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The behavior of maize hybrids generated from contrasting progenies regarding the use of nitrogen

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ABSTRACT. The purpose of this study was to evaluate the performance of maize hybrids synthesized from contrasting genotypes with regard to the use of nitrogen that were selected for their performance in topcrosses. Sixty-seven $S_{0:1}$ progenies derived from the germplasm bank of Ufla were evaluated in topcross combinations with two testers at two nitrogen levels. The six progenies with the greatest tolerance and responsiveness to nitrogen (RT) and the five with the least tolerance and responsiveness (R_nT_n) were selected and, were, afterwards, crossed in a complete diallel, for a total of 55 hybrid combinations. The following genetic parameters were estimated: genetic variance among the hybrids ($\hat{\sigma}^2_G$), broad sense heritability in the mean of the hybrids, and selective accuracy ($\hat{r}^2_{\hat{g}g}$). It was observed that the genetic parameters were greater in the environments with available nitrogen and that the early selection by performance in topcrosses of progenies tolerant to low N levels may not be made with high intensity. The hybrids tolerant to low N levels were obtained by crossing contrasting parents.

Keywords: plant breeding, abiotic stress, *Zea mays*.

Comportamento de híbridos de milho sintetizados a partir de progênies contrastantes em relação ao o uso do nitrogênio

RESUMO. O objetivo deste estudo foi avaliar o desempenho de híbridos de milho, selecionados pelo seu desempenho em topcrosses e sintetizados a partir de genitores contrastantes em relação ao uso do nitrogênio. Sessenta e sete progênies $S_{0:1}$ derivadas do banco de germoplasma da Ufla, foram avaliadas em combinações de topcross com dois testadores em dois níveis de nitrogênio. As seis progênies com maior tolerância e responsividade ao N (RT) e as cinco não tolerantes e não responsivas ao N (RnTn) foram selecionadas e posteriormente cruzadas em esquema de dialelo completo, resultando em 55 combinações híbridas. Os seguintes parâmetros genéticos foram estimados: variância genética entre os híbridos ($\hat{\sigma}^2_G$), herdabilidade em sentido amplo na média dos híbridos e acurácia seletiva ($\hat{r}^2_{\hat{g}g}$). Observou-se que os parâmetros genéticos foram maiores nos ambientes com disponibilidade de nitrogênio e que a seleção precoce com base no desempenho de híbridos topcrosses oriundos de progênies tolerantes a baixos níveis de N não pode ser feita com alta intensidade. Os híbridos tolerantes a baixos níveis de N foram obtidos pelo cruzamento de pais contrastantes.

Palavras-chave: melhoramento de plantas, estresse abiótico, *Zea mays*.

Introduction

The maize production system in Brazil is quite heterogeneous in regard to the technological level used. On many properties, modern production techniques are used with an intensive applications of inputs. Nevertheless, there are a large number of properties, typically family farms, that use little or no agricultural input. This difference in the management systems is quite evident with regard to fertilizer consumption, especially nitrogen fertilizers.

This nutrient is one of the most costly with respect to yield (DANGL et al., 2000) and leads to a greater risk of environmental contamination when applied in excessive doses. Lal (1998) reports that the transport of nitrogen derived from fertilizers and decaying matter used in agriculture is one of the factors that most affects water quality.

In this context, these aspects must be taken into account when developing new maize cultivars. For that reason, it is necessary to obtain plants that are efficient and tolerant to nitrogen (N) stress and that are responsive to applied N. This breeding strategy contributes to an increase in the sustainability of the agricultural system.

The concept of tolerance to nutrient stresses is often not a matter of consensus. For Blair (1993) and Oliveira et al. (1999), tolerance is related to the capacity of the genotype to develop and reproduce under nutrient-deficient conditions. Miti et al. (2010) relates the concept of tolerance to a smaller decrease in the yield of the genotype under conditions of nitrogen stress when compared to the yield of the same genotype grown in the ideal environment. This same concept of tolerance is used by Maia et al. (2011). These latter authors also work with the concept of the efficiency of nutrient use, defining the efficiency based on the relationship between the yield and the resources available to the plant. Blair (1993) also proposes the concept of responsiveness, which is the ability of the plant to respond favorably to an increase in nutrient supply.

