

GENETIC DIVERGENCE WITH AND WITHOUT USE OF *AZOSPIRILLUM BRASILENSE* IN CORN CULTIVARS

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Abstract–The present work aims to analyze the genetic divergence with and without the use of *Azospirillum brasilense* in maize cultivars. The experiment was carried out at the Federal University of Tocantins (UFT), Gurupi Campus. The design used was randomized blocks, with ten treatments and three replications, where the treatments are represented by ten maize cultivars. The variables were measured when the plant reached R6 stage. For the study of genetic divergence, the following variables were analyzed: The number of grains per row (NGPF), number of rows per spike (NFE), plant height (AP), the height of the spike (AE), moisture (UMID), grain yield (kg). The Tocher method was used to verify similar groups; for the verification of genetic distance, there was the foundation through the generalized distance of Mahalanobis (D^2) and Singh's criterion for quantifying the contribution of the variables evaluated. The characteristics of grain yield and number of grains per row were the ones that most contributed to the genetic divergence for *treatments with Azospirillum* and without *Azospirillum*. The crosses between cultivars aiming at promising combinations to obtain strains are BM3051 X CATIVERDE with *Azospirillum* and BM3051 X CATIVERDE without *Azospirillum*.

INTRODUCTION

One of the crops highlighted in grain production in the world is the corn crop (*Zea mays* L.). In Brazil, the area of grain cultivation is 22.1 million hectares and with an estimated production of 116.3 million tons, being the third largest producer in the world of the crop (Conab, 2022). Corn is a cereal of very nutritious quality, mainly used as an energy source because it is rich in starch (Borém *et al.*, 2015).

The use of biological agents in the crop has intensified aiming at reducing fertilizer costs, improving plant development, and consequently,

increasing the productivity of cereal production areas. For corn crops, inoculation with bacteria of the genus *Azospirillum* stands out, which contributes to plant development and increased grain yield of corn cultivars due to their performance in the plant stimulating the production of plant hormones such as gibberellins, auxins, and cytokinin's and their biological nitrogen fixation capacity (Assunção *et al.*, 2021; Galindo *et al.*, 2017; Garcia *et al.*, 2017; Gavilanes *et al.*, 2019; Portugal *et al.*, 2017).

Thus, breeding programs have been one of the main ones responsible for the success of corn crops, providing improved genotypes for various regions

of the country. To obtain improved maize materials, it is important to crossbreed genetically distant individuals to generate greater genetic variability of the segregating population to select superior genotypes.

The study of genetic divergence is fundamental for genetic improvement programs because it allows the knowledge of the genetic distance between individuals, aiming to obtain greater gain with the selection in the crosses of genetically different groups that have desirable characteristics.

Genetic divergence in corn crops has been studied by several authors (Santos *et al.*, 2014; Santos *et al.*, 2015; Silva *et al.*, 2015; Santos *et al.*, 2017; Sodre *et al.*, 2017; Santos *et al.*, 2018), however, there are few studies developed in the northern region of the country on the production of corn using bacterium.

Therefore, the present work aims to evaluate the genetic divergence in corn cultivars in the southern Tocantins with the inoculation of *Azospirillum* and without inoculation of the bacterium.

MATERIALS AND METHODS

The experiment was carried out at the Federal University of Tocantins (UFT), Campus from Gurupi (11°44' south latitude, 49°05' west longitude, and altitude of 280 meters) in the agricultural year 2019/20, the sowing on 02/11/2019.

The climate of the region is classified as AW, tropical, with moderate water deficiency, the temperature the average annual temperature is 33°C in the dry season and 26°C in the rainy season, with average annual precipitation of 1804 mm, with rainy summer and dry winter, according to the classification of Köppen (Dubreuil *et al.*, 2019).

