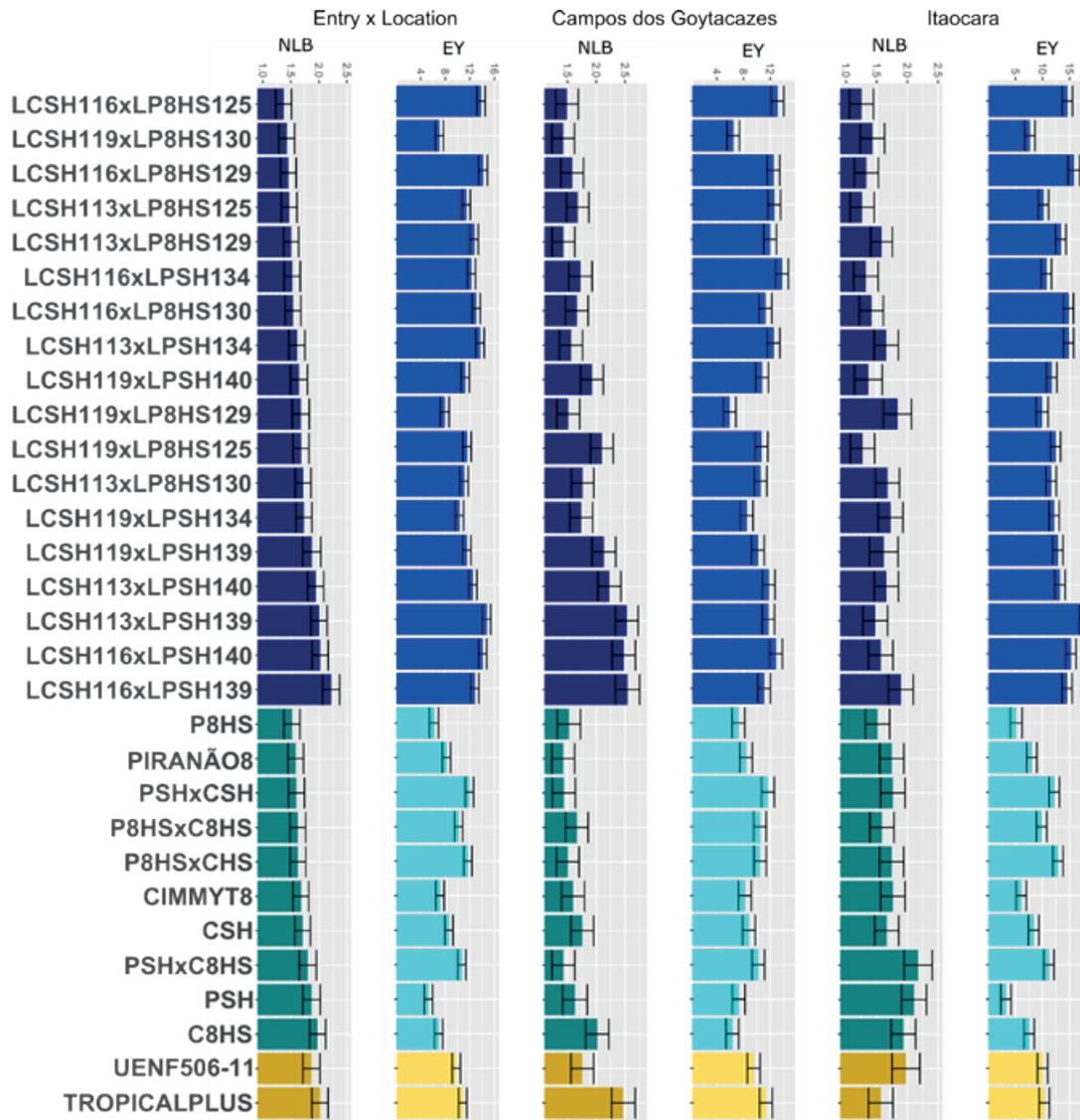
As additional information, the unhusked ear yield, evaluated at kernel milk stage (R3), about 20 days after silking, was analyzed following the same unfolded effects, except for the repeated measures, given that the yield analysis was performed with a single measure. According to Guan *et al.* (2013), about 20 days after silking fresh corn kernels have larger size and weight.

