Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 23, No. (4) : 2021 : 626-632

ISSN-0972-3005

AGRONOMIC PERFORMANCE OF CORN CULTIVARS WITH AND WITHOUT INOCULATION OF *AZOSPIRILLUM BRASILENSE*

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(Received 31 August, 2021; Accepted 4 November, 2021)

Key words: *Zea mays L. Growth-promoting bacteria, Nitrogen fertilization, Nitrogen fixation.*

Abstract–Maize plays an important role in the economy and food security of several countries and is highlighted as the main source of carbohydrates. Corn can be consumed in several ways: grains, starch, flour and oil. The products derived from the grains of this cereal have various applications in the food, chemical, pharmaceutical and cosmetic industries. In the search for a more sustainable agriculture, bacteria have been sought to make use of bacteria for biological nitrogen fixation. The genus *Azospirillum* are the bacteria that have obtained better results in corn crop for n fixation of the atmosphere, besides providing results in the promotion of growth and antagonistic in some phytopathogens. In view of the above, the objective of this work was to evaluate the agronomic performance of corn cultivars with grain aptitude on the effect of seed treatment with the bacterium *Azospirillum brasilense*. The experiment was carried out at the Federal University of Tocantins (UFT), Gurupi Campus in the agricultural year 2018/19, and the sowing in December. An experimental design was used in randomized blocks, with 3 replications, in a 2 x 10 factorial scheme, totaling 20 treatments. The first factor consisted of the use of seeds treated with *Azospirillum brasilense* and without *Azospirillum brasilense*. The second factor refers to the ten commercial maize cultivars. The agronomic characteristics evaluated were: number of grains per row, number of rows per ear, plant height, ear height and grain yield. The data were submitted to analysis of variance, after testing the normality of the data by the Shapiro-Wilk test, at 5% significance. Then, the Scott-Knott mean test was used at 5% probability. Statistical analyses were performed using the statistical program SISVAR 5.0. The application of *Azospirillum brasilense* applied in different maize cultivars was not significant at 5 % probability for any agronomic characteristic analyzed in this experiment, i.e., the interaction was not significant for these factors.

INTRODUCTION

The corn (*Zea mays* L.) plays an important role in the economy and food security of several countries and is highlighted as the main source of carbohydrate (Santos *et al.,* 2019). Cereal is also the main component in the manufacture of animal feed and has been standing out as a raw material for ethanol production both in the United States and here in Brazil (Corsini, 2018).

Corn can be consumed in several ways: grains, starch, flour and oil. The products derived from the grains of this cereal have various applications in the food, chemical, pharmaceutical and cosmetic industries (Timm, 2020). In the Brazilian Cerrado corn is the most used plant for crop rotation and succession in the no-tillage system and occupying the second position in the ranking of the most produced grains (Rosa, 2021).

With a planted area of approximately 18,525.8 million hectares of corn production in Brazil in the harvest 2020/2021 was of 102.5 million tons and average productivity of 5533 kg ha^{-1}, record production that kept the country in third position in the world ranking (Conab, 2020). Corn production in Tocantins in the harvest 2019/2020 had an increase in 20% compared to the crop 2018/19. The production was 1195.82 thousand tons harvested in confrontation 992.76 thousand tons in the past harvest. In relation to the area, the state also showed a significant increase, as it went from 201.92 thousand hectares for 240.69 thousand hectares, this increase represented 19.2% (Seagro, 2020).

In Tocantins, corn production cannot be considered high when compared to the national production average, this can be explained due to the presence of adverse climatic conditions, lack of genetic stuff adapted to the region and low technological level employed by producers. Among the adverse conditions we can mention climatic and nutritional variations mainly related to nitrogen (Peluzio, 2018). Corn crop needs approximately 20 kg from N soil, with this, alternatives have been sought to reduce the production costs of this crop (Guimarães, 2017).