According to soil analysis (Table 1), the dredging was first carried out with the application of two tons ha⁻¹ dolomitic limestone Filler, incorporated into the soil through the aration and grading operations in the surface layer (0-20 cm). Then, the resulting was performed in the area, followed by the application of fertilizer, and sowing which was performed manually.

Through the demands of culture (Ribeiro *et al.*, 1999), the recommendation of the basic fertilization was 500 kg ha⁻¹ of the formulated 5-25-15 and for cover fertilization 150 kg ha⁻¹ urea (43% from N), fractional in two applications, in the V4 (Fourth expanded sheet) and V6 (Sixth expanded sheet).

Inoculation with *Azospirillum brasilense* strain AbV5 and AbV6, in concentration 5 x 10⁸ Cells mL⁻¹ via soil, at the dose of 600 mL ha⁻¹ recommended by the manufacturer (Qualyfix Gramineas®).

The experimental design used was randomized blocks with 10 treatments and 03 replicates. The treatments were represented by the following cultivars: AG1051, AG8088, ANHEMBI, BM3051, BR205, BRS3046, BRS2022, CATIVERDE, M274, and PR27D28, as described (Table 2).

The experimental unit was composed of two rows of 3.0 m in length adopting spacing of 1.0 m between rows, with an experimental area of 6 m². In each linear meter, 5 seeds were sowed, obtaining a final population of 50,000 plants ha⁻¹.

The cultural treatments for the control of weeds, diseases, and pests were carried out according to the technical recommendations of the crop (Borém *et al.*, 2015).

The variables were measured when the plant reached the R6 stage. For the study of genetic divergence, the following variables were analyzed: The number of grains per row (NGPF), number of rows per spike (NFE), plant height (AP), the height of the spike (AE), moisture (UMID), grain yield (kg) (Embrapa, 2010).

Dissimilarities were determined in the presence of the *Azospirillum* - CA and the absence of *Azospirillum* - SA according to the multivariate analysis model, which allowed the obtaining of dissimilarities, residual covariances, and population means. Then, the Tocher grouping method was applied (Rao, 1952), with and without *Azospirillum*, using the generalized Mahalanobis distance (D²) (Mahalanobis, 1936), and Singh criterion (1981) to quantify the relative contribution of the characteristics.

Table 1. Results of the chemical analysis of the soil in the 0-20 cm layer for the experiment site. Gurupi- TO, 2020.

pH ¹	M.O. dag.kg ⁻¹	P ² mg dm ⁻³	K ²	K ²	Ca ³	Mg ³	Al ³	H+Al ³	SB	CTC	V %
5.2	1.7	2.2	30	0.08	1.2	0.7	0.0	2.50	1.98	4.48	44
	Clay(g kg ⁻¹) 275				Silt(g kg ⁻¹) 50					Sand(g kg ⁻¹) 675	

(1): CaCl₂ 0.01 mol L⁻¹; (2): Extractor Mehlich; (3) KCL 1mol.

Statistical analyses were performed using the Computational Genes program, version 2007 (Cruz, 2007).

RESULTS AND DISCUSSION

The measures of genetic dissimilarity estimated from the Mahalanobis distance (Table 3) presented a very significant magnitude (2.31 to 115.34 With *Azospirillum* - CA; 2.12 to 42.39 Without *Azospirillum* - SA), indicating that there is genetic variability among the evaluated cultivars. The amplitudes of the values of Mahalanobis dissimilarity estimates observed suggest that there is genetic variability, thus enabling the choice of contrasting parents for the extraction of desirable characteristics that can be used in future breeding programs (Santos *et al.*, 2014; Santos *et al.*, 2015; Silva *et al.*, 2015; Santos *et al.*, 2017; Sodre *et al.*, 2017; Santos *et al.*, 2018).

It is observed that longer distances (Table 3) represent cultivars that probably came from different germplasm banks, and shorter distances, cultivars that can probably be from the same germplasm bank (Santos *et al.*, 2015). The aid for the choice of parents to be used in future crossings can be done by analyzing genetic distance, this will save time, manpower, and financial resources (Silva *et al.*, 2015).

