

Cultivar x environment interaction on green ear yield in corn inoculated with *Azospirillum brasilense*, at low latitude

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Abstract — *The cultivation of green corn has been increasingly important for small farmers, due to its economic and social importance, derived from consumption in natura in the form of green ears. Thus, the present work was carried out to study the behavior of corn cultivars, in the presence and absence of the bacterium *Azospirillum brasilense*, under different nitrogen doses, aiming at the productivity of green ears in cultivation under low latitude. Two trials were installed, one in the agricultural year 2019/20 and the other in the agricultural year 2020/21, in the central region of the State of Tocantins. The experimental design used in each assay was randomized blocks, with three replications. The treatments were arranged in subdivided plots, where treatments involving seed inoculation with the bacterium were allocated in the plots *Azospirillum* (C Az) and without inoculation of seeds (S Az), in the subplots five doses of nitrogen (00, 30, 60, 90 and 120 kg ha⁻¹ N) and in the subplots eight maize cultivars. For each process (C Az and S Az), an adaptability and stability study were carried out using the Eberhart & Russell (1966) and environmental stratification by the method of Lin (1982), where the combination of each dose of N, in each assay and in each process (C Az e S Az) represented a distinct environment. There was a differential response of the cultivars between the processes with and without seed inoculation. Seed inoculation resulted in a higher increase in the productivity of green ears. The cultivar BRS-3046 and AG-1051 adapted to the environments.*

I. INTRODUCTION

The corn (*Zea mays* L) has aroused great economic interest due to its nutritional properties, being used in human food, mainly in natura like green corn (roasted, baked, porridge, pamonha, bled and other), which has

driven social, economic, and cultural development in small and medium-sized properties [1].

To obtain a high productivity of corn, nitrogen fertilization is indispensable, since nitrogen is the mineral nutrient required in greater quantity by the crop, because it

acts on root growth and vegetative development, directly participating in the biosynthesis of proteins and chlorophylls, which reflects in productivity gains [2].

However, due to the high cost of this intake, combined with the environmental risk arising from its use, there is a need to incorporate technologies for rationalization and awareness in the use of nitrogen fertilizers [3]. In this sense, one of the alternatives would be the use of diazotrophic bacteria capable of making atmospheric N available to the corn plant, enabling crop growth and increased grain yield [4], as well as a reduction in the use of nitrogen fertilizers and the final cost of crop implantation [1].

Second Moreira et al. [5], diazotrophic bacteria can contribute to plant growth through nitrogen supply, phosphate solubilization and increased nitrate reductase activity [6]. In addition, these bacteria may result in changes in the morphology of the root system, in the number of radículas and diameter of the roots, probably due to the production of growth-promoting substances: auxins, gibberelins and cytokinins [7].

Increases in grain yield in corn crop when inoculated with *Azospirillum brasilense* have been observed in several studies involving maize [8]. To produce green corn, Araújo et al. [9], when studying the effect of inoculation with *Azospirillum brasilense*, associated with nitrogen fertilization, there was a significant increase in the number and mass of commercial ears with the inoculation of *A. brasilense*, treatment without inoculation, and that the combination of inoculation with *A. brasilense* and nitrogen increases by more than 30% the production of green corn cobs.

In a series of environments represented by years, locations, sowing times, different forms of management, fertilization and others, cultivar interaction x environment (C x A) that influences the performance of cultivars, hindering the selection process of those with superior characteristics. Aiming to mitigate the effect of this interaction, the identification and use of genotypes with wide adaptability and stability [10] and the identification of similar environments, which makes the improvement program more agile and reduces costs [11], have been tools used.

In this sense, the identification of green corn cultivars with adaptability and specific stability to different environments, combined with the use of new technologies, such as nitrogen-fixing bacteria, could result in increases in current productivity indices, as well as promote a rationalization in the use of nitrogen fertilizers.

However, after the economic, social importance and they're in natura consumption in the form of green ears,

there are few studies involving the green corn crop, for this purpose, aiming at the identification of cultivars, associated with the use of new technologies, such as nitrogen-fixing bacteria, in the presence of different nitrogen doses, under low latitude conditions, to which the present study is proposed.