In the search for a more sustainable agriculture, bacterium have been sought to make use of bacteria for biological nitrogen fixation (Rosa, 2017). Research has been conducted in Brazil using bacterium diazotrophic in grasses, these microorganisms can be free-living, associated with plant species or also establish symbiosis with legumes, in addition, diazotrophic bacteria have the enzymatic complex dinitrogenase, that converts nitrogen gas (N_2) of the atmosphere in ammonia (NH_3) (Galeano *et al.*, 2019). Dartora *et al.* (2013) the genera *Azospirillum* are the bacterium that have obtained better results in corn crop.

Gender *Azospirillum,* is able to fix N of the atmosphere when associated with grasses, this is due to the modification of the morphology of the root system by the production of growth-promoting substances (Araújo, 2017). According to Costa Leite *et al.* (2018) growth promotion occurs due to higher production of gibereline hormones, auxins, cytokinins and ethylene. In addition, by penetrating the roots of plants these bacterium can help tolerance to temperature variations and provides antagonistic effect on some phytopathogens (Galeano *et al.*, 2019).

Several works have worked with bacterium *Azospirillum* over the last few decades especially in grasses such as: corn (Júnior *et al.*, 2020), sorghum (Schumacher *et al.*, 2021), wheat (Santos *et al.*, 2020), rice (Amaral *et al.*, 2020) forage plants (Domingues Duarte *et al.*, 2020) among other cultures. Rockenbach *et al.* (2017) cites that the barrier to the use of *Azospirillum* in maize crop is the lack of reluctance of scientific studies, considering that there are several studies confirming its use is beneficial in relation to corn yield, while others that do not report this effect and do not recommend its use for this purpose.

In view of the above, the objective of this work was to evaluate the agronomic performance of corn cultivars with grain aptitude on the effect of seed treatment with bacterium *Azospirillum brasilense*.

MATERIALS AND MÉTODOS

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 2018/19, being sowing on 02/11/2019.

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

According to soil analysis (Table 1) the dredging was carried out first with the application of two tons ha^{-1} of filler dolomystic limestone, proceeding with incorporation into the soil through the pheaking and grading operations in the surface layer (0-20 cm). Then, the sulcing was performed in the area, followed by the application of fertilizer and sowing that was performed manually.

Through the demands of culture (Ribeiro *et al.*, 1999), the recommendation of the base fertilization was 500 kg ha^{-1} of formulated 5-25-15 and for the cover fertilization 150 kg ha^{-1} of Urea (43% of N), fractionated in two applications, in stage V_4 (Fourth expanded leaf) and V_6 (Sixth expanded leaf).

An experimental design was used in randomized blocks, with 3 replications, in a 2 x 10 factorial scheme, totaling 20 treatments. The first factor consisted of the use of seeds treated with *Azospirillum brasilense* and without *Azospirillum brasilense.* In the treatment with *Azospirillum brasilense,* a proportion of 100 ml of inoculum to 50 kg of seed was homogenized in a plastic bag. The second factor refers to the ten commercial maize cultivars, which are listed below (Table 2) with their

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

pH[1]	M.O.	P[2]	K[2]	K[2]	Ca[3]	Mg[3]	Al[3]	H+Al[3]	SB	CTC	V
	dag.kg^{-1}	mg dm^{-3}		————————————			cmolc.dm^{-3}	————————————			%
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})	Silt(g kg^{-1})	Sand(g kg^{-1})
275	50	675

(1): CaCl2 0,01 mol L-1; (2): Extractor Mehlich: (3) KCL 1mol;

respective agronomic characteristics.

The experimental unit consisted of two rows of 3.0 m in length adopting spacing of 1 m between rows with an experimental area of 6 m^2. In each linear meter, 5 seeds were sowing, obtaining a final population of 50,000 plants ha^{-1}. The entire experimental area was used for the evaluations.

