ARTICLE

Genetic control of efficiency in association of tropical maize with *Azospirillum brasilense* for grain yield

Crop Breeding and Applied Biotechnology
25(3): e51622534, 2025
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332025v25n3a34

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Abstract: *This study aims to investigate the genetic control of the efficiency of maize association with* Azospirillum brasilense *for grain yield. A total of 48 experimental single-cross maize hybrids were evaluated in seven environments for the grain yield without nitrogen topdressing (Nt) and without* Azospirillum brasilense *(Az), with Nt but without Az, without Nt but with Az, and the efficiency of* Azospirillum brasilense itself*. The data were used to estimate the variance components and to perform a diallel analysis. The magnitudes of the genotypic determination coefficients indicate that selection is possible for all traits analyzed. Thus, breeding programs should contribute to developing maize genotypes that are more efficient in association with the bacteria. The inbred lines differed regarding the frequency of favorable alleles and contributed differently to the crosses. Therefore, since the* Azospirillum brasilense *efficiency is under genetic control, it can be improved through selection.*

Keywords: Zea mays *L., diazotrophic bacteria, nitrogen, inheritance, diallel analysis*

INTRODUCTION

High-yield maize crops increase the demand for nitrogenous fertilizers, which are typically applied on a large scale and supplied as chemical fertilizer. This raises production costs and may contribute to environmental problems. Alternatives for rationalizing and raising awareness about the use of nitrogen fertilizers in maize crops are essential. Among these, we highlight the use of plant-beneficial microorganisms, such as diazotrophic bacteria of the genus Azospirillum, that, when associated with the roots of plants of the *Poaceae* family, can promote plant growth (Döbereiner and Day 1976, Bashan et al. 2014, Carvalho et al. 2023) and biological nitrogen fixation (Hungria 2011).

Regarding efficiency, the different responses of maize genotypes to the association with *Azospirillum brasilense* have been constantly reported in field results. There are reports where this association is so efficient that inoculation with *Azospirillum* allows for a partial replacement of nitrogen fertilization (Lana et al. 2012, Bashan et al. 2014, Pereira et al. 2015, Buzinaro et al. 2018, Vidotti et al. 2019, Hungria et al. 2022, Carvalho et al. 2023, Viana et al. 2024). Therefore, identifying and selecting the most efficient maize genotypes when associated with the bacteria can enhance the response of maize plants and promote the adoption of inoculation by farmers (Hungria

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Received: 10 December 2024
Accepted: 09 April 2025
Published: 27 June 2025

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et al. 2010, Meneses et al. 2011, Alquéres et al. 2013, Beauregard et al. 2013, Espindula and Passaglia 2024), thus reducing the nitrogen use in maize crops.

Breeders need to understand the genetic control mechanisms of traits under selection to design appropriate breeding programs and develop superior genotypes more efficiently. Diallel analysis is an excellent tool for this purpose (Sprague and Tatum 1942, Griffing 1956, Vencovsky and Barriga 1992, Cruz et al. 2012).

Diallel analysis is a strategy commonly used to estimate useful parameters that allow breeders to understand the genetic control of traits and to adequately outline selection strategies in breeding programs designed for developing superior genotypes (Sprague and Tatum 1942, Griffing 1956, Geraldi and Miranda Filho 1988). This method also provides information that helps in selecting parents with greater potential for breeding programs, while also revealing insights into the genetic effects involved in the expression of these traits (Cruz et al. 2012, Oliboni et al. 2013) and facilitating the identification of heterotic groups (Hallauer et al. 2010). Knowledge of the parameters involved in trait control, such as genetic effects, heterosis, inbreeding depression, and general combining abilities (GCA) and specific combining abilities (SCA), allows breeders to efficiently apply breeding techniques that contribute to the development of genetically superior genotypes (Sprague and Tatum 1942, Vencovsky and Barriga 1992, Cruz et al. 2012).

Understanding the parameters involved in the genetic control of efficiency in the association of maize with *Azospirillum brasilense* should help breeders decide and design specific breeding programs to develop more efficient maize genotypes. This study aims to investigate the genetic control of efficiency in the association of tropical maize with *Azospirillum brasilense* regarding grain yield.