II. MATERIAL AND METHODS

The present study was carried out in the experimental area of the Federal University of Tocantins - UFT, campus of Palmas – TO (altitude of 230 m, latitude 10°12'54"S and longitude 48°20'02"W). Two tests were installed, the first season being in the agricultural year 2019/20, in sowing carried out on 12/04/2019, and the second season in the agricultural year 2020/21, in sowing carried out on 10/12/2020.

The soil of the experimental area, where the tests were carried out, according to the Brazilian Soil Classification System is considered as dystrophic Yellow Red Latosol. Soil samples collected at a depth of 0 to 20 cm showed, on average, the following characteristics: pH (CaCl₂) 6,0; Clay 15,5%; Silte 5,9%; Sand 78,6%; M.O 11,63 g dm⁻³; P (Mehlich⁻¹) 9,92 mg dm⁻³; K 0,2 cmol dm⁻³; Ca 1,90 cmol dm⁻³; Mg 1,12 cmol dm⁻³; S.B 3,22 cmol dm⁻³; CTC 5,02 cmol dm⁻³, e V 64,14%. It is emphasized that the two tests were performed in adjacent areas, in the same location.

Table 1 shows the average rainfall temperatures and precipitations recorded in the agricultural years 2019/2020 and 2020/2021 in the UFT experimental station [12].

Table 1. Average temperatures (°C) and rainfall (mm) in the conduction period of the tests in the 2019/2020 and 2020/2021 harvests in Palmas - TO.

Period	Crop 2019/2020		Crop 2020/2021	
	Temp. average (°C)	Precipitation (mm)	Temp. average (°C)	Precipitation (mm)
November	28.6 °C	198 mm	27.9 °C	52 mm
December	26.9 °C	298 mm	26.8 °C	258 mm
January	26.8 °C	308 mm	26.3 °C	349 mm
February	26.9 °C	342 mm	24.2 °C	485 mm
March	26.5 °C	420 mm	26.1 °C	511 mm
Average	27.0 °C	314 mm	26.4 °C	338 mm

Source: [12].

The experimental design used in each assay was randomized blocks, with three replications. The treatments were arranged in subdivided plots, where treatments with

seed inoculation were allocated in the plots with *Azospirillum* (C Az) and without inoculation of seeds (S Az), in the subplots five doses of nitrogen (00, 30, 60, 90 and 120 kg ha⁻¹ N) and in the subsubplots eight maize cultivars, three of which were simple hybrids (M-274, PR-27D28, AG 8088-PRO2), two double hybrids (BRS-2022, AG-1051), two triple hybrids (BRS-3046, BM-3061) and a variety of open pollination (Anhembí), all acquired in the local trade. The experimental plots consisted of four rows, with 3,0 m length, spaced by 1,0 m totaling an area of 12,0 m².

The tillage was in conventional cultivation, without the need for cathes. At sowing, fertilization was performed in the groove with 70 kg ha⁻¹ from P₂O₅, and 48 kg ha⁻¹ from K₂O potassium chloride.

Sowing was performed no-side in the groove, and the seeds were inoculated 30 minutes before planting with the bacterium *Azospirillum brasilense* (AbV5 and AbV6), being 100ml for each 25 kg as recommended by the manufacturer. Population density was 50,000 plants per hectare [13].

Weed control was performed using a post-emergent herbicide. It was not necessary to control pests and diseases. Cover fertilization was performed with ammonia sulfate (21% from N), in the doses (00, 30, 60, 90 and 120 kg ha⁻¹ N), between the lines of the plots, half of which

were applied to the V4 and half in V8 (four and eight true leaves, respectively) [14].

Based on the useful area of the plot (two central rows), green ears were collected as the grains were between the stages of milky grain (grain with about 80% moisture) and pasty grain [15]. Then the ears were scattered, and the weight of each parcel converted into kg ha⁻¹.