In pre-sowing, seeds with fungicide and insecticide (active ingredient Piraclostrobin, Methyl Tiofanato and Fipronil) were treated (Fernandes and Ávila, 2017). For cartridge Caterpillar control (*Spodoptera frugiperda*), Caterpillar elasmo (*Elasmopalpus lignosellus*) and Threaded Caterpillar (*Agrotis ípsilon*) throughout the crop cycle, insecticides were used: Deltametrina (200 ml ha^{-1}); Clorpirifós (1 L ha^{-1}); Lambda-Cialotrina + Clorantraniliprole (150 ml ha^{-1}) (Queiroz, 2018). For control of the Green-bellied Bedbug (*Dichelops furcatus*), Corn cigarrinha (*Dalbulus maidis*) and whitefly (*Bemisia argentifolii*) used: Tiametoxam + Lambda-Cialotrina (180 ml ha^{-1}), Imidacloprido + Bifentrina (400 ml ha^{-1}) e Acetamiprido + Alfa-Cipermetrina (250 ml ha^{-1}) (Borém *et al.*, 2017).

Some evaluations were carried out in the phenological stage R_3 (Pasty grain) considered ideal for green corn (Barros, 2019), the following agronomic characteristics: number of grains per row (NGF), occurred by choosing the most homogeneous and representative row of the ear; number of rows per spike (NFE), was held at the center of the ear. In the field, the height of the (AE) and plant height (AP), in cm, with a metric trena, considering the distance from the ground to the insertion of the first ear and last open leaf, respectively. At the end of the physiological maturation of the crop (R_6) five ears were collected from the second remaining half of the experimental unit. Then the ears were trodden, the grains weighed, the moisture corrected to 13% and grain yield (PG) transformed into kg ha^{-1} (Marafon *et al.*, 2015).

The data were submitted to analysis of variance, after testing the normality of the data by the Shapiro-Wilk test, at 5% significance.

Then, the Scott-Knott mean test was used at 5% probability. Statistical analyses were carried out using the statistical SISVAR 5.0 (Ferreira, 2011).

RESULTS AND DISCUSSION

In the analysis of variance (Table 3), the following coefficients of variation (CV) were observed for the

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

Trade name	Genetic basis	Transgenics	Cycle	Purpose of use	Technological level
AG8088 PRO2	HS	PRO2	P	G/SPI	A
M274	HS	C	P	G/SPI	B/M
ANHEMBI	PPA	C	P	G/SPI	B/M
AG1051	HD	C	SMP	G/MV/SPI	M/A
BR2022	HD	C	P	G/SPI	B/M
BR205	HD	C	P	G	B/M
BM3051	HS	C	P	MV/SPI	M/A
CATIVERDE	PPA	C	SMP	MV/SPI	M
PR27D28	HD	C	SP	G/SPI	B/M
BRS3046	HT	C	SMP	MV	M/A

HS: simple hybrid; HD: híbrido double; HT: híbrido triple; PRO2: technology VT PRO 2™; C: conventional; PW: technology Powercore™; P: precocious; SMP: semiprecocious; SP: Super precoceprecocious; G: grain; MV: corn green; SPI: silage of the whole plant; SGU: wet grain silage; A: high; M: medium and B: low.
Fonte: Cruz et al. (2015).

characteristics: 10.24% for the number of grains per row (NGF), 7.76% for number of rows per ear (NFE), 6.92% for plant height (AP), 16.41% for ear height (AE) and 23.68% for grain yield (PG). According to Pimentel-Gomes (2009) these data cause the NFE and AP to be classified as low CV and high experimental precision, already towards NGF and AE the experiment is classified as medium CV and average experimental precision and for PG the experiment is classified as high CV and low experimental precision.

The result of the variance analysis (Table 3) revealed a significant effect for all characteristics only for the cultivar. In this sense, the non-significance of the interaction *Azospirillum brasilense (A) x* cultivar (C) reveals that cultivars present similar behavior in the face of the use or not of *Azospirillum*.

Araújo (2017) also found similar results when evaluating the influence of *Azospirillum brasilense* application on corn crop. However, Hungary (2011) found yield rendiments higher than 7000 kg ha^{-1} when using N doses corresponding to less than 50% of that recommended for corn crop.

