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Maternal inheritance in the efficiency of use of *Azospirillum brasilense* in maize

Herança materna na eficiência de uso de *Azospirillum brasilense* em milho

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Abstract

The maize crop demands high nitrogen input. However, Brazilian soils are generally deficient in this element. The use of associative bacteria, such as *Azospirillum brasilense*, can benefit agricultural systems that use low nitrogen levels. This study analyzes the occurrence of maternal inheritance in traits of maize genotypes for the efficiency of response to *Azospirillum brasilense*. For that, we used 132 treatments consisting of 66 crosses in complete diallel scheme with reciprocals. We evaluated two experiments in the same area and under the same conditions. We performed one experiment without nitrogen topdressing / with *Azospirillum brasilense* inoculation and another with nitrogen topdressing / without *Azospirillum brasilense* inoculation. These experiments took place in the second crop of 2017, in a randomized block design with two replicates. After obtaining the data, we estimated the efficiencies for the use of *Azospirillum brasilense* by adapting the Fischer model. From these data, we conducted analysis of variance, t test, and diallel analysis, thus estimating maternal inheritance. The evaluated traits showed no maternal inheritance, being governed by nuclear genes. Most genotypes were efficient for the use of *A. brasilense*, for all traits.

Additional keywords: combining ability; diallel analysis; diazotrophic bacteria; *Zea mays* L.

Resumo

O nitrogênio é um dos elementos mais exigidos pela cultura do milho, sendo geralmente deficiente nos solos Brasileiros. O uso de bactérias associativas, como *Azospirillum brasilense*, pode ser benéfico para sistemas agrícolas sob baixa utilização de nitrogênio. O objetivo do trabalho foi verificar a ocorrência de herança materna para a eficiência de resposta ao *Azospirillum brasilense*, em caracteres de genótipos de milho. Foram utilizados 132 tratamentos, compostos por 66 cruzamentos obtidos em esquema dialélico completo e com recíprocos, sendo avaliados dois experimentos na mesma área e sob as mesmas condições, sendo um experimento sem adubação nitrogenada de cobertura e com inoculação com *Azospirillum brasilense* e outro com adubação nitrogenada de cobertura e sem aplicação de *Azospirillum brasilense*. Os experimentos foram conduzidos na segunda safra de 2017, no delineamento experimental de blocos ao acaso com duas repetições. Com os dados obtidos foram estimadas as eficiências ao uso de *Azospirillum brasilense*, com a adaptação do modelo de Fischer. A partir desses dados foram realizadas análise de variância, teste t e análise dialélica, estimando-se a herança materna. Não houve herança materna para as características avaliadas, sendo as mesmas governadas por genes nucleares. A maioria dos genótipos foi eficiente ao uso de *A. brasilense*, para todas as características.

Palavras-chave adicionais: análise dialélica; bactérias diazotróficas; capacidade de combinação; *Zea mays* L.

Introduction

Maize is a cereal of great importance worldwide, with application in human food and in industry. Its main purpose is to supplement animal diets. Brazil is the third largest maize producer in the world, with an average yield (including the first, second, and third crops) of 5,719 kg ha^{-1} (CONAB, 2020).

A proper supply of nutrients is necessary for this crop to express its maximum yield potential. Among these nutrients stand out nitrogen (Akhtar et al., 2015; Wasaya et al., 2017). Despite the abundance of atmospheric nitrogen, plants do not assimilate it naturally. However, this assimilation can take place from biological fixation through the action of microorganisms (Montañez et al., 2008). Associative bacteria, such as those of the genus *Azospirillum*, can benefit maize plants, including providing part of the necessary nitrogen (Hungria, 2011; Buzinaro et al., 2018; Revolti et al., 2018).

In genetic breeding programs, diallel crosses assist in determining the best combinations between parents and in population segregation through the recombination of genetic variability (Griffing, 1956). These crosses allow understanding the inheritance of traits. The transmission of traits to the progenies occurs from nuclear genes or cytoplasmic genes, the latter corresponding to maternal inheritance (Cruz & Regazzi, 2014). Knowledge of these effects is fundamental for the development and success of a genetic breeding program because, when there is maternal inheritance, the female parent will directly interfere with the expression of the traits of the descendants.

This study analyzes the occurrence of maternal inheritance in traits of maize genotypes for the efficiency of response to *A. brasilense*, addressing the genotypes most responsive to this bacterium.

Material and methods

We conducted the experiment in an experimental rural area in Jaboticabal city, São Paulo State, Brazil (21°15'22'' S latitude and 48°18'58'' W longitude; average altitude of 575 meters). According to the Köppen Classification (1948), the climate of the region is type Aw, tropical with rainy summers and dry winters. The average annual rainfall is 1,425 mm, with rainfall concentration in the summer. The relief is smooth wavy and the soil is a eutrophic Red Latosol.

We used 132 genotypes as treatments, consisting of 66 crosses in complete diallel scheme with reciprocals. For that, we used as parents 12 synthetics from the company Phoenix Agrícola Ltda. The 132 treatments took place in the second crop of 2017. The experimental design was a randomized block with two replicates, with experimental plots consisting of two 5-m long rows. Spacing was 0.50 meter between rows and 0.33 meter between plants, totaling a final population of 60,000 plants per hectare.

To evaluate the response to *Azospirillum brasilense*, we installed two experiments in the same area and under the same conditions, namely:

Experiment 1 - With *Azospirillum*: without nitrogen topdressing and with *A. brasilense* inoculation.

