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Selection of parents for low nitrogen stress through the combining ability of maize partially inbred lines

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ABSTRACT. The objective of this work was to estimate the general combining ability of partially inbred lines under high and low nitrogen (N) conditions and to select promising lines to obtain high yields for both conditions. Fifty-five partially inbred S1 progenies were evaluated using the top-cross method, where the lines were crossed with two testers. The 110 top-cross hybrids were evaluated for grain yield during the off-season of 2012 at two sites in the Brazilian Midwest. Partial diallel analysis was performed with the adjusted means of each of the individual analyses of the top crosses. The combining ability of partially inbred lines and testers as well as their interaction with N levels, was estimated for each site. The coefficient of concordance among 15 partially inbred lines selected by the highest general combining ability estimates for each N level was 0.60. The S1 lines 39, 11, 41, 55, 38, 37, 6, 8, and 14 were selected at both N levels. This indicates that these lines can be used to identify ideal hybrids for growing in the off-season in the Brazilian Midwest.

Keywords: diallel; hybrid combination; top-cross; *Zea mays* L.

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Introduction

Maize (*Zea mays* L.) is one of the most important cereals grown in the world for human and animal food. Nitrogen (N) is taken up in large amounts by the crop and has the greatest influence on crop yield (Al-Naggar, Shabana, Atta, & Al-Khalil, 2015; Rao, Miles, Beebe, & Horst, 2016). The use of nitrogen fertilizers can meet the demand of N by the maize crop; however, the high costs of this input and the uncertainty of the economic return, especially in tropical climate regions such as the Brazilian Cerrado, constitute high risk factors for farmers (Fidelis, Miranda, & Faluba, 2010).

Developing cultivars adapted to low N conditions in the soil is an economically viable and ecologically sustainable option to ensure higher yield in low input farming systems (Rao et al., 2016). In this sense, one of the objectives of maize breeding programmes for abiotic stress is to obtain genotypes showing high N use efficiency through satisfactory yield grains with low N requirements. This achievement can be made through the selection of superior genotypes since studies indicate the existence of genetic variability for nitrogen use efficiency in maize (Silva, Miranda, Cruz, Galvão, & Silva, 2008; Fidelis, Miranda, & Faluba, 2010; Al-Naggar et al., 2015).

One of the important decisions in maize breeding programmes is the choice of populations for inbred line extraction. Among the available germplasm sources, commercial hybrids with favourable alleles have already been established for the traits of interest (Pfann et al., 2009; Rodrigues, Von Pinho, Albuquerque, Faria Filho, & Goulart, 2009; Hallauer, Carena, & Miranda Filho, 2010). To select promising lines for high and low N conditions, it is necessary to estimate their general combining ability (GCA) in each environment (Oliboni et al., 2013). Parents with the highest GCA estimates are potentially favourable for their contributions to breeding programmes, depending on the interest in increasing or decreasing the trait (Ramalho, Abreu, Santos, & Nunes, 2012).

The step of obtaining and evaluating inbred lines is costly and time-consuming in any hybrid development programme. One way to accelerate the process and reduce programme costs is to obtain hybrids from partially inbred lines. Therefore, it is possible to reduce the time and cost of obtaining these hybrids because the production system requires fewer successive self-fertilizations and a smaller area for obtaining and multiplying the lines, reaching the market faster and maintaining higher yield when compared with inbred lines.

Given the above, the objective of this work was to estimate the general combining ability of partially inbred lines under high and low nitrogen (N) conditions and to select promising lines to obtain high-yielding hybrids for both conditions.

Material and methods

Five base populations were evaluated, from which partially inbred lines were extracted (Table 1).

Table 1. Characteristics of the materials and company holding the base populations used for extraction of inbred progenies.