After obtaining the productivity data of the green ears, statistical analyses were performed for each process, i.e., for the process with inoculation of the seeds with *Azospirillum* (C Az) and for the process without inoculation of seeds (S Az). Initially, individual variance analysis was performed and, later, joint analysis of the assays was performed, in which the smallest residual mean square did not differ by more than seven times the largest. Then, for each process, adaptability and stability analyses were performed according to Eberhart & Russel [16], as well as environmental stratification according to the method of grouping environments based on the Lin algorithm [17].

In statistical analysis, in each process, the combination of each dose of N (kg by ha⁻¹) in each of the trials (sowing time), represented a distinct environment. Thus, for each process (C Az and S Az), ten environments were obtained from the combination of the five doses of N with the two assays, as shown in Table 2.

Table 2. Environments derived from the combination of two assays (sowing times) and five nitrogen doses in cover (kg by ha⁻¹) in seed inoculation processes (C Az and S Az) for productivity of green ears in Palmas - TO.

Environment	Epoch 1	Dose N	Environment	Epoch 2	Dose N
1	04/12/2019	00	6	10/12/2020	00
2	04/12/2019	30	7	10/12/2020	30
3	04/12/2019	60	8	10/12/2020	60
4	04/12/2019	90	9	10/12/2020	90
5	04/12/2019	120	10	10/12/2020	120

Statistical analyses were performed using the statistical computer program Genes [18].

III. RESULTS AND DISCUSSION

For the process without inoculation of seeds (S Az) the analysis of joint variance showed significant effect of environment, interaction Cultivars x Environments, and not significant effect for cultivars. On the other hand, for the process with inoculation of seeds (C Az) there was significant effect for cultivars, environments, and cultivars x environments (Table 3).

Table 3. Summary of the analysis of joint variance to produce green ears in two seed inoculation processes S Az and C Az, and in eight maize cultivars submitted to five levels of N in the agricultural years 2019/20 and 2020/21. Palmas - TO.

Source Variation	Degree of freedom	Square medium	
		S Az Epoch 1 and 2	C Az Epoch 1 and 2
Blocks/Environment	18 18	37066	97448

Cultivars	7	7	2456682 ^{ns}	6674529*	1 x 5	-4.23	104.23	1 x 5	18.57	81.43
Environments	9	9	83624940*	43307681*	1 x 6	1.93	98.07	1 x 6	4.89	95.11
					1 x 7	-24.72	124.73	1 x 7	-18.37	118.37
					1 x 8	-20.16	120.16	1 x 8	-19.89	119.89
Cult. x Env.	47	44	1828492**	2194987**	1 x 8	-20.16	120.16	1 x 8	-19.89	119.89
Residue	101	93	64984	98807	1 x 9	-26.00	126.00	1 x 9	-23.89	123.89
CV (%)			2.97	3.49	1 x 10	-29.99	129.99	1 x 10	-28.95	128.95
General Average			8.571	9.006	2 x 3	32.78	67.22	2 x 3	24.29	75.71

ns, **, *: not significant and significant to 1% and 5%, respectively, by the F test.

The significant effect of cultivars, only for the process with inoculation of the process seeds (C Az), indicates that the bacterium was able to promote conditions for the differentiation of cultivars. Second Hungria [19] the effects of inoculation of maize seeds on grain yield depend on plant genetic characteristics, strains, and environmental conditions. Towards Quadros et al. [20], the success of inoculation will be as a function of the site, soil type, climate of the region and genotype of the plants.

The coefficients of variation (CV) obtained were 2.97 the 3.49% (S Az and C Az) respectively (Table 3) and are in line with the studies carried out by Gurgel et al. [21] and corn experiments.

For the vast majority of pairs of environments, in both processes, the interaction was of the complex type (% FC) (Table 4), indicating that cultivars exhibit different behaviors due to environmental factors arising from years of and doses of N Distinct. Thus, studies of stability, adaptability and environmental stratification were carried out.

When the fraction of the complex type (%FC) has a very large weight over the C x A interaction, the great difference between environments is evident, and reinforces how much it is necessary to evaluate cultivars in various conditions [10].

Table 4. Estimates of the simple (%FS) and complex (%FC) fractions of the cultivar x environments interaction, between pairs of evaluation environments, in two inoculation processes of seeds S Az and C Az, evaluated for green ear yield, in ten environments, according to the method of Cruz & Castoldi [11].