Experiment 2 - Without *Azospirillum*: with nitrogen topdressing and without *A. brasilense* inoculation.

We used a plot seeder for sowing, applying 350 kg ha^{-1} of 8-28-16 (NPK) as base fertilizer. We controlled pests and weeds according to the needs and recommendations for maize cultivation. For nitrogen topdressing, we used urea in the amount necessary to supply 140 kg ha^{-1} nitrogen. For the inoculation of genotypes with *Azospirillum brasilense*, we applied the commercial product (Qualifix Gramínea®) via soil, at a dose of 600 mL ha^{-1}.

In each experimental plot, we assessed the following characteristics:

a) Plant height, measuring the distance from the ground to the flag leaf insertion (cm);

b) Ear height, measuring the distance from the ground to the main ear insertion (cm);

c) Relative ear position, resulting from the relationship between ear height and plant height;

d) Grain yield, correcting the quantity of grains produced in the plot to 13% moisture and converting the values to tons per hectare (t ha^{-1}).

From these data, we performed analysis of variance, estimating the efficiency of the response to inoculation with *Azospirillum brasilense* for each genotype and for all traits by applying the Student t test and the diallel analysis.

We estimated the efficiency in the use of *Azospirillum brasilense* (E) for the traits by contrasting the data from the two experiments. For that, we used the same principles of Fischer et al. (1983), who estimated the efficiency in the use of nutrients. Thus, we obtained the following expression:

$$EY_n = \left[\frac{Y_n(\frac{C}{N})}{Y_n(\frac{C}{Az})}\right] \times \left[\frac{Y(\frac{C}{N})}{Y(\frac{C}{Az})}\right] \quad (1)$$

Wherein: EY_n is the efficiency of response to *A. brasilense* for the *Y-th* trait of the *n-th* genotype;

$Y_n\left(\frac{C}{N}\right)$ is the mean for the *Y-th* trait of the *n-th* genotype, with application of nitrogen topdressing;

$Y_n\left(\frac{C}{Az}\right)$ is the mean for the *Y-th* trait of the *n-th* genotype, with *A. brasilense* inoculation;

$Y\left(\frac{C}{N}\right)$ is the general mean for the *Y-th* trait, with application of nitrogen topdressing;

$Y\left(\frac{C}{Az}\right)$ is the general mean for the *Y-th* trait, with *A. brasilense* inoculation.

We determined the efficiency for the traits from the nitrogen: *Azospirillum brasilense* ratio. To assess the reliability of the genotypes in being efficient to *A. brasilense*, we used the t test, looking for differences between the means of the genotypes and the comparative parameters 0 and 1. Values less than or equal to 1 indicate efficient genotypes, and higher values indicate nonefficient genotypes.

We performed the diallel analysis following the model I of method 3 of Griffing (1956), estimating the general and specific combination ability and the reciprocal effect. The statistical model is given by:

$$Y_{ij} = m + g_i + g_j + s_{ij} + r_{ij} + e_{ij} \quad (2)$$

Wherein: Y_{ij} is the mean value of F_1 hybrids and reciprocals (*i, j = 1, 2, ..., p*); m is the general mean; g_i and g_j are the effects of the general combining ability of the *i-th* and *j-th* parent, respectively; s_{ij} is the effect of the specific combining ability for crosses between parents of order *i* and *j*; r_{ij} is the reciprocal effect that measures the differences provided by the parent *i* or *j*, when used as male or female in the *ij* cross, allowing to infer about the existence of maternal inheritance; e_{ij} is the mean experimental error. By this methodology, the comparison of crosses with their reciprocals allows inferring maternal inheritance for the trait when the source of variation of the reciprocal effect is significant (Griffing, 1956).

Results and discussion

In the analysis of variance, the results of the F test did not differ for the 132 genotypes regarding the efficiency of use of *Azospirillum brasilense* (E) for all traits under study. The coefficients of variation were acceptable for all traits, with minimum and maximum values of 9.17 and 32.81, respectively, for the efficiency for relative ear position (ERP) and the efficiency for grain yield (EGY) (Table 1).

Table 1 - Summary of the analysis of variance of the efficiency of use of *Azospirillum brasilense* for plant height, ear height, ear position and grain yield of 132 maize genotypes.

[1]SV	[2]DF	Mean squares			
		[4]EPH	[5]EEH	[6]ERP	[7]EPG
Treatment	131	00.0087^{ns}	00.0209^{ns}	00.0071^{ns}	00.4180^{ns}
Error	131	00.0107	00.0240	00.0087	00.4062
Mean	-	01.1234	01.1463	01.0191	01.9425
[3]CV (%)	-	09.2281	13.5214	09.1778	32.8107

[1]SV: sources of variation; [2]DF: degrees of freedom; [3]CV: coefficient of variation; [4]EPH: efficiency of use of *A. brasilense* for plant height; [5]EEH: efficiency of use of *A. brasilense* for ear height; [6]ERP: efficiency of use of *A. brasilense* for relative ear position; [7]EGY: efficiency of use of *A. brasilense* for grain yield. ^{ns}, not significant by F test ($p > 0.05$).