Studies have focused on identifying and transferring genes, by means of genetic modification that are, related to the efficiency of nitrogen use in maize. Nevertheless, the success of this technology is still quite limited due to the quantitative nature of the efficiency of N use. This fact limits the use of this technique because it handles only one or a few genes (PRAKASH, 2001). Another alternative technique is conventional breeding, which allows for the simultaneous handling of thousands of genes (RAMALHO; FURTINI, 2009).

In this context, it is fundamental to generate more detailed information regarding a germoplasm with yield potential in environments with an N deficit. The identification of sources of favorable alleles and crosses that provide good genetic complementation for the development of genotypes adapted to stress conditions is another alternative. It is a promising strategy for increasing maize yields under N-deficient conditions (MANSKE et al., 2001).

The purpose of this study was to evaluate the performance of synthesized hybrids based on contrasting genotypes with regard to the use of nitrogen that were selected for their performance in topcrosses.

Material and methods

The experiments were conducted in two locations, Amb 1 and Amb 2. Amb 1 is located at an altitude of 951 m, 21° 10' S and 44° 55' W, and Amb 2 is located at an altitude of 918 m, 21° 14' S and 45° 00' W.

Initially, 67 $S_{0:1}$ progenies were evaluated, which were derived from the Ufla germplasm bank, in topcross combinations with two testers (one simple hybrid and one mixture of the 67 progenies) at two nitrogen levels (high and low N). Among the progenies, the six greatest tolerance and responsiveness to nitrogen (RT) and the five with the least tolerance and responsiveness (R_nT_n) were selected based on topcross performance (GUEDES et al., 2011).

The 11 progenies selected were crossed in a complete diallel, synthesizing 55 hybrid combinations. Of these hybrids, 15 were derived from crosses among the RT progenies, 30 from among the progenies RT x R_nT_n and 10 were derived from the R_nT_n progenies.

The 55 hybrids, plus the 11 $S_{0:2}$ progenies derived from the self-fertilization of the $S_{0:1}$ progenies were evaluated in experiments with different nitrogen levels. For all the experiments, a randomized block design was used, which was composed of three replications, with plots of two rows that were three meters in length, with a spacing of 0.6 m between rows and 0.25 m between plants, thus, the density was approximately 66,666 plants ha^{-1}.

For the differentiation of experiments with regard to the nitrogen level, the following strategy was adopted: For the experiments with a high availability of nitrogen (high N), fertilization at planting was performed as recommended for the high-technology by placing a dosage of 350 kg ha^{-1} of the formulation (N-P-K) 8-28-16 in the planting row; the first topdressed fertilization for the nitrogen supply was performed with 200 kg ha^{-1} ammonium sulfate (21-0-2) at the V4 phenological stage, and the second topdressed fertilization was performed with 200 kg ha^{-1} of urea (45-0-0) at the V8 stage. Therefore, approximately 160 kg ha^{-1} of N was added in these experiments. For the experiments with a low availability of nitrogen (low N), the planting fertilization was the same as the aforementioned experiments, and the topdressed fertilization was not performed, thus, only approximately 30 kg ha^{-1} of N was added (ALVES et al., 1999).

The grain yield trait was evaluated in all experiments. The data obtained were balanced and met the prerequisites for an analysis of variance. The individual analyses of variance were performed in accordance with the procedures described by Ramalho et al. (2005), and, afterwards, two joint analyses were performed, one for the N level and the other considering the two N levels and the two environments. In all analyses, the effects of the models were considered to be random, except for the general mean value.

After obtaining the mean values, the hybrids were classified into four categories in accordance with their yields under stress conditions, and under ideal conditions according to Blair (1993): I – non-responsive and tolerant cultivars (R_nT): are those that do not respond to the nutrient supply and have a high yield under low nutrient availability; II – responsive and tolerant cultivars (RT): are those that respond positively to the supply and have a high yield under low nutrient availability; III – responsive and non-tolerant cultivars (RT_n): are those that respond positively to the nutrient supply and have a low yield under low nutrient availability; and IV – non-responsive and non-tolerant cultivars (R_nT_n): are those that do not respond to the nutrient supply and have a low yield under low nutrient availability.