Par	S Az		C Az		Par	C Az		Par	C Az		
	%FS	% FC	%FS	% FC		%FS	% FC		%FS	% FC	
1 x 2	-4.24	104.24	1 x 2	-15.52	115.52	7 x 8	42.59	57.41	7 x 8	83.18	16.82
1 x 3	0.85	99.14	1 x 3	-8.51	108.51	7 x 9	51.89	48.11	7 x 9	72.43	27.57
1 x 4	-11.95	111.95	1 x 4	6.11	93.89	7 x 10	56.52	43.48	7 x 10	55.48	44.52
						5 x 6	9.32	90.68	5 x 6	6.91	93.09
						5 x 7	2.63	97.37	5 x 7	-6.70	106.70
						5 x 8	10.63	89.37	5 x 8	-5.07	105.07
						5 x 9	-8.25	108.25	5 x 9	2.69	97.32
						5 x 10	-13.00	113.00	5 x 10	-7.95	107.95
						6 x 7	40.65	59.35	6 x 7	37.38	62.62
						6 x 8	19.99	80.01	6 x 8	43.21	56.79
						6 x 9	24.97	75.03	6 x 9	23.60	76.40
						6 x 10	39.46	60.54	6 x 10	43.37	56.63

8 x 9	67.56	32.44	8 x 9	76.37	23.63
8 x 10	40.69	59.31	8 x 10	68.63	31.37
9 x 10	62.50	37.50	9 x 10	60.65	39.35

S Az: without inoculation of seeds; C Az: with inoculation of seeds, Environments: First Season (1, 00 kg ha⁻¹ N; 2, 30 kg ha⁻¹ N; 3, 60 kg ha⁻¹ N; 4, 90 kg ha⁻¹ N, and 5, 120 kg ha⁻¹ N); Second Season (6, 00 kg ha⁻¹ N; 7, 30 kg ha⁻¹ N; 8, 60 kg ha⁻¹ N; 9, 90 kg ha⁻¹ N, and 10, 120 kg ha⁻¹ N), agricultural years 2019/20 and 2020/21.

The environmental index, for the two processes (S Az and C Az) evaluated in the ten environments are presented in Table 5. Second method of Eberhart & Russel [16], favorable environment is one in which its average is higher than the general average of all environments studied, resulting in a positive index. On the other hand, unfavorable environment is one whose average is lower than the general average, thus being negative index.

In the agricultural year 2019/20, all environments (environments of 1 the 5) without *Azospirillum* (S Az) and with *Azospirillum* (C Az), classified as unfavorable. In the agricultural year 2020/21, all environments (environments 6 to 10), for the processes (S Az) and (C Az), were classified as favorable. Thus, within each process in each of the agricultural years, the doses of N used in coverage (30, 60, 90 and 120 kg from N by ha⁻¹) were not able to cause changes in the classification of environments, so that their classification in favorable and unfavorable occurred mainly due to climatic fluctuations between agricultural years.

In the agricultural year 2020/21, the environments were classified as favorable due, mainly, to the occurrence of more regular rainfall in the grain filling phase (February 2021) (Table 1), when compared with the environments from the agricultural year 2019/20.

The occurrence of lower water availability during the grain filling phase promotes changes in metabolic routes [22], reducing the number of grains per m², the number of ears per m² [23], length of internodes, the storage capacity of sugars in the stem, in addition to resulting in thinner stems, smaller plants and smaller leaf area, which can impair the development of plants [24].

In all environments (1 to 10), whether favorable or unfavorable, seed inoculation (C Az), promoted a greater gain in the productivity of green ears. This fact may have occurred due to diazotrophic bacteria contributing to plant growth, through the supply of nitrogen via symbiotic fixation [5] and to promote an increase in the availability of N from mineral fertilization to plants, through the

incorporation of inorganic nitrogen into complex molecules, resulting from the increase in nitrate reductase enzyme activity [6].