Regarding the efficiency for plant height (EPH), the t test showed that 120 treatments were not significant. In other words, their values did not differ from 1, which points to efficiency for that trait in these genotypes. The other 12 treatments showed significance, differing from 1 at 5% significance. These treatments had higher mean efficiency values, being thus inefficient for the use of *Azospirillum brasilense* for the trait. For parameter 0, all genotypes showed significant results at 1% significance (Table 2).

Table 2 - Number of efficient genotypes for the use of *Azospirillum brasilense* for plant height, ear height, relative position of the ear and grain yield, using Student's t test, in 132 maize genotypes, using 0 and 1 as comparative parameters.

[1]EPH	0	1	[2]EEH	0	1	[3]ERP	0	1	[4]EGY	0	1
120	**	ns	127	**	ns	132	**	ns	012	ns	ns
012	**	*	005	**	*	-	-	-	044	*	ns
-	-	-	-	-	-	-	-	-	054	**	ns
-	-	-	-	-	-	-	-	-	019	**	*
-	-	-	-	-	-	-	-	-	003	**	**

[1]EPH: efficiency of use of *A. brasilense* for plant height; [2]EEH: efficiency of use of *A. brasilense* for ear height; [3]ERP: efficiency of use of *A. brasilense* for relative ear position; [4]EGY: efficiency of use of *A. brasilense* for grain yield. By t test: ** significant to 1% probability, * significant to 5% of probability, ^{ns} not significant

Regarding the efficiency for ear height (EEH), 127 genotypes were not significant, not differing from parameter 1 and therefore showing efficiency for the trait. The other 5 treatments differed from 1 at 5% significance, being higher than 1 and indicating inefficiency for the trait. For parameter 0, all genotypes showed a significant difference at 1% significance (Table 2).

Regarding ERP, all genotypes were not significant, not differing from parameter 1 and showing efficiency for the trait. Parameter 0 reaffirmed these results for all genotypes, at 1% significance (Table 2).

For EGY, 110 genotypes were not significant, not differing from parameter 1 and thus being efficient for the use of *Azospirillum brasilense*. The other 22 genotypes differed from parameter 1, with 3 of them (54, 60, and 108) at 1% significance, corresponding to the highest values. Parameter 0 showed 120 genotypes with significant differences, 76 at 1% significance and 44 at 5% significance. The other 12 genotypes (6, 11, 15, 17, 31, 56, 92, 102, 103, 105, 118, and 121) did not show significance for parameters 0 and 1 simultaneously (Table 2).

The genotypes that did not show a significant difference in both parameters are those with lower mean values for EGY, indicating good yield in experiment 1 with *Azospirillum brasilense*. This confirms the efficiency of these genotypes for that trait (Table 3). The use of *A. brasilense* in maize favors the production of phytohormones that stimulate root growth. This improves plant nutrition and photosynthetic activities, increasing chlorophyll content, biomass production, and plant height (Hungria, 2011; Buzinaro et al., 2018; Revolti et al., 2018).

Table 3 - Student's t test of the efficiency of use of *Azospirillum brasilense* for relative position of the ear and grain yield in 132 maize genotypes.