Population	Material	Type	Cycle	Grain color	Type of grain	Company
BP (01)	UFGD 1	V	E	YE/OR	Semi-dent	-
BP (02)	BRS Sol-da-manhã	V	E	OR	Flint	Embrapa
BP (05)	BRS 3035	TH	SE	OR	Semi-dent	Embrapa
BP (07)	DKB 789	DH	E	YE/OR	Semi-flint	Dekalb
BP (13)	AG 30A91	SH	E	OR	Semi-flint	Agromen

Type: V – variety, SH – single hybrid, DH – double hybrid, TH – triple hybrid; Cycle: E– early, SE – super-early; Grain color: OR – orange, YE – yellow.

From these populations, 11 partially inbred S1 progenies were extracted from each base population. To obtain the S1 progenies of each studied population, a self-fertilization method was used, as described by Borém (2009). In each population, one hundred S0 plants were selected for vigour and type of plant and later self-fertilized, harvesting only erect plants. In each population, the eleven best S1 lines were selected to be evaluated at the top-cross level. The process of self-fertilization of the plants was carried out during the off-season of 2011.

Top-crosses were obtained from two isolated crosses in the field, where the lines were intercalated with the testers. Two testers (T1 - a commercial single hybrid with good yield potential and T2 - a fair mix of S1 progenies) were used. Top-cross hybrids were obtained during the 2011/2012 harvest. We obtained 110 hybrid progenies, called top-crosses, which together with the five base populations and six controls were used in the evaluation trials. The controls used were the variety BR 106 and the hybrids BRS 1010, XB 9003, XB 8010, DKB 390, and Omega, in addition to the populations originally used.

The trials were installed during the 2012 harvest in the municipalities of Dourados and Caarapó in the State of Mato Grosso do Sul, Brazil. In Dourados, the tests were installed at the Experimental Farm of Agricultural Sciences of the Federal University of Grande Dourados (UFGD), located at latitude 22° 14’ 02” S, longitude 54° 59’ 17” W and 406 m of altitude. In Caarapó, the trials were conducted at Urtigão Farm, located at latitude 22° 38’ 45” S, longitude 55° 00’ 28” W and 482 m of altitude. The regional climate according to Köppen‘s classification is Cwa, which is characterized as a humid mesothermic climate with hot summers and dry winters, with a temperature of the coldest month lower than 18°C and the warmest month higher than 22°C and an average cumulative rainfall of 1,427 mm.

To characterize the high N environment, a rate of 120 kg ha^{-1} of N was used by applying 20 kg ha^{-1} at sowing and 100 kg ha^{-1} in top dressing. For the low N environment, a rate of 20 kg ha^{-1} at sowing was applied. The experimental design was an 11 x 11 lattice with two replicates. The experimental unit consisted of five-meter rows spaced 0.90 m between them and 0.20 m between plants in the trials conducted in Dourados. In Caarapó, the spacing was 1.0 m between rows and 0.18 m between plants.

The experimental area of Dourados was prepared in a conventional manner, and the sowing was performed manually on February 15, 2012, using two seeds per hole. The experimental area of Caarapó was cultivated under a no-tillage system, and sowing was carried out in succession to soybean cultivation. Seeding was performed manually on March 9, 2012, using two seeds per hole. In both sites, sowing fertilizer was used with 20 kg ha^{-1} of N, 50 kg ha^{-1} of potassium and 50 kg ha^{-1} of phosphorus, by using the formula 08-20-20 + 0.4% Zn. Thinning of the crop was carried out to maintain a stand of 55,000 plants ha^{-1}. The first nitrogen top dressing was performed when the maize plants had four to five fully expanded leaves, and the second when the plants had eight to ten fully expanded leaves. Other cultural practices were carried out according to the technical recommendations for maize crops.

For the evaluation of grain yield (YIE, kg ha^{-1}), the ears from the central rows were harvested manually and threshed to determine the weight and moisture of grains, correcting the moisture to 13%.