In addition, these bacteria may result in changes in the morphology of the root system, in the number of radícelas and diameter of the roots, probably due to the production of growth-promoting substances (auxins, giberelinas and cytokinins) [25]. Thus, with the use of these bacteria, it would be possible to reduce the use of nitrogen fertilizers, reducing the cost of production and contamination of the environment resulting from the leaching of this element [1].

Chavarria & Melo [26], report that the use of micro-organisms (FBN) in agricultural practices has become increasing, as nitrogen fertilization is an important element in production costs, reduces environmental damage and reduces the greenhouse effect.

Increases in grain yield in corn crop when inoculated with *Azospirillum brasilense* have been observed in several studies [8].

Table 5. Environmental index (Ij) of ten environments, for productivity of green ears (kg ha⁻¹), in processes without inoculation of seeds (S Az) and inoculating the seeds (C Az), according to the Eberhart & Russell [16] method, in the agricultural years 2019/20 and 2020/21, in Palmas – TO.

Environment	S Az		C Az	
	Average	Index (Ij)	Average	Index (Ij)
1	6,140	-2431	8,258	-748
2	6,565	-2006	7,218	-1788
3	7,532	-1039	7,592	-1414
4	6,906	-1926	7,241	-1765
5	8,094	-477	8,422	-584
6	8,966	395	9,274	268
7	9,947	1376	10,136	1130
8	10,152	1581	10,304	1298
9	10,354	1783	10,484	1478
10	11,050	2479	11,130	2124
General Average	8,571	---	9,006	---

Environments: Agricultural Year 2019/20, sowing on 04/12/2019: (Environment 1, 00 kg ha⁻¹ N; Environment 2, 30 kg ha⁻¹ N; Environment 3, 60 kg ha⁻¹ N; Environment 4, 90 kg ha⁻¹ N, and Environment 5, 120 kg ha⁻¹ N).

Agricultural Year 2020/21. Sowing on 10/12/2020: (Environment 6, 00 kg ha⁻¹ N; Environment 7, 30 kg ha⁻¹ N; Environment 8, 60 kg ha⁻¹ N; Environment 9, 90 kg ha⁻¹ N, and Environment 10, 120 kg ha⁻¹ N).

The averages and parameters of adaptability and stability of each cultivar, for each of the processes (S Az and C Az) for the productivity of green ears, by the Method of Eberhart & Russell [16], are represented in Table 6.

All cultivars showed significant regression deviations ($S^2d \neq 0$), in both processes (S Az and C Az), indicating the non-predictability of behavior (instability), i.e., they present variations in the productivity of green ears depending on the environment.

The cultivars BRS-3046 and AG-1051, in the processes S Az and C Az, presented regression coefficient greater than the unit ($\beta_1 > 1$) and average higher than the general average of the group, being considered adapted to favorable environments, that is, where the technological level employed is high.

AG 8088-PRO2 and BRS-2022, in both cases, they presented specific adaptation to unfavorable environments ($\beta_1 < 1$), that is, with low investment in cultivation technology. In this environment, however, only the cultivar AG 8088-PRO2, in the process S Az, averaged higher than the general average and can be classified as well adapted.

The other cultivars presented different classifications when comparing the different inoculation processes (S Az and C Az), indicating their differential behavior when submitted to different seed inoculation processes. Thus, while PR-27D28 presented $\beta_1 < 1$, in the process S Az, and $\beta_1 > 1$, in the process C Az; BM-3061 presented $\beta_1 > 1$, in

the process S Az, and $\beta_1 < 1$, in the process C Az. On the other hand, M-274, presented β_1 not differing from the

unit, in the process S Az, and $\beta_1 < 1$, in the process C Az and Anhembi presented $\beta_1 < 1$, in the process S Az, and β_1 not differing from the unit, in the process C Az.

The Cultivars M-274, in the process S Az, and Anhembi, in the process C Az, presented regression coefficient equal to the unit ($\beta_1 = 1$), that is, they were adapted to favorable and unfavorable environments. These M-274 average dwelled above the overall average. These cultivars are responsive to improving the environment, but require an adequate positioning, because if grown in unfavorable environments, where the technological level is low and face adverse climatic conditions, usually present reduction in productivity [27].