[1]G	[2]C	[3]ERP	0	1	[4]EGY	0	1	[1]G	[2]C	[3]ERP	0	1	[4]EGY	0	1
001	01x02	1.141	**	ns	1.685	*	ns	067	02x01	1.105	**	ns	2.255	**	ns
002	01x03	1.098	**	ns	2.020	**	ns	068	03x01	0.924	**	ns	2.257	**	ns
003	01x04	1.054	**	ns	1.920	**	ns	069	04x01	1.037	**	ns	1.995	**	ns
004	01x05	1.102	**	ns	2.672	**	*	070	05x01	1.051	**	ns	2.287	**	ns
005	01x06	0.930	**	ns	1.681	*	ns	071	06x01	1.064	**	ns	1.932	**	ns
006	01x07	0.942	**	ns	1.094	ns	ns	072	07x01	0.980	**	ns	1.860	**	ns
007	01x08	1.004	**	ns	1.834	**	ns	073	08x01	1.037	**	ns	1.814	**	ns
008	01x09	1.128	**	ns	1.354	*	ns	074	09x01	1.023	**	ns	1.718	*	ns
009	01x10	1.026	**	ns	1.927	**	ns	075	10x01	0.989	**	ns	2.089	**	ns
010	01x11	1.047	**	ns	1.910	**	ns	076	11x01	0.989	**	ns	2.178	**	ns
011	01x12	0.954	**	ns	1.288	ns	ns	077	12x01	1.034	**	ns	1.511	*	ns
012	02x03	1.022	**	ns	1.866	**	ns	078	03x02	1.049	**	ns	2.007	**	ns
013	02x04	0.994	**	ns	2.578	**	*	079	04x02	1.055	**	ns	2.006	**	ns
014	02x05	0.901	**	ns	1.358	*	ns	080	05x02	1.023	**	ns	2.365	**	*
015	02x06	1.043	**	ns	1.280	ns	ns	081	06x02	1.032	**	ns	2.012	**	ns
016	02x07	1.072	**	ns	1.721	*	ns	082	07x02	0.968	**	ns	2.012	**	ns
017	02x08	1.091	**	ns	1.275	ns	ns	083	08x02	1.035	**	ns	1.684	*	ns
018	02x09	1.023	**	ns	2.506	**	*	084	09x02	0.930	**	ns	1.554	*	ns
019	02x10	1.054	**	ns	2.526	**	*	085	10x02	1.002	**	ns	1.843	**	ns
020	02x11	1.050	**	ns	2.382	**	*	086	11x02	1.010	**	ns	2.034	**	ns
021	02x12	1.053	**	ns	2.365	**	*	087	12x02	1.007	**	ns	1.676	*	ns
022	03x04	0.935	**	ns	2.073	**	ns	088	04x03	1.090	**	ns	1.521	*	ns
023	03x05	0.976	**	ns	1.369	*	ns	089	05x03	1.000	**	ns	1.395	*	ns
024	03x06	1.010	**	ns	1.388	*	ns	090	06x03	0.985	**	ns	1.603	*	ns
025	03x07	0.908	**	ns	2.039	**	ns	091	07x03	1.032	**	ns	1.577	*	ns
026	03x08	1.051	**	ns	1.737	*	ns	092	08x03	1.006	**	ns	1.171	ns	ns
027	03x09	1.017	**	ns	2.720	**	*	093	09x03	1.055	**	ns	1.698	*	ns
028	03x10	0.964	**	ns	2.148	**	ns	094	10x03	0.996	**	ns	1.491	*	ns
029	03x11	0.947	**	ns	1.311	*	ns	095	11x03	1.016	**	ns	1.434	*	ns
030	03x12	1.056	**	ns	1.347	*	ns	096	12x03	0.882	**	ns	1.363	*	ns
031	04x05	1.024	**	ns	1.219	ns	ns	097	05x04	1.026	**	ns	2.341	**	*
032	04x06	0.952	**	ns	1.382	*	ns	098	06x04	0.991	**	ns	1.642	*	ns
033	04x07	1.065	**	ns	2.333	**	*	099	07x04	1.001	**	ns	1.388	*	ns
034	04x08	0.999	**	ns	1.998	**	ns	100	08x04	1.053	**	ns	2.228	**	ns
035	04x09	0.988	**	ns	2.008	**	ns	101	09x04	1.028	**	ns	1.645	*	ns
036	04x10	1.034	**	ns	1.612	*	ns	102	10x04	1.003	**	ns	1.294	ns	ns
037	04x11	0.958	**	ns	1.340	*	ns	103	11x04	1.022	**	ns	1.233	ns	ns
038	04x12	1.043	**	ns	1.897	**	ns	104	12x04	1.015	**	ns	2.240	**	ns
039	05x06	0.910	**	ns	2.471	**	*	105	06x05	1.049	**	ns	1.261	ns	ns
040	05x07	1.064	**	ns	2.050	**	ns	106	07x05	1.088	**	ns	1.957	**	ns
041	05x08	0.985	**	ns	1.712	*	ns	107	08x05	1.036	**	ns	1.933	**	ns
042	05x09	0.981	**	ns	1.501	*	ns	108	09x05	0.971	**	ns	2.996	**	**
043	05x10	0.896	**	ns	1.636	*	ns	109	10x05	1.049	**	ns	2.162	**	ns
044	05x11	1.053	**	ns	1.927	**	ns	110	11x05	0.904	**	ns	2.263	**	ns
045	05x12	0.924	**	ns	2.007	**	ns	111	12x05	0.953	**	ns	2.175	**	ns
046	06x07	0.991	**	ns	1.705	*	ns	112	07x06	1.072	**	ns	2.519	**	*
047	06x08	0.949	**	ns	2.470	**	*	113	08x06	1.065	**	ns	1.918	**	ns
048	06x09	1.030	**	ns	1.682	*	ns	114	09x06	1.127	**	ns	2.271	**	ns
049	06x10	0.996	**	ns	2.631	**	*	115	10x06	0.825	**	ns	1.781	**	ns
050	06x11	1.026	**	ns	1.407	*	ns	116	11x06	1.001	**	ns	1.954	**	ns
051	06x12	0.942	**	ns	2.404	**	*	117	12x06	1.106	**	ns	2.055	**	ns
052	07x08	1.051	**	ns	2.032	**	ns	118	08x07	1.066	**	ns	1.235	ns	ns
053	07x09	1.005	**	ns	1.357	*	ns	119	09x07	1.043	**	ns	1.654	*	ns
054	07x10	1.112	**	ns	2.861	**	**	120	10x07	1.046	**	ns	2.264	**	ns
055	07x11	1.032	**	ns	2.495	**	*	121	11x07	0.988	**	ns	1.035	ns	ns
056	07x12	0.999	**	ns	1.239	ns	ns	122	12x07	0.977	**	ns	1.466	*	ns

Table 3 - Cont…

1G	[2]C	[3]ERP	0	1	4EGY	0	1	[1]G	[2]C	[3]ERP	0	1	4EGY	0	1
057	08x09	0.969	**	ns	1.867	**	ns	123	09x08	1.006	**	ns	2.314	**	*
058	08x10	1.049	**	ns	2.160	**	ns	124	10x08	1.046	**	ns	1.695	*	ns
059	08x11	1.021	**	ns	2.638	**	*	125	11x08	1.103	**	ns	1.793	**	ns
060	08x12	1.001	**	ns	2.777	**	**	126	12x08	1.134	**	ns	1.871	**	ns
061	09x10	0.975	**	ns	1.815	**	ns	127	10x09	1.040	**	ns	2.057	**	ns
062	09x11	0.990	**	ns	1.999	**	ns	128	11x09	1.139	**	ns	1.996	**	ns
063	09x12	1.133	**	ns	1.646	*	ns	129	12x09	1.162	**	ns	1.670	*	ns
064	10x11	1.012	**	ns	1.920	**	ns	130	11x10	0.843	**	ns	1.412	*	ns
065	10x12	1.060	**	ns	2.511	**	*	131	12x10	0.983	**	ns	1.436	*	ns
066	11x12	1.112	**	ns	1.678	*	ns	132	12x11	1.013	**	ns	2.070	**	ns