Based on the expectations of the mean squares of the analyses of variance, the genetic variance among the hybrids ($\hat{\sigma}^2_G$),broad sense heritability in the mean of the hybrids, and selective accuracy ($\hat{r}^2_{\hat{g}g}$) were estimated (RAMALHO et al., 2005; RAMALHO et al., 2012).

Results and discussion

This study differs from the others found in the literature due to the selection of the progenies, based on their performance in topcrosses with two testers in two contrasting environments with regard to N availability (GUEDES et al., 2011). This strategy (of selecting progenies based on topcrosses) is currently used in most of the maize breeding companies (TROYER; WELLIN, 2009). In a great deal of the studies related to nitrogen stress, progenies were selected based on their performance *per se* under soils deficient in this nutrient (MEDICI et al., 2005).

In general, good experimental precision for the grain yield trait in the two N levels was observed through the coefficient of variation (CV). Except for the low N level, the CV estimates were below 20% (Table 1). Greater coefficients of variation in environments under stress were obtained by Bänziger and Lafitte (1997).

Table 1. Summary of the joint analyses of variance per N level and general group, coefficient of variation (CV%), phenotypic mean, significance of the effects of genotypes (G), environments (Amb), levels of nitrogen (N) and interactions (GxAmb) and (NxG) for the grain yields evaluated in the 2010/2011 growing season.

Joint Analyses	Grain yield (kg ha^{-1})						
	G	Amb	N	NxG	GxAmb	CV (%)	Mean
High N	**	**	-	-	NS	18.11	9496.29
Low N	**	*	-	-	NS	22.16	8199.80
General Group	**	NS	**	NS	NS	19.41	8848.05

*, ** and NS Significant at the levels of 5 % and 1 % and not significant, respectively.

The greater CV values for the high N levels are mainly attributable to the heterogeneity of the soil; in other words, the greater the amount of fertilizer applied to the area, the smaller the soil heterogeneity becomes, which lessens the effect of the environment improves the experimental precision, and allows for more reliable estimates (RAMALHO et al., 2012). Another parameter that is used to indicate experimental precision is selective accuracy, which had estimates in agreement with those of the CV for the grain yield trait; in other words, the favorable environments had better experimental conditions (Table 2). It is notable that all experiments had an accuracy above 80 %, which is considered high, and, for that reason, show good experimental precision (RESENDE; DUARTE, 2007).

Table 2. Estimates of the components of variance for the grain yield trait (kg ha^{-1}) in the two environments (Amb) and in the two N levels.

Environments	Components of variances		
	Genetic Variance ($\hat{\sigma}^2_G$) (IC)[¥]	Heritability ($\hat{h}^2_a$) (LI – LS)[¥]	Selective Accuracy ($\hat{r}^2_{\hat{g}g}$)
Amb 1 High N	4.39 (3.74 – 7.69)	84.45 (76.4 – 89.5)	91.92
Amb 1 Low N	2.67 (2.27 – 4.68)	76.29 (63.93 – 83.97)	87.34
Amb 2 High N	5.02 (4.27 – 8.80)	83.93 (75.56 – 89.14)	91.62
Amb 2 Low N	2.33 (1.98 – 4.08)	67.66 (50.72 – 78.10)	82.22

[¥]Confidence interval. [¥]Lower and upper limits of the estimate of heritability.

In the joint analysis per N level, the grain yield variable was significant ($p < 0.01$) for the source of genotype variation (G) in the two N levels. This significance demonstrates the existence of differences among the genotypes, which provides a favorable condition for selection (Table 1).

In the joint analysis, considering the N levels and the environments, the N level variation source was observed to be significant, which indicated the existence of differences among the high and low N levels. This fact may be observed in the mean grain yields in each N level, the high N level had a 13.65 % higher yield than the low N level (Table 1).

A significant interaction was not detected for the sources of genotype variation by the N levels (G x N) in the joint analyses. In other words, on average, the genotypes maintained a similar classification in the environments with low and high supplies of N. In this type of situation, the best option would be to select in the environment with high N levels, where the estimates of heritability were greater (Table 2). It is valid to emphasize that, in the literature, some studies report results that are different from those presented here (MAIA et. al., 2011; MEDICI et al., 2005). The interaction between the genotypes and environments was also not detected (Table 1). This lack of interaction demonstrates the consistent behavior of the genotypes in the two N levels and in the two environments; this condition is favorable for the selection of genotypes.