MATERIAL AND METHODS

Forty-eight experimental single-cross maize hybrids were obtained by partial diallel crossing between eight and six inbred lines (S6) extracted from synthetics IG-3 and IG-4, respectively. Synthetics IG-3 and IG-4 were obtained from a Reciprocal Recurrent Selection Program applied to populations BR-105 and BR-106, which belong to distinct heterotic groups. These populations were obtained from the Maize Breeding Program of the Genetics Department of ESALQ/USP (Souza Júnior et al. 1993, Rezende and Souza Júnior 2000, Souza Júnior et al. 2010).

The 48 experimental hybrids and one commercial single-cross maize hybrid (DKB 390) were evaluated across different sowing times during two crop seasons at the Sertãozinho Experimental Station (lat 22°43’ 04” S, long 47° 37’ 07” W) and the Genetics Department (lat 22° 42’ 23” S, long 47° 38’ 15” W), both located at ESALQ/USP in Piracicaba, State of São Paulo, as well as at the Teaching, Research and Extension Farm (FEPE) (lat 21° 14’ 54” S, long 48° 17’ 13” W) of the FCAV/UNESP in Jaboticabal, State of São Paulo. Each combination of crop season (1 or 2) x site (Sertãozinho, Genetics, or FEPE) x sowing time (1st or 2nd) was considered a distinct environment, totaling seven evaluation environments.

Three experiments were established at the same site for each environment: (1) without nitrogen topdressing and without *Azospirillum brasilense* inoculation (-a-n); (2) with nitrogen topdressing but without *Azospirillum brasilense* inoculation (-a+n); and (3) without nitrogen topdressing but with *Azospirillum brasilense* inoculation (+a-n). Conventional soil preparation was performed in all trials, consisting of basal fertilization following the requirements and recommendations of each environment. In the experiment that required nitrogen (N) topdressing, enough urea was applied to provide 170 kg N ha^{-1} during the V3–V5 developmental stages. Qualyfix Gramíneas (containg a mix of the Abv-5 and Abv-6 strains of *Azospirillum brasilense*) was applied to the soil at a rate of 600 mL ha^{-1} during the V3–V5 plant developmental stages. Other crop practices and treatments were carried out similarly across all three locations, following technical recommendations for each environment, with supplementary irrigation provided only at the Genetics Department of the Experimental Station.

Each experimental design consisted of a 7x7 lattice design, with two replications. Each plot consisted of 4.0-m-long rows spaced 0.80 m apart with 0.20 m between plants, maintaining 20 plants after thinning, equivalent to a population of 62,500 plants ha^{-1}. For each plot, the following traits were evaluated: grain weight, measured by threshing and weighing the grains of the ears harvested; plant stand, determined by counting plants at harvest; and grain moisture at harvest. For statistical analysis, grain yield was obtained for each plot with grain weight corrected to 13% moisture content, while average stand-by covariance was converted to t ha^{-1}. From the grain yield data collected from each plot and all three experiments, without nitrogen topdressing and without *Azospirillum brasilense* inoculation ($GY - a + n$); with nitrogen topdressing but without inoculation ($GY - a + n$); and without topdressing but with *Azospirillum brasilense* inoculation ($GY + a - n$). The *Azospirillum brasilense* efficiency (EAz) was calculated using the following expression, adapted from Parentoni et al. (2011): $EAz = (GY + \mathrm{a} - \mathrm{n})^2/(GY - a - n.GY - a + n)$.

All statistical analyses were performed using the SAS software (V8). The traits considered were: grain yield without *Azospirillum brasilense* and without nitrogen topdressing (GY-a-n, t ha^{-1}); grain yield without *Azospirillum brasilense* but with nitrogen topdressing (GY-a+n, t ha^{-1}); grain yield with *Azospirillum brasilense* but without nitrogen topdressing (GY+a-n, t ha^{-1}); and efficiency in response to *Azospirillum brasilense* (*EAz*). These data were first subjected to individual variance analyses for each environment following the lattice design, with hybrids treated as fixed effects (Cochran and Cox 1966). Subsequently, joint variance analyses were performed using the adjusted means from the individual analysis, considering the hybrids as fixed effects and environments as random effects.