[1]G: Genotype; [2]C: Crossing; [3]ERP: efficiency of use of *A. brasilense* for relative ear position; [4]EGY: efficiency of use of *A. brasilense* for grain yield. By t test: ** significant to 1% probability, * significant to 5% of probability, ^{ns} not significant

Pereira & Tozoni (2017) used a maize hybrid that showed higher grain yield in the treatment with *Azospirillum* inoculation. This increase, however, only occurred when the treatment included nitrogen fertilization. Several studies addressing the response of maize genotypes to inoculation with *Azospirillum brasilense* have identified that genotypes interact with the microorganism (Pereira et al., 2015). This differential response of the genotypes also occurs in their response to nitrogen (Han et al., 2015; Amaral et al., 2018).

In general, one can infer that genotypes that are more responsive to *Azospirillum brasilense* favor nutrient use efficiency, which is positive for trait enhancement through genetic breeding programs.

The experiment by Pereira & Tozoni (2017) indicates favorable results depending on the affinity of the cultivar with the microorganism strain. This corroborates Hungria (2011) and other authors who observed functional nitrogenase activity depending on the bacterial strain and the genetic material of maize (Brusamello-Santos et al., 2017; Pereira-Defilippi et al., 2017; Buzinaro et al., 2018; Revolti et al., 2018).

In the diallel analysis, the results of the Griffing test (1956) for general combining ability (GCA), specific combining ability (SCA), and reciprocal effects (RE) did not differ among themselves for the 132 genotypes, for all traits. As the reciprocal effects were not significant, it appears that there is no maternal inheritance for efficiency regarding these traits and, therefore, the genes that affect them are probably nuclear (Cruz & Regazzi, 2014) (Table 4).

Table 4 - Summary of diallel analyzes of the efficiency of use of *Azospirillum brasilense* for plant height, ear height, relative ear position and grain yield of 132 maize genotypes.

[1]SV	[2]DF	Mean squares			
		[5]EPH	[6]EEH	[7]ERP	[8]EGY
Treatment	131	0.0212^{ns}	0.0495^{ns}	0.0163ns	1.0964^{ns}
[3]GCA	11	0.0266^{ns}	0.0595^{ns}	0.0189ns	0.9028^{ns}
[4]SCA	54	0.0242^{ns}	0.0528^{ns}	0.0161ns	1.1605^{ns}
Reciprocal	66	0.0178^{ns}	0.0451^{ns}	0.0160ns	1.0763^{ns}
Error	131	1.0000	1.0000	1.0000	1.0000
Total	393	-	-	-	-
Mean	-	1.1359	1.1655	1.0246	2.2071

[1]SV: sources of variation; [2]DF: degrees of freedom; [3]GCA: general combining ability; [4]SCA: specific combining ability; [5]EPH: efficiency of use of *A. brasilense* for plant height; [6]EEH: efficiency of use of *A. brasilense* for ear height; [7]ERP: efficiency of use of *A. brasilense* for relative ear position; [8]EGY: efficiency of use of *A. brasilense* for grain yield. ^{ns} not significant by F test ($p > 0.05$)

Also in the diallel analysis, the quadratic components of GCA and SCA indicate the type of gene effect favored by gene actions, for each trait (Griffing, 1956; Cruz & Regazzi, 2014). A higher value for the quadratic components of GCA in relation to those of SCA favors the occurrence of additive gene effects. In turn, a higher value for the quadratic components of SCA in relation to those of GCA favor nonadditive gene effects (Ferreira et al., 2002). Quadratic values differed from zero only in EGY, with SCA being higher than GCA, thus favoring nonadditive gene effects (Table 5).

Table 5 - Quadratic components of the combining capabilities for the efficiency of use of *Azospirillum brasilense* of plant height, ear height, relative position of the ear and grain yield of 132 maize genotypes.

Components	Fixed model			
	[3]EPH	[4]EEH	[5]ERP	[6]EGY
[1]GCA	0	0	0	0
[2]SCA	0	0	0	0.0401

[1]GCA: general combining ability; [2]SCA: specific combining ability; [3]EPH: efficiency of use of *A. brasilense* for plant height; [4]EEH: efficiency of use of *A. brasilense* for ear height; [5]ERP: efficiency of use of *A. brasilense* for relative ear position; [6]EGY: efficiency of use of *A. brasilense* for grain yield.

The estimates of the GCA effects of parents did not differ for the traits under study (Table 6), with values very close to zero. The minimum and maximum values were, respectively: -0.0448 (parent 10) and 0.0395 (parent 01) for EPH; -0.056 (parent 10) and 0.0507 (parent 09) for EEH; -0.0433 (parent 03) and 0.0367 (parent 09) for ERP; -0.2718 (parent 11) and 0.1751 (parent 05) for EGY. The largest interval between the minimum and maximum values occurred for EGY, corresponding to 0.447.

Table 6 - Estimation of the effects of the general combining ability regarding the efficient use of *Azospirillum brasilense* for plant height, ear height, relative ear position and grain yield for 12 maize parents.