Revolti (2014) it was not possible to generalize the recommendation of the most appropriate form of inoculation since there is a genotype interaction x inoculation form. Therefore, it is necessary to develop cultivars, aiming at the production of green ears, through breeding programs aimed specifically at the processes S Az or C Az.

Already Quadros et al. [20], when evaluating the field agronomic performance of corn hybrids inoculated with *Azospirillum brasilense*, verified the effect of the interaction between hybrids and treatments on productivity, indicating that inoculation may be more efficient in certain hybrids. According to these authors, the benefit of inoculation, depending on the maize genotype, can be observed in different parts of the plant, such as the grains, shoots, or stems.

Table 6. Adaptability Parameters (β_1) and stability (S^2d), for productivity of green ears (kg ha⁻¹), in eight maize cultivars, according to the method of Eberhart & Russell [16], in agricultural years 2019/20 and 2020/21, in Palmas – TO.

Cultivate	S Az			C Az		
	Average	β_1	$s^2 d$	Average	β_1	$s^2 d$
BRS-3046	8,926	1.16**	472407**	9,716	1.33**	287478**
Anhembi	8,431	0.85**	166164**	8,316	1.00ns	431036**
M-274	8,637	1.00ns	228198**	8,890	0.70**	384264**
PR-27D28	8,147	0.79**	116181**	8,568	1.19**	236571**
BRS-2022	8,382	0.91*	293953**	8,814	0.88*	135527**
BM-3061	8,425	1.19**	281028**	9,457	0.79**	332616**
AG-1051	9,000	1.14**	266382**	9,360	1.34**	420721**

AG 8088-PRO2	8,621	0.95*	100670**	8.928	0.78**	683710**
General Average	8,571			9,006		

β_1 = Regression coefficient; S²d= regression deviations; **, *, ns= significant to 1%, 5% and not significant respectively by the test t.

The results of the clusters of the environments, according to the method of Lin [17] (Table 7), for the process C Az, revealed the formation of a single group composed of environments 7 (sowing 10/12/20, 30 kg ha⁻¹ N) and 9 (sowing 10/12/20, 90 kg ha⁻¹ N). In this case, aiming at the optimization of human and financial resources in breeding programs, it would be possible to conduct only the test with the lowest nitrogen fertilization, that is, from 30 kg from N ha⁻¹.

On the other hand, in the absence of *Azospirillum brasilense* (S Az), no group with similar environment was formed (Table 7). Therefore, it can be inferred that the doses of N used (00, 30, 60, 90 and 120 kg ha⁻¹ N) and climatic factors (precipitation and temperature), arising from different agricultural years, promoted significant changes in the environments. In this way, for this feature S Az, it is recommended to conduct a larger number of trials represented by the combination of years with different doses of N in coverage.

Table 7. Grouping of the ten evaluation environments for green ear productivity (kg ha⁻¹), by the method of Lin [17], in agricultural years 2019/20 and 2020/21, in Palmas – TO.

S Az		C Az	
Group	Environments	Group	Environments
I	---	I	7; 9

Environments: Rehearsal First Season (Environment 1, 00 kg ha⁻¹ N; Environments 2, 30 kg ha⁻¹ N; Environments 3, 60 kg ha⁻¹ N; Environments 4, 90 kg ha⁻¹ N, and Environments 5, 120 kg ha⁻¹ N), in 04/12/2019.

Rehearsal Second Season (Environment 6, 00 kg ha⁻¹ N; Environment 7, 30 kg ha⁻¹ N; Environments 8, 60 kg ha⁻¹ N; Environments 9, 90 kg ha⁻¹ N, and Environment 10, 120 kg ha⁻¹ N, in 10/12/2020.

IV. CONCLUSION

There was differential response of cultivars between processes with and without seed inoculation.

Seed inoculation resulted in a higher increase in the productivity of green ears.

BRS-3046 and AG-1051 presented broad adaptation to the environments.

Due to the differential behavior of cultivars, in the presence and absence of *Azospirillum brasilense*, there is a need to conduct specific improvement programs for each process.

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