To understand the relation that the variables have with each other, we estimated the level of Pearson's phenotypic correlation between NLB and EY variables and DTS covariable. This correlation was calculated from raw data of EY and DTS and estimated data of NBL (mean of measures of repeated measure analysis).

The analyses were conducted in the R environment, using the "ASREML 3.0" package for model adjustment, and the "Hmisc" package (Harrell Jr 2019) for estimating the correlation between the traits and the significance level of the correlation coefficient.

#### **Estimation of statistical genetic parameters**

The statistical genetic parameters were estimated for the effects of SC, Population, and Entry. Both the coef-



**Fig. 3 - Best Linear Unbiased Estimator of Northern Leaf Blight (NLB, Score) and Ear Yield (EY, t ha<sup>-1</sup>) of 27 sweet corn and three field corn genotypes. The colors were used to differentiate single-crosses, populations and control; bars represent the mean of each genotypes with the standard error.**

ficient of variation,  $((SE) / (\mu) \times 100)$ , in which SE is the standard error and  $\mu$  the overall mean, and the selective accuracy  $(1 - MS_e / MS_g)^{0.5}$ , in which  $MS_g$  is the mean square genotypes and  $MS_e$  is the mean square error, were evaluated. Regarding the analyses carried out for disease evaluation (repeated measures), as there was an error related to them, the SE was considered using the SE mean of the genotype means.

**Results and Discussion**

**Phenotypic correlation**

The variable and covariable unhusked ear yield (EY) and days to silk (DTS), showed a moderate and signi-

ficant correlation between the two environments ( $r = 0.55$  and  $r = 0.54$ , respectively), while the NLB variable showed no correlation between the environments, that is, Itaocara (ITA) and Campos dos Goytacazes (CGZ) ( $r = 0.09$ ).

When evaluated in CGZ, the correlation between NLB and DTS was stronger ( $r = -0.42$ ) than the correlation estimated in ITA ( $r = -0.01$ ). The result in CGZ suggests that, with higher precocity, NLB severity tends to be higher, which is expected, as diseases usually start at a more advanced stage of plant development and worsen in senescence. This is not a sign they are more susceptible to NLB, as they are being evaluated at dif-

ferent stages of development. On the other hand, it is an indication that the covariable DTS is important in improving this type of analysis. Argillier *et al.* (1994) related yield with silking date, depending of the location. Other authors related the relationship between NLB and DTS (Aziz *et al.* 1992; Kumar *et al.* 2018)

This negative correlation is supported by the studies of Poland *et al.* (2011), who achieved a higher correlation between DTS and NLB ( $r = -0.59$ ), while Balint-Kurt *et al.* (2010) noticed low correlations in all environments evaluated, although with the same negative sign.

The correlation between EY and DTS was  $-0.52$  in ITA, higher than the  $-0.35$  observed in CGZ (Table 1). The negative correlations between EY and DTS point to higher productivity in earlier genotypes – which is in line with the results of Sesay *et al.* (2017) and Musimwa and Derera (2017).

Despite having fewer leaves in earlier plants and, consequently, less ability to produce photoassimilates (Colasanti and Muszynski, 2009), the correlation between precocity and yield may be linked to the single-cross hybrids evaluated, since, according to studies, heterosis may influence the increase in yield, precocity and plant height (Dickert and Tracy, 2002).

The correlation between NLB and EY severity was very weak, pointing to a good level of resistance and a low influence of the fungus on genotype yield.

### Statistical Genetic Parameters

The coefficients of variation (CV) were relatively low in the NLB severity evaluations, with 8.24% in CGZ, 3.94% in ITA, and 6.27% in the joint analysis (Table 2). In the EY variable, CV was higher, with 14.26% in CGZ; 20.05% in ITA; and 17.60% in the joint analysis. Although the CV of EY was high, it is a value which is consistent with the trait evaluated in a prior study that used sweet corn genotypes of the same origin and an analysis based on mixed models (Entringer *et al.* 2016).

The means based on predictions (SC and Population means) and estimations (Entry) point to the influence of the environment on the NLB severity of genotypes, with broad genetic base genotypes (populations) presenting lower severity in CGZ, and SC presenting lower severity in ITA. Nevertheless, in ITA, the Tropical Plus (TP) control was more resistant to the fungus than the SC group (Table 2).