Parents	[1]GCA			
	[2]EPH	[3]EEH	[4]ERP	[5]EGY
01	0.0395	0.0499	0.0086	0.0172
02	0.0183	0.0417	0.0195	0.1470
03	-0.0047	-0.0550	-0.0433	-0.2377
04	-0.0329	-0.0306	0.0053	-0.1079
05	0.0029	-0.0151	-0.0181	0.1751
06	-0.0244	-0.0330	-0.0068	0.0893
07	0.0373	0.0265	-0.0106	-0.0428
08	-0.0120	0.0206	0.0291	0.1700
09	0.0096	0.0507	0.0367	0.0079
10	-0.0448	-0.0560	-0.0083	0.1085
11	0.0061	-0.0012	-0.0070	-0.2718
12	0.0051	0.0014	0.0051	-0.0550
SD[6](Gi)	0.1513	0.1513	0.1513	0.1513
SD(Gi-Gj)	0.2236	0.2236	0.2236	0.2236

[1]GCA: general combining ability; [2]EPH: efficiency of use of *A. brasilense* for plant height; [3]EEH: efficiency of use of *A. brasilense* for ear height; [4]ERP: efficiency of use of *A. brasilense* for relative ear position; [5]EGY: efficiency of use of *A. brasilense* for grain yield; [6]SD: standard deviation

According to Cruz & Regazzi (2014), more extreme GCA values indicate whether the parent will have more or less favorable alleles. Results close to zero indicate nondifferential behavior in relation to the general mean of the parents, meaning that these parents do not stand out positively or negatively with regard to the number of favorable alleles. Depending on the trait, extreme positive values represent a greater number of favorable alleles. This occurred for all traits under study.

Among the 12 parents, 7 showed positive values and 5 showed negative values for EPH. For EEH and ERP, 6 parents showed positive values and 6 showed negative values. For EGY, 7 parents showed positive values and 5 showed negative values.

The SCA effects for crosses and reciprocals did not differ for the traits under study (Tables 7 and 8). For these effects, which refer to nonadditive gene effects, nonsignificance of the traits prevents the determination of superior combinations of parents according to their mean. In Tables 7 and 8, the minimum and maximum values of the crosses were, respectively: -0.1402 (cross between 09x10) and 0.1635 (cross between 01x08) for EPH; -0.2025 (01X07) and 0.3011 (06x11) for EEH; -0.1196 (01x07) and 0.1681 (09x12) for ERP; -1.1005 (03x05) and 1.3465 (05x07) for EGY. The largest range of SCA occurred for EGY and had a very low value, 2.4471.

Table 7 - Estimates of the effects of the specific combining ability and the reciprocal effect, regarding the efficiency of use of *Azospirillum brasilense* for plant height and ear height for 12 parents in maize.

Crossing	[2]EPH		[3]EEH	
	[1]SCA	Reciprocal	[1]SCA	Reciprocal
01x02	0.0186	0.0395	0.0937	0.0090
01x03	0.0162	-0.1020	-0.0299	0.0215
01x04	0.0114	-0.0280	0.0325	-0.0025
01x05	0.1000	0.0485	0.2206	0.1260
01x06	-0.0815	-0.0175	-0.1015	-0.0520
01x07	-0.0623	-0.0235	-0.2025	-0.0535
01x08	0.1635	-0.0120	0.1673	-0.0295
01x09	-0.1011	0.1420	0.0112	0.2105
01x10	0.0088	0.0345	-0.0060	0.0045
01x11	-0.1021	-0.0285	-0.1462	-0.0670
01x12	0.0283	0.0110	-0.0389	-0.0040
02x03	-0.1080	0.0885	-0.0002	0.1820
02x04	-0.0333	0.0450	-0.0542	0.0555
02x05	0.0062	-0.1115	-0.0951	-0.1530
02x06	0.1181	0.0850	0.1401	0.1085
02x07	0.0328	-0.0485	0.0496	-0.0285
02x08	-0.0482	0.0360	-0.0719	0.1000
02x09	-0.0239	-0.0520	-0.1135	-0.0145
02x10	0.0895	0.1260	0.0716	0.1150
02x11	0.0225	-0.0330	0.0404	0.0875
02x12	-0.0744	0.2250	-0.0602	0.2975
03x04	-0.0432	0.0960	-0.0214	0.0745
03x05	0.0509	0.0480	0.0421	0.0105
03x06	-0.0102	-0.0155	-0.0980	-0.0215
03x07	-0.0644	-0.0670	-0.1010	-0.1820
03x08	0.0208	0.0100	0.0518	0.0780
03x09	0.0871	0.0530	0.1937	0.0830
03x10	-0.0168	0.0325	-0.0075	0.0320
03x11	0.0271	0.0155	0.0247	-0.0130
03x12	0.0402	0.0235	-0.0539	0.1310
04x05	-0.1279	0.0350	-0.1378	0.0380
04x06	0.0564	0.1070	0.0245	-0.0135
04x07	-0.0473	-0.0640	-0.0290	-0.0385
04x08	-0.0404	-0.0075	-0.0495	-0.0780
04x09	0.0588	0.0315	-0.0127	-0.0470
04x10	0.0733	-0.0155	0.1090	0.0210
04x11	0.0748	-0.0440	0.0722	-0.0440
04x12	0.0173	0.0595	0.0665	0.0720
05x06	-0.0679	-0.0895	-0.0314	-0.0750
05x07	0.0503	0.0125	0.1830	-0.0880
05x08	-0.0503	0.0665	-0.0665	0.0895
05x09	0.0644	0.1410	-0.0186	0.1855
05x10	0.0509	-0.0340	-0.0144	-0.2210
05x11	0.0419	0.0160	0.0938	0.0760
05x12	-0.1189	-0.0400	-0.1753	-0.0435
06x07	0.1141	-0.1250	0.0363	-0.2645
06x08	-0.1274	-0.0420	-0.1531	-0.0940
06x09	-0.0436	0.1555	0.0046	0.1460
06x10	-0.0841	0.0155	-0.0750	-0.0055
06x11	0.1473	0.0410	0.3011	0.0155
06x12	-0.0211	-0.0165	-0.0475	-0.1485
07x08	0.0197	0.0200	0.0432	0.0420
07x09	0.0365	0.0025	0.0456	-0.0225
07x10	-0.0374	0.0120	0.0738	0.0620
07x11	0.0040	0.0785	0.0081	0.0290