From the expected mean squares of the joint variance analyses, the phenotypic variance at the mean level ($\hat{\sigma}^2_{\bar{F}}$), the variance of the interaction between the hybrids and the environment ($\hat{\sigma}^2_{HxE}$), the residual variance ($\hat{\sigma}^2$), the genetic quadratic component of the hybrids ($\hat{V}_H$), and the genotypic coefficient of determination of the hybrids at the mean level (R^2_H), were estimated, using the following expressions, respectively: $\hat{\sigma}^2_{\bar{F}} = QM_H/re$; $\hat{\sigma}^2_{HxE} = (QM_{HxE} - QM_R)/r$; $\hat{\sigma}^2 = QM_R$; $\hat{V}_H = (QM_H - QM_{HxE})/re$; $R^2_H = \hat{V}_H/\hat{\sigma}^2_{\bar{F}}$, where QM_H is the mean square of the hybrids; QM_{HxE} is the mean square of the interaction of the hybrids with the environments; QM_R is the mean square of the residue; r and e are the number of replications and environments, respectively. The confidence intervals for the variance components were estimated with 95% probability, according to Burdick and Graybill (1992) while the confidence interval for the genotypic determination coefficient of the hybrids was also estimated at 95% probability using the methodology proposed by Knapp et al. (1985).

The adjusted means of the single-crosses obtained from the individual variance analyses were used to perform diallel analyses for the considered traits. These analyses followed Griffing's model I, method IV (Griffing 1956), adapted for partial diallel (Geraldi and Miranda Filho 1988) in multiple environments (Ferreira et al. 1993), as follows:

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_k + (ge)_{ik} + (ge)_{jk} + (se)_{ijk} + \bar{\varepsilon}_{ijk}$, where Y_{ijk} is the mean value of the hybrid between lines i and j in environment k; μ is the general mean; g_i is the GCA of line i extracted from population IG-3 ($i = 1, 2, \ldots, 8$); g_j is the GCA of line j extracted from population IG-4 ($j = 1, 2, \ldots, 6$); s_{ij} is the effect of SCA of the cross between lines i and j; e_k is the effect of environment k ($k = 1, 2, \ldots, 7$); $(ge)_{ik}$ the interaction between the GCA of line i and environment k; $(ge)_{jk}$ is the interaction between the GCA of line j and environment k; $(se)_{ijk}$ is the effect of the interaction between the SCA of the cross between lines i and j and environment k, and $\bar{\varepsilon}_{ijk}$ is the mean experimental error.

The relative contribution of general (%*GCA*) and specific (%*SCA*) combining ability was estimated by: $\%GCA = [(SQ_{GCA(IG3)} + SQ_{GCA(IG4)})/(SQ_{GCA(IG3)} + SQ_{GCA(IG4)} + SQ_{SCA})]100$ and $\%SCA = [(SQ_{SCA})/(SQ_{GCA(IG3)} + SQ_{GCA(IG4)} + SQ_{SCA})]100$, where $SQ_{GCA(IG3)}$ and $SQ_{GCA(IG4)}$ are the sums of squares of the GCAs of the lines extracted from populations IG-3 and IG-4, respectively, and SQ_{SCA} is the sum of squares of the SCA.

The GCAs of lines extracted from populations IG-3 ($\hat{g}_i$) and IG-4 ($\hat{g}_j$), and the SCAs ($\hat{s}_{ij}$) were estimated by: $\hat{g}_i = (Y_{i.}/N_1) - (Y_{..}/N_1\,N_2)$; $\hat{g}_j = (Y_{.j}/N_2) - (Y_{..}/N_1\,N_2)$; $\hat{s}_{ij} = Y_{ij} - (Y_{i.}/N_1) - (Y_{.j}/N_2) + (Y_{..}/N_1\,N_2)$, where Y_{ij} is the hybrid value from the cross between lines i and j; $Y_{..}$ is the sum of all hybrids between lines i and j; $Y_{i.}$ is the sum of the combinations of line i with line j; $Y_{.j}$ is the sum of the combinations of line j with line i; N_1 and N_2 are the numbers of lines i and j, respectively.