Joint analysis of variance was performed according to the statistical model (Equation 1):

$$Y_{ijkl} = \mu + T_i + E_l + B_{j(kl)} + R_{k(l)} + T \times E_{il} + \varepsilon_{ijkl} \qquad (1)$$

where: Y_{ijkl} is the observation in the j-th block within the k replicate evaluated in the i-th treatment and l-th environment; μ is the overall mean of the experiments; T_i is the fixed effect of the i-th treatment; E_l is the random effect of the l-th environment; $B_{j(kl)}$ is the effect of the j block within the k replicate within the j environment; $R_{k(l)}$ is the effect of the k replicate within the l environment; $T \times E_{il}$ is the random effect of the interaction between i treatments and the l environment; and ε_{ijkl} is the random error associated with Y_{ijkl} observation.

In the joint analysis, the squared sums of treatment were partitioned into top-cross, control, their contrast and the interaction of these with the environments. In addition, the squared sums of the top cross were unfolded for each tester and the contrasts between them. The interactions of these with N levels were performed. We also unfolded the squared sums of the environment in sites and levels and their contrasts.

For each site, a diallel analysis was performed with the adjusted means from each of the individual analyses of the top-crosses, except for the controls. The analysis was performed according to Griffing's (1956) model I, method 4, adapted for partial diallel in multiple environments (Ferreira, Rezende, & Ramalho, 1993). This method is used when there is a set of hybrids without their reciprocal. In this case, the statistical model used is described in Equation 2:

$$Y_{rsl} = \mu + a_l + g_r + g'_s + s_{rs} + (ag)_{rl} + (ag')_{sl} + (as)_{rsl} + \varepsilon_{rsl} \qquad (2)$$

where: Y_{rsl} is the mean of the cross between the r-th tester and the s-th line at the l nitrogen level; μ is the overall mean of the diallel; a_l is the effect of the l nitrogen level; g_r is the effect of the general combining ability of the r-th tester; g'_s is the effect of the general combining ability of the s-th progeny; s_{rs} is the effect of the specific combining ability between the r tester and s progeny; $(ag)_{rl}$ is the effect of the interaction between the general combining ability of the r-th tester and the effect of the nitrogen level; $(ag')_{sl}$ is the effect of the interaction between the general combining ability of the s-th progeny and the effect of the level nitrogen; $(as)_{rsl}$ is the effect of the interaction between the specific combining ability between the r and s progeny and the nitrogen level (i.e., the specific combining ability between the lines from groups 1 and 2); and ε_{rsl} is the mean experimental error.

Analysis of variance was performed using SAS software (Statistical Analysis System [SAS], 2018), while the diallel analysis was performed using Genes software (Cruz, 2013).

Results and discussion

There was significance ($p < 0.01$) for the effects of environment and treatment (Table 2). Effects of treatments (T) were partitioned into top-crosses (TC), (tester top-cross 1 (TCt1) and tester top-cross 2 (TCt2)), controls and their contrasts (TC vs. control (C), TCt1 vs TCt2) and general (GCA) and specific (SCA) combining ability. There was a significant effect for top-crosses, TCt1, TCt2, GCA, SCA and controls, which indicates the existence of genetic variability. Cancellier, Afférri, Carvalho, Dotto, and Leão (2011) and Guedes et al. (2014) found similar results.

The mean YIE was 4,748.09 kg ha^{-1}, where the grain yield in the environment with high N was 10.9% higher than the mean YIE found in low N, confirming the high significance of the N level effect for this trait. Considering that the difference in grain yield between the two environments was approximately 548 kg ha^{-1} and that the difference of N applied was 100 kg, the mean response of kg of grains produced per kg of N applied was approximately 5.5 kg. These results are a magnitude lower than those found by Guedes et al. (2014), who found a mean response of 17 and 24 kg of grains per kg of N applied in maize crops, respectively. However, these results can be attributed to low water availability and stressful factors present in the off-season, which reduces the potential of the crop response to N application.