The combination of cultivars BM3051 and CATIVERDE showed the most divergent ($D^2 = 115.34$ CA; $D^2 = 42.39$ SA) (Table 3). Next, the combination of AG1051 and CATIVERDE ($D^2 = 101.84$ CA; $D^2 = 34.07$ SA), BRS3046 and CATIVERDE ($D^2 = 83.45$ CA; $D^2 = 38.23$ SA), and

BR205 and BM3051 ($D^2 = 71.40$ CA; $D^2 = 34.88$ SA) cultivars had the highest distances.

The shortest distance was obtained between the combination of cultivars ANHEMBI X BRS2022 ($D^2 = 2.31$ CA) and AG1051 X BM3051 ($D^2 = 2.12$ SA). The shorter the distance between cultivars, the greater the genetic similarity between cultivars, a factor capable of reducing the probability of success in obtaining hybrids (Santos *et al.*, 2014).

Using the method of optimizing Tocher (Rao, 1952), that separates the cultivars into distinct groups, so that there is intragroup homogeneity and intergroup heterogeneity (Cruz *et al.*, 2014) based on the divergence obtained through Mahalanobis distance, and it is possible to observe how the evaluated cultivars were distributed (Table 4).

The cultivars were divided into five groups, in Group I was the cultivars with the greatest similarity to each other genetically, totaling four SACultivars (ANHEMBI, BRS2022, AG8088, and M274) and three SACultivars (AG1051, BM3051, and PR27D28). Group II (CA and SA) and III (CA and SA), present two cultivars. Group IV (CA) and V (CA and SA) with only one cultivar, indicating greater divergence about the other cultivars, thus facilitating the projection of a breeding program (Santos *et al.*, 2014; Santos *et al.*, 2015; Santos *et al.*, 2017; Santos *et al.*, 2018; Rotili *et al.*, 2012; Silva *et al.*, 2019).

When analyzing the grouping of ten maize cultivars CA and SA, changes in cultivars within the groups were observed through the inoculation process, inoculation with *Azospirillum* provided changes in the distances between cultivars. The *Azospirillum* when inoculated in the plant can cause

Table 2. Agronomic characteristics of the ten corn cultivars used in the experiment.

Trade name	Genetic basis	Transgenic	Cycle	Purpose of use	Technological level
AG 1051	HD	C	SMP	G/SPI/MV	M/A
AG 8088 PRO2	HS	PRO2	P	G/SPI	A
ANHEMBI	PPA	C	P	G/SPI	B/M
BM3051	H	C	P	MV/SPI	M/A
BR 205	HD	C	P	G/SPI	B/M
BRS 3046	HT	C	SP	MV/SPI	M/A
BRS2022	HD	C	P	G/SPI	B/M
CATIVERDE	PPA	C	SMP	MV/SPI	M
M274	HD	C	P	G/SPI	B/M
PR27D28	HD	C	P	G/SPI	M/A

HS: simple hybrid; HD: double hybrid; HT: triple hybrid; PRO2: technology VT PRO 2™; C: conventional; P: precocious; SMP: semi-early; SP: Superprecocious; G: grain; MV: green corn; SPI: silage of the whole plant; SGU: wet grain silage; A: high; M: medium e B: low.

Source: Cruz *et al.* (2015).

Table 3 - Estimation of distances from Mahalanobis (D^2) maximum and a minimum of the 10 maize cultivars.