T-test was used to assess the significance of the general ($\hat{g}_i$ and $\hat{g}_j$) and specific ($\hat{s}_{ij}$) combining abilities. The variances of these estimates were obtained by: $\hat{\sigma}^2_{\hat{g}i} = [(N_1 - 1)/N_1\,N_2](QM_{GCA(IG3)xE}/re)$; $\hat{\sigma}^2_{\hat{g}j} = [(N_2 - 1)/N_1\,N_2](QM_{GCA(IG4)xE}/re)$; $\hat{\sigma}^2_{\hat{s}ij} = [(N_1 - 1)(N_2 - 1)/N_1\,N_2](QM_{SCAxE}/re)$, where $\hat{\sigma}^2_{\hat{g}i}$ is the variance of the overall combining ability of line i; $\hat{\sigma}^2_{\hat{g}j}$ is the variance of the overall combining ability of line j; $\hat{\sigma}^2_{\hat{s}ij}$ is the variance of the SCA of the cross between lines i and j; N_1 and N_2 are the numbers of lines i and j, respectively; $QM_{GCA(IG3)xE}$ is the mean square of the interaction of the GCA of the lines extracted from IG-3 population with the environments; $QM_{GCA(IG4)xE}$ is the mean square of the interaction of the GCA of the lines extracted from IG-4 population with the environments; QM_{SCAxE} is the mean square of the interaction of the SCA of the lines with the environments; and r and e are the numbers of replications and environments, respectively.

RESULTS AND DISCUSSION

In the joint variance analysis, the F-test was significant for considered variation sources: environments, hybrids, and the interaction of hybrids with environments for all traits considered (data not shown). These results indicate, respectively, variability among the environments where the experiments were conducted; that at least one of the hybrids was different from the others; and that the hybrids responded differently depending on the variations in the environments. The estimates of the variance components were all positive and significantly different from zero ($p \leq 0.05$), since the confidence intervals do not include negative or zero values (Burdick and Graybill 1992). These estimates were higher for GY-a+n and lower for GY-a-n, while GY+a-n presented intermediate values, except for the residual variance, which was higher for GY+a-n and lower for GY-a+n (Table 1). The observed values are in agreement with those reported in the literature, considering the studied genotype and traits (Halauer et al. 2010, Koltun et al. 2018, Buzinaro et al. 2018).

Table 1. Estimates of phenotypic variance at the mean level ($\hat{\sigma}^2_{\bar{F}}$), variance of the interaction between hybrids and environment ($\hat{\sigma}^2_{HxE}$), residual variance ($\hat{\sigma}^2$), quadratic genetic component of hybrids ($\hat{V}_H$), and genotypic coefficient of determination of hybrids at the mean level (R^2_H) and mean, with the respective confidence intervals[1], for maize grain yields and efficiency of the association with *Azospirillum brasilense*

Parameter	GY-a-n	GY+a-n	GY-a+n	EAz
$\hat{\sigma}^2_{\bar{F}}$	0.24 [0.17; 0.38]	0.41 [0.28; 0.64]	0.51 [0.35; 0.80]	0.06 [0.04; 0.10]
$\hat{\sigma}^2_{HxE}$	0.60 [0.44; 0.88]	0.14 [0.05; 1.21]	0.28 [0.18; 0.55]	0.14 [0.11; 0.21]
$\hat{\sigma}^2$	1.02 [0.86; 1.23]	1.38 [1.16; 1.65]	0.97 [0.82; 1.16]	0.26 [0.22; 0.31]
$\hat{V}_H$	0.08 [0.04; 0.54]	0.29 [0.18; 0.58]	0.40 [0.26; 0.75]	0.02 [0.01; 0.12]
R^2_H	0.35 [0.02; 0.60]	0.71 [0.56; 0.82]	0.79 [0.68; 0.87]	0.38 [0.07; 0.61]
Mean	6.31 [4.32; 8.30]	6.69 [4.38; 9.00]	7.45 [5.51; 9.38]	1.14 [0.14; 2.15]

GY-a-n (grain yield, t ha^{-1} without *Azospirillum brasilense* and Nitrogen topdressing); GY+a-n (grain yield, t ha^{-1} with *Azospirillum brasilense* but without Nitrogen topdressing); GY-a+n (grain yield, t ha^{-1} without *Azospirillum brasilense* but with Nitrogen topdressing); EAz (Efficiency of *Azospirillum brasilense* for grain yield).