According to Repke *et al.* (2013) the factors that influence the response of the crop to the inoculation of *Azospirillum brasilense* are not fully understood. The successful results found in the literature when talking about plant combination – *Azospirillum brasilense* are mostly linked to characteristics of the bacterium itself, such as the ideal number of cells per seed, choice of strain and its viability (Tonial, 2018).

Junior (2019) comments that the concentration of *the bacterium Azospirillum brasilense* in the solution of the inoculant is more important than the dose and that the optimal bacterial concentration that promotes the best development of corn plants is 10 million viable cells ml^{-1}, that is, approximately 17,000 seed colony forming $units^{-1}$. Levels above the optimum present an inhibiting effect of the growth of inoculated plants, while low concentrations simply have no effect on the vegetative phase (Barros, 2019).

The differences between the cultivars, for all traits evaluated, are presented in Tables 4, 5 and 6.

The mean number of grains per row (NGF) (Table 4) of cultivars ranged from 40.11 (BRS3046) to 27.94 (CATIVERDE) thus enabling separation into two groups of mean. The group with the lowest averages was composed of the cultivars CATIVERDE (28.78), BR205 (27.11) and ANHEMBI (30.94) the rest of the cultivars evaluated are included in the group with the highest averages. Corsini (2018) found similar results when evaluating the influence of different methods of application of *Azospirillum brasilense.*

Table 4 also shows the number of rows per ear (NFE) of cultivars ranging from 16.78 (BR205) to 14.33 (PR27D28) and thus classified into two groups of means. The group with the highest averages was composed of the cultivars CATIVERDE (16.11), BRS3046 (16.33), AG8088 (16.67) and BR205 (16.78) and the rest of the cultivars were grouped in the group with the lowest averages. NFE and NGF are agronomic characteristics that directly influence corn crop yield (Guimarães, 2017).

In Table 5, plant height (PA) resulted in two distinct groups. The group with the highest mean was the group composed of cultivars M274 (203.56), AG1051 (206.39) and BM3051 (210.67) the rest of the cultivars were grouped in the group with the lowest averages. In this same table, the height of ears (AE) also promoted the formation of two groups, with cultivars with the highest averages AG 1051 (109.28),

Table 3. Summary of the Janalysis of variance of the agronomic characteristics of grain number per row (NGF), number of rows per ear (NFE), ear height (AE), plant height (AP) and grain yield (PG), of eight corn cultivars with and without seed treatment with *Azospirillum brasilense.* Gurupi - TO, 2020.

Source of variation	Degree of freedom	Middle square				
		NGF	NFE	AP	AE	PG
Block	2	3.22^{ns}	0.16^{ns}	13.63^{ns}	153.88^{ns}	4905760.21^{ns}
Azospirillum (A)	1	10.73^{ns}	0.89^{ns}	15.64^{ns}	366.70^{ns}	2598690.38^{ns}
Cultivate (C)	9	91.57^{*}	4.22^{*}	649.85^{*}	712.14^{*}	4776581.33^{*}
Interaction A x C	9	16.36^{ns}	2.61^{ns}	154.35^{ns}	150.12^{ns}	876036.69^{ns}
Residue	38	12.32	1.47	178.26	224.29	1735480.80
Total	59	3.22	0.16	13.63	153.88	4905760.21
Average	34,29	15.63	192.83	91.24	5564.18	
Coefficient of variation (%)	10,24	7.76	6.92	16.41	23.68	

* Significant and ^{ns} not significant by the F test to the 5% of significance.

Table 5. Average plant height (AP) and ear height (AE) in cm, of ten maize cultivars with and without seed treatment with *Azospirillum brasilense.* Gurupi – TO, 2020.