The existence of genetic variability among the hybrids for the grain yield trait may be verified by estimates of the genetic parameters (Table 2). The estimates of the genetic variances among the hybrids ($\hat{\sigma}_G^2$) were all non zero because the estimates of the lower limit were always greater than zero. It is clear that the estimates for the high N level in the two environments were always greater than the estimates for the low N level. This result clearly demonstrates that the hybrids expressed greater variability in favorable environments. The estimates of the heritability were greater than 67%, and the confidence intervals show positive lower limits. The high N level always showed the greatest estimates of heritability associated with high selective accuracy (Table 2). This observation indicates the importance of always evaluating contrasting environments, because the favorable environments allow for a better discrimination of the genotypes and selection for responsiveness to N whereas the unfavorable environments allow for the identification of tolerant genotypes.

Considering that the progenies selected as RT have a greater frequency of favorable alleles in relation to the R_nT_n, progenies, it was expected that the hybrids synthesized within the RT group would present a better performance than the hybrids synthesized by the crosses between RT x R_nT_n and also the hybrids within the R_nT_n group in accordance with the results found in the literature (BALKO; RUSSEL, 1980; TSAI et al., 1991).

In this context, it was observed that of the 15 hybrids that were expected to show an RT phenotypic performance, only 20% maintained this classification, 60% showed an R_nT_n performance, and 20% showed an intermediate performance (RT_n or R_nT). Of the 30 hybrids expected to show an intermediate performance, only 30% maintained this classification, 40% showed an RT performance, and 30% showed an R_nT_n performance. Of the 10 hybrids derived from the R_nT_n progenies, only 10% maintained this classification, 50% showed an intermediate phenotypic performance (R_nT or RT_n), and 40% inverted the classification, and showed an RT phenotypic performance (Table 3). In general, there was an equitable distribution of the hybrids in relation to the use of N, with 34.5% RT, 34.5% R_nT_n and 31% with intermediate performance (Figure 1).

Table 3. Classification of the synthesized hybrids in the complete diallel in relation to what was expected and what was observed with regard to the use of N, according to Figure 1.

Treatment	Parents		Hybrids	
			Expected	Observed
2	1	2	RT	R_nT_n
3	1	3	RT	R_nT_n
4	1	4	RT	R_nT
5	1	5	RT	RT
6	1	6	RT	R_nT
13	2	3	RT	R_nT
14	2	4	RT	R_nT_n
15	2	5	RT	R_nT_n
16	2	6	RT	R_nT_n
23	3	4	RT	RT
24	3	5	RT	RT
25	3	6	RT	R_nT
32	4	5	RT	R_nT_n
33	4	6	RT	R_nT_n
40	5	6	RT	R_nT_n
7	1	7	R_nT or RT_n	R_nT_n
8	1	8	R_nT or RT_n	R_nT
9	1	9	R_nT or RT_n	RT
10	1	10	R_nT or RT_n	R_nT
11	1	11	R_nT or RT_n	R_nT_n
17	2	7	R_nT or RT_n	RT_n
18	2	8	R_nT or RT_n	RT_n
19	2	9	R_nT or RT_n	RT
20	2	10	R_nT or RT_n	R_nT_n
21	2	11	R_nT or RT_n	RT
26	3	7	R_nT or RT_n	R_nT_n
27	3	8	R_nT or RT_n	R_nT
28	3	9	R_nT or RT_n	RT
29	3	10	R_nT or RT_n	R_nT_n
30	3	11	R_nT or RT_n	RT
34	4	7	R_nT or RT_n	RT
35	4	8	R_nT or RT_n	R_nT_n
36	4	9	R_nT or RT_n	RT_n
37	4	10	R_nT or RT_n	R_nT
38	4	11	R_nT or RT_n	RT
41	5	7	R_nT or RT_n	RT
42	5	8	R_nT or RT_n	R_nT_n
43	5	9	R_nT or RT_n	RT
44	5	10	R_nT or RT_n	RT_n
45	5	11	R_nT or RT_n	RT
47	6	7	R_nT or RT_n	R_nT_n
48	6	8	R_nT or RT_n	R_nT
49	6	9	R_nT or RT_n	RT
50	6	10	R_nT or RT_n	R_nT_n
51	6	11	R_nT or RT_n	RT
53	7	8	R_nT_n	R_nT_n
54	7	9	R_nT_n	RT_n
55	7	10	R_nT_n	RT
56	7	11	R_nT_n	R_nT
58	8	9	R_nT_n	RT_n
59	8	10	R_nT_n	RT_n
60	8	11	R_nT_n	RT
62	9	10	R_nT_n	RT
63	9	11	R_nT_n	RT
65	10	11	R_nT_n	R_nT