[1] Confidence interval at 0.95

The genotypic determination coefficients were different from zero for all traits considered (Knapp et al. 1985), with the lowest values observed for GY-a-n and EAz, and the highest values for GY-a+n and GY+a-n (Table 1). These results indicate that selection for the studied traits is feasible. While the greatest gains were recorded for nitrogen topdressing and *Azospirillum brasilense* inoculation, specific breeding programs can be designed to increase the efficiency of the maize and *Azospirillum brasilense* association (Döbereiner and Day 1976, Fehr 1998, Hungria et al. 2010, Buzinaro et al. 2018, Vidotti et al. 2019, Espindula and Passaglia 2024, Takahashi et al. 2024), thus allowing the development of efficient genotypes for this association.

The diallel analysis was significant for the GCA for the traits GY+a-n, GY-a+n, and EAz in population IG-3 lines, and for GY-a-n, GY+a-n, and GY-a+n in population IG-4 lines (Table 2). This indicates that the lines differed regarding the frequency of favorable alleles, thus contributing differently to the breeding crosses when present (Robinson et al. 1949, Griffing 1956, Geraldi and Miranda Filho 1988, Hallauer et al. 2010). The SCA were significant for GY+a-n, GY-a+n, and EAz (Table 2), which suggests a difference in the complementarity of the hybrids for these traits as they perform differently from what would be expected based solely on GCA effects (Robinson et al. 1949, Griffing 1956, Geraldi and Miranda Filho 1988, Hallauer et al. 2010). These results allow us to conclude that both additive and non-additive effects (dominance and epistasis) act on the expression of the analyzed traits (Robinson et al. 1949, Vencovsky and Barriga 1992).

The GCA of population IG-3 and IG-4 lines, along with the SCA, showed significant interaction with the environment for all traits except the SCA of GY+a-n (Table 2). This suggests that both the GCA and SCA effects varied differently concerning the evaluation environments (Ferreira et al. 1993). Since the evaluation environments are random and were used to increase the traits estimation precision, the general average of all environments will be considered for obtaining and discussing the estimates of the GCA and SCA effects, disregarding the specificities that may have occurred in each evaluated environment, since they are not reproducible (Vencovsky and Barriga 1992, Ramalho et al. 2012).

The contribution of GCA and SCA varied among the studied traits. For yields, GCA effects were dominant, accounting for 54% in GY-a-n, 69% in GY+a-n, and 79% in GY-a+n. in contrat, SCA effects were dominant for EAz, accounting for 59% of the observed variation (Table 2). These results indicate that the additive effects were more important than the non-additive effects (dominance and epistasis) for yields across all three studied situations (combinations with nitrogen topdressing, with *Azospirillum brasilense* inoculation, and without either input). However, for the efficiency of *Azospirillum brasilense* (EAz) association, the non-additive effects were more important in the manifestation of this trait.

Table 2. Source of variation (SV), degrees of freedom (DF), mean squares with respective significances and contribution of general (GCA) and specific (SCA) combining abilities of the joint diallel analyses of the experimental maize hybrids for grain yields and efficiency to *Azospirillum brasilense.*

Sources of variation	**df**	Mean Squares [1]			
		GY-a-n	**GY+a-n**	**GY-a+n**	**EAz [2]**
Environments (E)	6	459.14 **	384.13 **	494.23 **	68.60 **
Hybrids (H)	47	3.41 *	5.68 **	7.15 **	8.77 **
GCA I (IG-3)	7	4.33	11.20 **	18.43 **	10.46 $^{+}$
GCA II (IG-4)	5	11.25 *	21.02 **	27.34 **	18.91
SCA	35	2.10	2.39 *	2.01 *	6.98 $^{+}$
H x E	282	2.22 **	1.65 $^{+}$	1.53 **	5.46 **
GCA I x E	42	2.56 **	2.11 *	2.53 **	5.39 **
GCA II x E	30	3.16 **	2.20 *	1.69 *	9.46 **
SCA x E	210	2.02 **	1.48 ^{ns}	1.31 **	4.90 **
Effective Error	252	1.02	1.38	0.97	2.60
GCA (%)		54,04	68.73	79.08	40.70
SCA (%)		45,96	31.27	20.92	59.30

[1] GY-a-n (grain yield, t ha-1; without *Azospirillum brasilense* and Nitrogen topdressing); GY+a-n (grain yield, t ha-1; with *Azospirillum brasilense* but without Nitrogen topdressing); GY-a+n (grain yield, t ha-1; without *Azospirillum brasilense* but with Nitrogen topdressing); EAz (Efficiency of *Azospirillum brasilense* for grain yield)

[2] Mean square multiplied by 10.