Table 2. Joint diallel analysis for grain yield (kg ha^{-1}) of 110 top-crosses and six controls evaluated under low and high nitrogen (N) conditions in Dourados-Mato Grosso do Sul and Caarapó-Mato Grosso do Sul, Brazil.

Sources of variation	Degrees of freedom	Mean square
Environment (E)	3	65381495.40**
Sites (S)	1	110377265.75**
Levels (N)	1	79751670.13**
S vs N	1	6015550.41^{ns}
Treatments (T)	120	4078829.50**
Top-crosses (TC)	109	2564267.90**
GCA (Testers)	1	24632343.59**
GCA (Progenies)	54	970938.70**
SCA	54	246247.70*
TC tester$_1$	54	2141687.90**
TC tester$_2$	54	3034334.40**
TC t$_1$ vs TC t$_2$	1	1273424.00^{ns}
Control (C)	10	20995433.00**
TC vs C	1	54038598.05**
T x E	360	982137.10^{ns}
Top-crosses x E	327	896282.61^{ns}
CGC (Testers) x E	3	5715252.90**
CGC (Progenies) x E	162	935759.19*
CEC x E	162	767565.83^{ns}
Control x E	30	742637.10^{ns}
Error	480	1242473.00
Site		Low N
Caarapó		4069.54
Dourados		4887.92
Mean		4478.73
Site		High N
Caarapó		4767.61
Dourados		5287.69
Mean		5027.65
Overall mean		4748.09
Coefficient of variation (%)		19.47
Lattice efficiency	Site	High/Low (N)
	Caarapó	129.73/136.72
	Dourados	121.91/116.22

^{ns}, ** and *: not significant and significant at 1 and 5% probability level by F test, respectively; t1: tester 1; t2: tester 2; GCA: general combining ability; SCA: specific combining ability.

On average, the testers did not differ from the top-crosses regarding the YIE, verified by the contrast TCt1 vs. TCt2 not being significant. This may be because tester 2 is a fair mix of tested progenies themselves, i.e., the tester is the population itself, and the top-cross performance with this tester is equivalent to the performance of the progenies per se. Thus, the performance of progenies per se was not changed when the single hybrid was used as a tester. This fact is important because it allows optimizing the top-cross method because the evaluation of progenies per se can be performed in the same field, which would save resources and reduce the time for obtaining hybrids.

The effect between treatments and environments (E) was not significant. However, it is important to mention that this effect was tested with 11 commercial controls recommended for the region, which did not interact with the environments. Therefore, this interaction was unfolded in effects due to top-crosses x E, GCA x E and SCA x E. The interaction between CEC x E was not significant and indicates that hybrids have a similar behaviour across the tested environments. There was a significant interaction between GCA x E, which allows the selection of parents for each N condition.

Thus, a joint diallel analysis of variance was performed for each site, investigating the interaction between GCA and SCA effects with N (Table 3). There were significant GCA effects of testers (GCA Test) and progenies (GCA Prog) at each site. It is known that parents with significant GCA estimates contribute a greater quantity of favourable alleles transmitted to the descendants (Ramalho et al., 2012). In this sense, it can be inferred that the parents differ regarding the presence of favourable alleles, where there are more promising parents for the formation of new populations. Rodrigues et al. (2009) found similar results in a complete diallel between eight lines and twenty-eight hybrids, aiming at producing green corn.

Table 3. Summary of diallel analysis of variance for grain yield (kg ha^{-1}) evaluated in 110 top-crosses hybrids in Caarapó-Mato Grosso do Sul and Dourados-Mato Grosso do Sul, Brazil.

Sources of variation	DF	Caarapó	Dourados
Levels (N)	1	53603015.22**	17580149.60**
Top-crosses (TC)	109	946035.50**	1037904.46**
GCA (Testers)	1	26801507.61**	8790074.80**
GCA (Progenies)	54	1109178.89**	1325193.92**
SCA (Testers x Progenies)	54	304087.08*	607056.30*
TC x N	109	647827.15**	979736.23**
GCA (Testers) x N	1	7533000.48**	1976218.74*
GCA (Progenies) x N	54	608174.16**	1214112.60**
SCA (Testers x Progenies) x N	54	559976.93*	726906.48**
Error	218	394159.84	500424.95

^{ns}, ** and *: not significant and significant at 1 and 5% probability level by F test, respectively; DF: degrees of freedom.