Regarding EY, genotypes belonging to the SC group had a higher mean in the joint analysis, even though in CGZ, on average, it was lower than the TP control. The means close to or even higher than the controls, however, suggest the presence of upper and lower genotypes within each group and potential for selection.

Selection accuracy (Table 2) was high in all analyses, and all estimates were higher than 0.86, which suggests a good possibility of selecting superior genotypes.

There was substantial variation between the estimated genetic variance in the different environments. This difference can be explained by the expression of the phenotype in different environments and the presence of GxE interaction. In Table 3, the presence of GxE interaction is displayed as significant in both NLB and EY variables.

In the Likelihood Ratio Test (for random effects) and Wald (for fixed effects), the effects of Block; Location; Measures; Measures x Entry; Measures x Location; Entry; Entry x Location; Line x Location; Control x Location; and Interclasses x Location were significant for NLB severity, while for EY, the significant effects were Location; Entry; Single-Cross; Population; Interclass; Entry x Location; Single-Cross x Location; and Interclass x Location (Table 3).

While Table 3 indicated no difference in severity between genotype classes, considering the mean value between measures in the joint analysis, as shown in Figures 1 and 2 and based on the means of predicted (SC and Population) and estimated (Control) values, there is a separation between the curves and between the standard error of each of the measures. Furthermore, the Interclass x Location interaction effect suggests this difference between genotype groups within environments.

As shown in Figure 1, which presents the NLB disease progress curves of the three genotype groups in CGZ, the broad genetic base genotypes (populations) are separated, on average, from the other genotypes when the second evaluation of the disease occurs, presenting a lower severity from the end of the evaluations. It should be stressed that, according to Pataky (1992), the influence of NLB severity on yield is linked more to the early onset of its manifestation. In Figure 2, in ITA, the SCs display a curve below the other two groups but differentiate themselves only in the second evaluation. This difference in means results from the significant Interclass x Locations interaction presented in Table 3.

Figure 3 depicts the estimated individual NLB and EY means, separated by treatment groups. In line with Table 1, results do not suggest any correlation between NLB severity and EY in the genotypes under evaluation. However, according to the joint analysis means, there is a relationship between the origin of the inbred lines (population) and the severity level to the NLB. Results show that the P8HS population and crosses with lines derived from it (LP8HS125; LP8HS130; LP8HS129) exhi-

bited lower NLB severity than the PSH population and crosses with lines derived from it (LPSH134; LPSH139; LPSH140).

These data point to the fact that, even after five backcross cycles, there is enough effect of  $sh_2$  donor populations on recurrent genotypes to differentiate themselves from each other on NLB resistance. Regarding the super sweet corn populations derived from the Piranão8 population, the donor background was evident with respect to the severity level of NLB disease in the joint analysis and the Itaocara environment. In this case, the PSH population was more susceptible.

With respect to sweet corn populations derived from the CIMMYT8 population, the population backcrossed with the  $sh_2$  donor population, named CSH, showed a higher mean for EY in CGZ and the joint analysis.

The results suggests that the introduction of the mutant gene did not interfere in the NLB severity when comparing with the severity level of the original common corn populations with the backcrossed near-isogenic populations carrying the  $sh_2$  gene in recessive homozygosis. This is a sign that the presence of the mutant gene does not interfere with the susceptibility to NLB.

### Conclusions

As expected, the use of the first-order autoregressive covariance structure of the mixed model was the one that offered the best fitted model due to the correlation between the measures of NLB severity. In addition, the covariate days to silk was also significant due to its influence on disease onset, which may play an important role in the adjustment of means.

Just as the P8HS population showed lower severity to NLB than the other populations and interpopulation hybrids tested, the crosses with inbred lines derived from this population are similarly less damaged by NLB. Therefore, the breeding program should favor lines derived from P8HS population when selecting genotypes that are more resistant to NLB and high EY.

Single-crosses had a higher ear yield than the others in the joint analysis, with many genotypes showing higher means than the controls. Although, there is a strong influence of environments on the expression of genotypes, which results in the genotype x environment interaction and interclass x environment interaction, both for NLB severity and for EY.

The similarity between genotypes without the  $sh_2$  mutant gene and its near-isogenic genotypes backcrossed (sweet corn), suggests that the  $sh_2$  mutant gene did not influence the severity of NLB.

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### Conflict of interest

No conflict exists for any author and disclosure of potential conflicts of interest.

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