$^{+}$, * and ** - significant at 0.10, significant at 0.05, and significant at 0.01 probability by F test, respectively.

Studies in the literature generally report a similar contribution of additive and dominant effects to maize grain yield (Hallauer et al. 2010, Souza Júnior et al. 2010, Buzinaro et al. 2018). Still, few investigations have examined the yield of maize crops inoculated with *Azospirillum brasilense*, unlike this study. The observed predominance of non-additive effects in EAz provides an important indicator for maize breeders, as it shows the potential to exploit the effects of heterosis through hybrid development in breeding programs, in addition to signaling to breeders the necessity to select lines based on their crosses performance rather than their performance *per se* (Fehr 1998, Hallauer et al. 2010, Buzinaro et al. 2018, Vidotti et al. 2019).

The diallel analysis results for GCA and SCA are presented in Table 3. For grain yield without nitrogen topdressing and without *Azospirillum brasilense* inoculation, the GCA of IG-4 population lines ranged from -3.39 (line 4) to 4.88 (line 3), indicating that these lines presented, respectively, the poorest and best average performance across all their crosses for this trait (Robinson et al. 1949, Griffing 1956, Geraldi and Miranda Filho 1988). The results indicate that, under these conditions, lines 5 and 4 are the most and the least promising for this trait, respectively.

For grain yield with *Azospirillum brasilense* inoculation and without nitrogen topdressing, the GCA ranged from -5.12 (line 4) to 4.43 (line 5) in the IG-4 population and from -4.28 (line 4) to 6.09 (line 1) in the IG-3 population. The IG-4 population's lines 5 and 6 and the IG-3 population's lines 1 and 2 exhibited the best average performances for this trait, with a higher frequency of favorable alleles. In contrast, the IG-4 population's lines 3 and 4 and IG-3 line 4 had the poorest performances, with a lower frequency of favorable alleles for this trait (Robinson et al. 1949, Griffing 1956, Geraldi and Miranda Filho 1988).

The SCA ranged from -10.01 (IG-3's line 3 x IG-4's line 4) to 10.68 (IG-3's line 3 x IG-4's line 1). Specifically, the combinations of the lines 3 (IG-3) x 1 (IG-4), 1 (IG-3) x 4 (IG-4), 2 (IG-3) x 4 (IG-4), and 6 (IG-3) x 4 (IG-4) had the best performance, and the crosses between the lines 6 (IG-3) x 1 (IG-4), 8 (IG-3) x 1 (IG-4), 7 (IG-3) x 2 (IG-4), and 3 (IG-3) x 4 (IG-4) had the poorest performance.

Grain yield with nitrogen topdressing and without inoculation exhibited GCA ranging from -5.20 (line 4) to 6.38 (line 5) in population IG-4 and from -5.10 (line 5) to 6.70 (line 2) in population IG-3. For the average of all crosses, the best performers were lines 1, 5, and 6 of population IG-4 and lines 1 and 2 of IG-3, whereas the worst performance was observed for lines 2, 3, and 4 of population IG-4 and lines 3, 5, 6, and 8 of IG-3. The SCA estimates ranged from -7.05 (IG-3's line 6 x IG-4's line 5) to 6.17 (IG3's line 4 x IG-4's line 1 and IG-3's line 7 x IG-4's line 4), while crosses between lines 4 (IG-3) x 1 (IG-4), 7 (IG-3) x 4 (IG-4), and 1 (IG-3) x 4 (IG-4) were superior, and combinations of lines 6 (IG-3) x 5 (IG-4), 4 (IG-3) x 4 (IG-4), 8 (IG-3) x 1 (IG-4), and 3 (IG-3) x 4 (IG-4) were inferior.