Cultivate	With <i>Azospirillum</i>			Without <i>Azospirillum</i>		
	Bigger	Cultivate	Minor	Cultivate	Minor	Cultivate
BRS3046	83.45	(CATIVERDE)	7.71	(CATIVERDE)	3.69	(AG1051)
AG1051	101.84	(CATIVERDE)	7.71	(BRS3046)	2.11	(BM3051)
AG8088	38.56	(BM3051)	6.67	(BRS2022)	3.94	(M274)
ANHEMBI	36.17	(CATIVERDE)	2.31	(BRS2022)	4.35	(BRS2022)
BM3051	115.34	(CATIVERDE)	8.25	(AG1051)	2.12	(AG1051)
BRS2022	34.87	(BM3051)	2.31	(ANHEMBI)	4.35	(ANHEMBI)
BR205	71.4	(BM3051)	7.77	(CATIVERDE)	2.67	(CATIVERDE)
CATIVERDE	115.34	(BM3051)	7.77	(BR205)	2.67	(BR205)
M274	50.79	(CATIVERDE)	2.53	(ANHEMBI)	3.94	(AG8088)
PR27D28	66.83	(BM3051)	10.1	(BRS2022)	2.33	(BM3051)
Biggerdistance	115.34	(BM3051 and CATIVERDE)			42.39	(BM3051 and CATIVERDE)
Minordistance	2.31	(ANHEMBI and BRS2022)			2.12	(AG1051 and BM3051)

In parentheses are represented cultivars.

several physiological changes, bringing a beneficial relationship to the plant by providing better root development, improving its absorption of water and nutrients, and promoting vegetative growth (Assunção *et al.*, 2021).

Of the six characteristics evaluated in Table 6, the one that most contributed to the divergence was the PRODG (35.13% CA; 27.06% SA) and NGPF (22.73% CA; 25.90 SA), and the smallest contributions were NFE (3.11% CA; 9.83 SA) and UMID (4.68% CA and 10.20% SA). Therefore, in the present work, the

Table 4. Grouping of the ten maize cultivars by the Tocher method (Rao, 1952), based on the generalized Mahalanobis distance.

Group	Without <i>Azospirillum</i>	
	With <i>Azospirillum</i>	Cultivate
I	ANHEMBI, BRS2022, AG8088 and M274	AG1051, BM3051, and PR27D28
II	BRS3046 and AG1051	BR205 and Cativeverde
III	BR205 and CATIVERDE	AG8088 and M274
IV	PR27D28	Anhembí and BRS 2022
V	BM3051	BRS3046

Table 5. Average distances between groups formed by genetic divergence analysis in maize cultivars.

Groups	Without <i>Azospirillum</i>	
	With <i>Azospirillum</i>	Sum
I x II	152.16	188.87
I x III	237.16	54.84
I x IV	48.50	85.41
I x V	120.10	13.19
II x III	292.81	51.30
II x IV	92.90	37.47
II x V	21.87	67.58
III x IV	37.76	29.09
III x V	186.74	18.60
IV x V	66.83	41.91

Table 6. Relative contribution of the characteristics of the ten maize cultivars, following Singh criterion (1981).

Variable	Without <i>Azospirillum</i>	
	With <i>Azospirillum</i>	Value in %
NGPF	22.73	25.90
NFE	3.11	9.83
AP	23.53	7.34
AE	10.82	19.67
UMID	4.68	10.20
PRODG	35.13	27.06

characteristics of NFE and UMID can be discarded in future evaluations, because their contribution is little to discriminate the evaluated cultivars, and can then reduce time, labor, and costs in the new breeding programs (Coelho *et al.*, 2019; Santos *et al.*, 2014; Santos *et al.*, 2015; Santos *et al.*, 2017; Santos *et al.*, 2019).

Conflict of Interest

There is no conflict of interest between the authors. all authors contributed directly to the article.

CONCLUSION

The characteristics of grain yield and number of grains per row were the ones that most contributed to the genetic divergence for *treatments with Azospirillum* and without *Azospirillum*.

Azospirillum influenced the expression of genetic variability.

The crosses between cultivars aiming at promising combinations to obtain strains are BM3051 X CATIVERDE with *Azospirillum* and BM3051 X CATIVERDE without *Azospirillum*.

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