Nevertheless, a great deal of variation is observed in the results. In particular, the substantial difference between what was expected and what was observed and the high percentage of classification inversion are noteworthy. This lack of consistency suggests that there is a dominance deviation and/or epistasis; in other words, the performance of the hybrids in relation to the use of N may be more related to the alleles in heterozygosis and gene interaction than to additive effects.

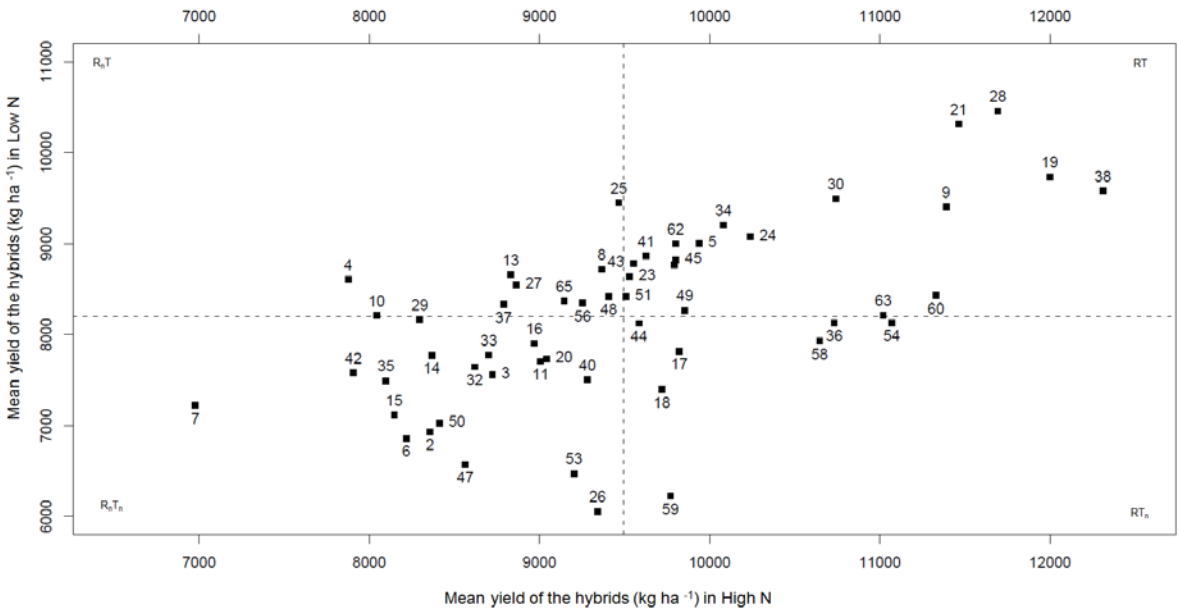

Figure 1. Behavior with regard to tolerance to low N levels and responsiveness to high N levels of the 55 hybrids (identification of the treatments in Table 3) derived from the complete diallel between contrasting.

Therefore, the strategy of high intensity early selection in $S_{0:1}$ progenies that are efficient and responsive to N, as determined by their performance in topcrosses under high and low N, is not the best approach.

The results show a tendency for the best hybrids to be synthesized between RT and R_nT_n progenies (Figure 1). In light of this fact, it is reasonable to suppose that in the synthesis of a hybrid that is responsive and tolerant to low N levels, at least one parent must be responsive when the nutrient is present, and tolerant in its absence.

Conclusion

Early selection for performance in the topcrosses of progenies tolerant to low N levels may not be made with high intensity.

Estimates of genetic variances, heritabilities and selective accuracy are greater in environments with nitrogen availability.

Hybrids that are tolerant to low N levels are derived from contrasting parents.

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