Considering the efficiency of *Azospirillum brasilense*, line 5 of population IG-3 exhibited the highest GCA estimate of 1.26, whereas the lowest GCA value of -2.00 was obtained for line 4. The SCA ranged from -3.43 (IG-3's line 4 x IG-4's line 2) to 5.09 (IG-3's line 1 x IG-4's line 2), and the best crosses were obtained between line 1 (IG-3) x 2 (IG-4), 3 (IG-3)

Table 3. GCA and SCA of the diallel analyses for grain yield (t ha^{-1}) without *Azospirillum brasilense* application and without nitrogen topdressing (GY-a-n), with *Azospirillum brasilense* and without Nitrogen topdressing (GY+a-n), without *Azospirillum brasilense* and with Nitrogen topdressing (GY-a+n) and for the efficiency of *Azospirillum brasilense* association on grain yield in maize crops (EAz)

IG-3/IG-4	s_{ij}^{1}						
	1	**2**	**3**	**4**	**5**	**6**	g_j^{1}
			GY-a-n				
1	-3.17	-4.82	6.11	2.75	0.02	-0.88	3.42
2	7.66	0.01	-1.72	6.43	-8.05	-4.33	1.77
3	-4.29	4.35	-3.29	-1.61	1.27	3.57	-1.81
4	-1.32	4.49	-4.14	-3.23	3.05	1.15	1.11
5	1.04	-1.27	1.57	-1.74	-0.84	1.25	-2.15
6	1.43	1.30	0.93	0.42	-2.77	-1.31	-3.23
7	0.26	-5.07	-2.49	2.14	3.05	2.11	-0.38
8	-1.61	1.03	3.04	-5.17	4.26	-1.55	1.26
g_i^{1}	1.81	-3.18^{+}	-0.94	-3.39^{+}	4.88**	0.81	
			GY+a-n				
1	-2.90	2.29	1.91	6.01*	-4.11	-3.20	6.09**
2	0.52	-1.33	0.61	5.83*	-1.83	-3.79	5.22**
3	10.68**	-0.90	-2.69	-10.01**	1.91	1.01	-1.58
4	0.57	-0.06	-1.73	-3.82	3.76	1.28	-4.28**
5	-1.44	2.17	-1.33	0.25	-1.84	2.20	-1.67
6	-5.20^{+}	3.84	-0.60	5.06^{+}	-4.33	1.23	-1.23
7	2.41	-5.15^{+}	0.55	-2.63	3.35	1.48	-1.85
8	-4.63^{+}	-0.85	3.29	-0.69	3.08	-0.20	-0.69
g_i^{1}	2.41	-0.64	-5.08**	-5.12**	4.43**	4.01*	
			GY-a+n				
1	2.58	-3.43	-0.25	-3.87	5.24*	-0.28	6.38**
2	-4.03	-4.34	3.90	2.68	2.33	-0.53	6.70**
3	0.93	2.69	-1.02	-4.96^{+}	-1.73	4.09	-3.47*
4	6.17*	4.97	-2.38	-5.05*	-0.22	-3.48	1.64
5	-0.83	-4.11	3.71	3.07	-0.09	-1.75	-5.10**
6	0.84	2.60	-0.01	0.54	-7.05**	3.08	-4.36**
7	-0.64	-2.72	-0.99	6.17*	-0.19	-1.63	0.94
8	-5.02*	4.32	-2.95	1.43	1.72	0.50	-2.74*
g_i^{1}	2.53^{+}	-3.05*	-4.71**	-5.20**	6.38**	4.06**	
			EAz				
1	-1.18	5.09**	-0.47	2.26	-2.85^{+}	-2.84^{+}	0.58
2	-1.70	-0.71	-1.03	-0.48	1.79	2.13	1.00
3	4.67**	-3.02	0.23	-2.41	1.62	-1.08	0.02
4	-1.30	-3.43*	1.46	1.20	0.59	1.48	-2.00**
5	0.22	2.95^{+}	-1.94	-0.65	-0.67	0.09	1.26*
6	-1.32	-1.19	-1.55	2.74^{+}	0.37	0.95	0.37
7	1.31	1.34	1.72	-3.19*	0.37	-1.55	-1.29*
8	-0.70	-1.03	1.58	0.54	-1.21	0.82	0.06
g_i^{1}	-0.31	2.11*	-1.34	-0.65	-0.82	1.01	

[1] Estimates multiplied by 10

$^{+}$, * and ** - significant at 0.10, significant at 0.05 and significant at 0.01 probability by F test, respectively.

x 1 (IG-4), 5 (IG-3) x 2 (IG-4), and 6 (IG-3) x 4 (IG-4), and the worst between lines 3 (IG-3) x 2 (IG-4), 7 (IG-3) x 4 (IG-4), 1 (IG-3) x 5 (IG-4), and 1 (IG-3) x 6 (IG-4).