Table 7 - Cont…

Crossing	[2]EPH		[3]EEH	
	[1]SCA	Reciprocal	[1]SCA	Reciprocal
07x12	-0.0463	0.0320	-0.1070	0.0935
08x09	0.0789	0.0805	-0.0084	0.0415
08x10	0.0269	-0.0550	0.0708	-0.0640
08x11	-0.1030	-0.0310	-0.0884	-0.1555
08x12	0.0594	-0.0405	0.1048	-0.1265
09x10	-0.1402	-0.0255	-0.1658	-0.1025
09x11	-0.0357	-0.0290	-0.1550	0.0480
09x12	0.0187	-0.0655	0.2192	-0.1640
10x11	-0.0722	-0.0040	-0.1498	0.2255
10x12	0.1012	0.0025	0.0934	0.0625
11x12	-0.0047	-0.0545	-0.0007	-0.0290
SD[4](Sij)	0.4522	-	0.4522	-
SD(Sij-Sik)	0.6708	-	0.6708	-
SD(Sij-Skl)	0.6324	-	0.6324	-
SD(Rij)	-	0.5000	-	0.5000

[1]SCA: specific combining ability; [2]EPH: efficiency of use of *A. brasilense* for plant height; [3]EEH: efficiency of use of *A. brasilense* for ear height; [4]SD: standard deviation.

Among the 66 crosses, 36 showed positive values and 30 showed negative values for EPH. For EEH, 30 crosses showed positive values and 36 showed negative values. For ERP, 32 crosses showed positive values and 34 showed negative values. For EGY, 33 crosses showed positive values and 33 showed negative values.

Table 8 - Estimates of the effects of the specific combining ability and the reciprocal effect, regarding the efficiency of use of *Azospirillum brasilense* for relative position of the ear and grain yield for the 12 maize parents.

Crossing	[2]ERP		[3]EGY	
	[1]SCA	Reciprocal	[1]SCA	Reciprocal
01x02	0.0615	-0.0295	0.2510	-0.4725
01x03	-0.0294	0.1005	0.1343	-0.0630
01x04	0.0167	0.0235	0.2500	0.0925
01x05	0.0942	0.0565	-0.0070	0.3815
01x06	-0.0164	-0.0320	0.0602	-0.6300
01x07	-0.1196	-0.0280	-0.6370	-0.3975
01x08	-0.0054	-0.0130	0.2140	0.3835
01x09	0.1029	0.0400	-0.7758	-0.3505
01x10	-0.0120	-0.0260	0.3780	-0.3330
01x11	-0.0383	-0.0360	0.3509	0.4965
01x12	-0.0542	-0.0120	-0.2188	-0.1235
02x03	0.0975	0.0815	0.3010	0.2085
02x04	-0.0186	0.0080	0.7597	1.0170
02x05	-0.0876	-0.0415	-0.6338	-0.5205
02x06	0.0145	0.0150	-0.5850	-0.3575
02x07	0.0148	0.0185	0.1231	-0.2125
02x08	-0.0194	0.0570	-0.6152	-0.2060
02x09	-0.0750	0.0330	0.0923	0.6075
02x10	-0.0149	-0.0110	-0.1607	0.2060
02x11	0.0192	0.1035	0.6321	0.5215
02x12	0.0078	0.0570	-0.1646	0.2805
03x04	0.0182	-0.0210	0.2760	0.3855
03x05	-0.0022	-0.0300	-1.1005	0.0270
03x06	-0.0824	-0.0070	-0.2812	-0.3735
03x07	-0.0391	-0.1085	-0.1570	0.0045
03x08	0.0270	0.0595	-0.2389	0.4565
03x09	0.0839	0.0200	0.9371	0.0835