The significant effect for SCA (Testers x Progenies) at both sites was significant and indicates that the type of tester used interferes with the top-cross hybrid yield. This shows that the parents have an appreciable degree of gene complementation in relation to the allele frequencies in the loci that exhibit dominance (Cruz, Regazzi, Carneiro, & Souza, 2012).

The significance of the effect of GCA (Progenies) x N at both sites reveals that parental lines contribute differently to trait expression under high and low N availability. Top-crosses x N interaction showed a significant effect, which is indicative of a difference in the favourable allele frequencies among the top-cross parents evaluated in contrasting environments regarding nitrogen availability. Similar results were found by Médici et al. (2005), who reported the existence of the "genotype x N levels" interaction for maize grain yield. According to these authors, in environments with high N availability, additive genetic effects are slightly more important than non-additive genetic effects, and additive and non-additive genetic effects show similar importance in environments with low availability of N.

The SCA (Testers x Progenies) x N interaction was significant in both sites (Tables 2 and 3), which allows us to infer that there was a differentiated performance of the hybrid combinations in response to the N levels. Oliboni et al. (2013) concluded that the evaluation in distinct environments provides an easiness in the identification of variability among the genotypes regarding the traits plant height, ear height and grain yield resulting from the effects of combining ability; hence, it is possible to predict the phenotype of new hybrids generated from combinations with the parents used in this study. Moreover, according to Miti, Tongoona, and Derera (2010), many N use traits are under genetic control, and physiological processes limiting yield differ according to the N level.

Table 4. Estimates of general combining ability for grain yield (kg ha^{-1}) of two testers and the 15 partially inbred lines selected for high and low nitrogen (N).

Tester	High N		Low N
1	10.38^{ns}		-197.759**
2	-10.38^{ns}		197.759**
Line	**High N**	**Line**	**Low N**
39	1740.68**	39	1210.78**
40	894.15**	41	917.02**
13	854.85**	8	865.34**
11	731.33**	9	774.23**
35	698.54*	38	746.90**
41	685.38*	6	739.83**
55	611.06*	45	631.10*
38	591.99*	55	621.13*
34	510.12*	28	547.97*
37	506.80*	14	420.77*
6	488.09*	3	417.00*
21	476.10*	54	401.31*
8	435.30*	52	351.90*
14	363.26*	37	332.61*
36	345.73*	11	328.53*

^{ns}, ** and *: not significant and significant at 1 and 5% probability level by F test, respectively.

The combining ability of partially inbred lines and testers, as well as their interaction with N levels, was estimated for each site. Table 4 contains the estimates of the testers and the top 15 partially inbred lines for each site. Under high N, GCA estimates of the testers did not differ from zero by the t-test. For low N, tester 2, which corresponds to a fair mix of the tested progenies themselves, provided means higher than the top-crosses.

The coefficient of concordance among the 15 partially inbred lines selected by the highest GCA estimates for each N level was 0.60. The S1 lines 39, 11, 41, 55, 38, 37, 6, 8, and 14 were selected at both N levels. This indicates that these lines can be used for identifying hybrids ideal for growing in the off-season in the Brazilian Midwest. These hybrids would be characterized by satisfactory grain yield in environments with low N availability but would be highly responsive to N increase. In addition, the partially inbred lines selected for each N level can be used in recurrent selection programmes aimed at developing maize hybrids with high yield for each environment.

Conclusion

The S1 lines 39, 11, 41, 55, 38, 37, 6, 8, and 14 were selected at both N levels. These lines can be used to identify ideal hybrids for growing in the off-season in the Brazilian Midwest.

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