Cultivate	AP			AE		
	Com	Sem	Average	Com	Sem	Average
AG 8088	178.44	181.89	180.16 b	65.67	84.67	75.17 b
M 274	203.78	203.33	203.56 a	82.34	100.00	91.17 b
ANHEMBI	198.00	182.56	190.27 b	88.00	84.67	86.33 b
AG 1051	205.11	207.67	206.39 a	113.11	105.44	109.28 a
BR 2022	185.00	196.11	190.55 b	80.22	90.22	85.22 b
BR 205	179.44	192.56	186.00 b	84.22	89.44	86.83 b
BM 3051	219.22	202.11	210.67 a	109.78	106.33	108.06 a
CATIVERDE	186.89	177.44	182.17 b	86.22	79.33	82.78 b
PR 27D28	188.44	189.78	189.11 b	82.55	96.33	89.44 b
BRS 3046	189.11	189.78	189.44 b	95.56	100.67	98.11 a
Average	193.34	192.32		88.76	93.71	

Averages followed by different letters, uppercase in rows and lowercase in columns, differ significantly, by the Scott-Knott test, at 5% probability.

BM3051 (108.06) and BRS3046 (98.11).

Caprio and Môro, (2017), when evaluating maize cultivars under inoculation *with Azospirillum brasilense* at different sowing times, they also found non-significant differences between them regarding AP and AE.

The average result for PG (Table 6), with the application of *Azospirillum brasilense* was 5,772 kg ha^{-1} and without the application of *Azospirillum brasilense* was 5,356 kg ha$^{-1,}$however, despite a difference of 416 kg ha^{-1}, that is, 6.93 bags, this result was not significant.

Segundo Junior; Freitas; Rezende, (2021); Matos *et al.,* (2017); Rockenbach *et al.,* (2017) the application of *Azospirillum brasilense* also did not influence PG in their experiments. On the other hand, Parente, (2019) found difference of 909 kg ha^{-1} when evaluating their experiment with and without the use of *Azospirillum brasilense,* however, this difference was not significant according to the test used by him.

Average grain production (PG) ranged from 6,868 kg ha^{-1} (AG1051) to 3,968 kg ha^{-1} (CATIVERDE), which resulted in the formation of two groups. The group with the highest averages consisted of AG1051 (6,868 ha^{-1}), BRS3046 (6,332 kg ha^{-1}), BM3051 (6,116 kg ha^{-1}), AG8088 (5,852 kg ha^{-1}) and M274 (5,800 kg ha^{-1}). With the lowest averages, the group was: ANHEMBI, BR2022, BR205, CATIVERDE and PR27D28.

Means followed by different letters, uppercase in rows and lowercase in columns, differ significantly, by the Scott-Knott test, at 5% probability.

Although in the present study no advantages were observed in the *use of Azospirillum brasilense* in agronomic characteristics, as well as in grain yield, the use of *Azospirillum brasilense* is a relevant tool for sustainable agriculture, as recommended by (Fukami *et al.,* 2016).

Thus, due to factors such as soil, fertilization, soil microbiota, climate and cultivars used to interfere in the inoculation result, further research on the subject is needed, aiming at a better understanding of the effect of this bacterium on corn yield and other agronomic, biometric, nutritional and physiological characteristics.

Table 6. Average grain yield (PG) in kg ha^{-1}, of ten maize cultivars with and without seed treatment with *Azospirillum brasilense.* Gurupi – TO, 2020.

Cultivate	PG		
	Com	Sem	Average
AG 8088	5,853	5,851	5,852 a
M 274	5,842	5,757	5,800 a
ANHEMBI	5,760	4,472	5,116 b
AG 1051	7,424	6,312	6,868 a
BR 2022	5,949	6,012	5,981 b
BR 205	5,004	3,763	4,383 b
BM 3051	6,172	6,061	6,116 a
CATIVERDE	4,526	3,411	3,968 b
PR 27D28	4,684	5,764	5,224 b
BRS 3046	6,508	6,157	6,332 a
Average	5,772	5,356	

Conflict of Interest

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

CONCLUSION

The use of Azospirillum *brasilense* did not promote a significant increase in characteristics.

The most productive cultivar with and without the use of *Azospirillum brasilense* was the double hybridAG1051, BRS3046, BM3051, AG8088 and M274.

Further research is needed to better understand the effect of bacterium on corn yield.

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