Table 8 - Cont…

Crossing	ERP[2]		EGY[3]	
	SCA[1]	Reciprocal	SCA[1]	Reciprocal
03x10	0.0064	0.0005	0.5775	1.2905
03x11	-0.0008	-0.0245	-0.1945	0.1030
03x12	-0.0792	0.0930	-0.2538	-0.0965
04x05	-0.0084	0.0035	-0.3923	-0.1370
04x06	-0.0207	-0.1065	0.1414	-0.1320
04x07	0.0180	0.0255	0.3411	0.4545
04x08	-0.0077	-0.0665	-0.0097	-0.0315
04x09	-0.0638	-0.0670	0.3098	-0.0690
04x10	0.0282	0.0330	-0.8207	0.1320
04x11	-0.0055	0.0005	-0.7713	0.2040
04x12	0.0435	0.0075	-0.0841	0.0340
05x06	0.0392	0.0170	0.5863	2.1180
05x07	0.1130	-0.0830	1.3465	-0.1430
05x08	-0.0112	0.0195	-0.1798	-0.6655
05x09	-0.0733	0.0400	0.1412	-0.2325
05x10	-0.0602	-0.1650	-0.3248	-0.2570
05x11	0.0464	0.0500	-0.0269	-0.5745
05x12	-0.0499	-0.0055	0.5912	-0.5095
06x07	-0.0726	-0.1165	-0.1046	-0.2860
06x08	-0.0204	-0.0525	0.2704	0.9410
06x09	0.0504	-0.0240	-0.0439	0.0445
06x10	0.0094	-0.0220	0.1239	0.1900
06x11	0.1221	-0.0240	-0.4421	-0.3195
06x12	-0.0232	-0.1205	0.2745	0.2070
07x08	0.0198	0.0180	-0.0578	0.5755
07x09	0.0057	-0.0205	-0.4772	-0.2470
07x10	0.1027	0.0445	0.3491	0.3170
07x11	0.0084	-0.0425	-0.0374	0.3030
07x12	-0.0514	0.0555	-0.6887	0.0695
08x09	-0.0755	-0.0330	0.3229	-0.6980
08x10	0.0399	-0.0035	-0.3247	0.7400
08x11	0.0171	-0.1190	0.6531	-0.0465
08x12	0.0357	-0.0695	-0.0346	0.5525
09x10	-0.0201	-0.0800	-0.4766	0.1230
09x11	-0.1034	0.0680	0.5652	0.2155
09x12	0.1681	-0.0715	-0.5955	-0.2175
10x11	-0.0738	0.2235	-0.6123	0.2465
10x12	-0.0057	0.0505	1.2913	1.4980
11x12	0.0087	0.0230	-0.1167	-0.4495
SD[4](Sij)	0.4522	-	0.4522	-
SD(Sij-Sik)	0.6708	-	0.6708	-
SD(Sij-Skl)	0.6324	-	0.6324	-
SD(Rij)	-	0.5000	-	0.5000

[1]SCA: specific combining ability; [2]ERP: efficiency of use of *A. brasilense* for relative ear position; [3]EGY: efficiency of use of *A. brasilense* for grain yield; [4]SD: standard deviation.

Reciprocal diallel crosses had no significant effect on the traits under study. This prevented the determination of the genotypes to be used as male and female parents in hybridization aiming to obtain efficient genotypes regarding the use of *Azospirillum* (Tables 7 and 8). The results of the crosses and their reciprocals were statistically equal, meaning that nuclear genes probably control the inheritance of these traits. Therefore, there is no maternal inheritance in their determination (Cruz & Regazzi, 2014). The minimum and maximum values for the reciprocal crosses were, respectively: -0.125 (06x07) and 0.225 (02x12) for EPH; -0.182 (03x07) and 0.2975 (02x12) for EEH; -0.165 (05x10) and 0.2235 (10x11) for ERP; -0.698 (08x09) and 2.118 (05x06) for EGY. The highest amplitude value is 2.816, corresponding to EGY.

Among the 66 crosses, 36 showed positive values and 30 showed negative values for EPH. For EEH, 34 crosses showed positive values and 32 showed negative values. For ERP, 32 crosses showed positive values and 34 showed negative values. For EGY, 37 crosses showed positive values and 29 showed negative values.

In the experiment by Buzinaro et al. (2018), the estimates for the effects of GCA for efficiency to *Azospirillum* in plant height, ear height, and grain yield, as well as for the effects of SCA for efficiency in grain yield, were significant. The authors concluded that additive effects influence the genetic control of the three traits in question, and that nonadditive effects influence the genetic control of the trait grain yield.

These contrasting results are understandable since GCA and SCA estimates are relative measures of the genotypes. These estimates thus depend on the population groups and evaluation conditions of each experiment, and may also suffer from environmental interference (Griffing, 1956).

Taken in isolation, the results of the present study are interesting for genetic breeding programs that aim at efficiency in the use of *Azospirillum brasilense*. As there is no maternal inheritance for these traits, only half of the genotypes need to be evaluated in the field since the study design can exclude reciprocal crosses (Bordallo, 2005; Cruz & Regazzi, 2014).

Paternianini & Campos (2005) described all the stages of a maize breeding program, highlighting the evaluation of the combining ability of genotypes. This stage includes the use of many materials in the field, demanding more investment, labor, and time. Removing the reciprocal of each cross for field evaluations optimizes costs and time.

Conclusions

There is no maternal inheritance for the efficiency of use of *Azospirillum brasilense* regarding plant height, ear height, relative ear position, and grain yield, indicating that nuclear genes govern these traits.

For these traits, there is no need to evaluate reciprocal crosses in the field, which optimizes resources and time in genetic breeding programs.

This study identified efficient genotypes for the use of *Azospirillum brasilense* for all traits under study. The most responsive genotypes regarding grain yield efficiency were: 6, 11, 15, 17, 31, 56, 92, 102, 103, 105, 118, and 121.

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