The positive and significant GCA estimates discriminate lines with a high concentration of favorable alleles for that trait, indicating their superior average performance across all crosses (Robinson et al. 1949, Griffing 1956, Geraldi and Miranda Filho 1988, Hallauer et al. 2010). Although this study did not observe the ideal line, that with a high and significant GCA for both EAz and yield with nitrogen and with *Azospirillum*, the selection of parents based on GCA for EAz allows for improving this trait and facilitate development of genotypes with efficient microbial association. Crosses with high and significant SCA are due to the divergence and complementarity of the parents for the trait considered, being favorable for exploring hybrids (Robinson et al. 1949, Griffing 1956, Geraldi and Miranda Filho 1988, Hallauer et al. 2010, Buzinaro et al. 2018, Vidotti et al. 2019, Carvalho et al. 2022).

Based on the SCA estimates, we can highlight the combinations between lines 3 (IG-3) x 1 (IG-4) and 6 (IG-3) x 4 (IG-4). These combinations showed significant and positive results for both EAz and yield in the presence of *Azospirillum brasilense*, indicating that these crosses are suitable for further research in breeding programs that aim to develop hybrids appropriate for inoculation with *Azospirillum brasilense*.

Upon comparing the association between *Azospirillum brasilense* and maize with that existing between *Rhizobium* and soybean, it becomes evident that achieving similar replacement levels of nitrogen fertilization in both crops is challenging, since the mechanism of association of these microorganisms occurs differently. The association of bacteria of the genus *Rhizobium* with vegetables occurs in an endosymbiotic manner, while the association of bacteria of the genus *Azospirillum* with grasses occurs mostly on the surface of the roots (Döbereiner and Day 1976, Hungria et al. 2010, Hungria 2011, Carvalho et al. 2023, Zhao et al. 2023, Viana et al. 2024). However, the results of this study and those available in the literature indicate that the *Azospirillum* association with maize is advantageous and can partially replace the nitrogen fertilization in maize crops while maintaining acceptable yield levels (Döbereiner and Day 1976, Hungria 2011, Oliveira et al. 2017, Revolti et al. 2018, Koltun et al. 2018, Buzinaro et al. 2018, Araújo et al. 2023, Silva et al. 2024).

A strategy that should be implemented to increase the efficiency of maize genotypes is to select those that are more efficient in association with *Azospirillum brasilense*, from the initial phases of breeding programs. Currently, maize breeding programs generally select genotypes in early breeding stages by applying high doses of nitrogen and, after the selection of superior hybrids, an attempt is made to partially replace this fertilization with *Azospirillum* inoculation. However, these hybrids have not undergone any selection for this association during their development. Drawing parallels from soybean crops, in all phases of breeding programs, the genotypes are already evaluated without nitrogen topdressing and with *Rhizobium* inoculation, and, consequently, genotypes that are not efficient and have lower yields for *Rhizobium* are discarded by breeders. This same strategy applied by maize breeders could accelerate the development and obtainment of genotypes that associate more efficiently with the *Azospirillum brasilense*.

The efficiency of maize with *Azospirillum brasilense* is under genetic control, with predominantly non-additive effects. Although there are also additive effects, breeding programs should be directed to exploit heterosis for this trait. The efficiency between maize and *Azospirillum brasilense* association can be improved through selection, and the design of breeding programs seeking to develop genotypes that are efficient in this association may bring benefits to both maize cultivation and agriculture in general. Other studies must be conducted to increase the number of genotypes and evaluation conditions, allowing the results obtained with this research to be confirmed.

ACKNOWLEDGMENTS

This research was supported by the Brazilian National Council for Scientific and technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico) and the Coordination for the Improvement of Higher Education Personnel (CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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