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Effect of *Azospirillum brasilense* on yield components of maize

Efeito do *Azospirillum brasilense* nos componentes da produção do milho

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Abstract

Among the countless operations involved in the maize production system, nitrogen fertilization is essential for the crop yield and development. However, due to the costs and negative environmental impacts caused by chemical fertilization, the use of the bacterium *Azospirillum brasilense* is an alternative to reduce costs and increase yield. Thus, this study evaluates the effect of inoculation of *Azospirillum brasilense* on the yield components of synthetic varieties of maize. The experiments were carried out at UNESP – Jaboticabal Campus, state of São Paulo, Brazil. The randomized block design was used with two replicates, testing forty-six maize genotypes: forty-four synthetic genotypes and two checks. Experiments were performed without nitrogen topdressing and with *Azospirillum brasilense* inoculation and, with nitrogen topdressing and without *Azospirillum brasilense* inoculation. Grain yield, average weight of grains, prolificacy, ear length, ear diameter, number of rows and number of grains per row were evaluated. The analysis of combined variance of experiments was performed considering the effects of genotypes, experiments, and the Genotype x Experiment interaction. Using the means from the analysis of variance, the Scott-Knott test was applied at 5% probability for the cases in which the F test was significant. It was found that bacterial inoculation increased yield, average weight of grains, ear diameter, and number of rows, while nitrogen fertilization increased prolificacy, ear length, and number of grains per row.

Additional keywords: biological N fixation; diazotrophic bacteria; nitrogen fertilization; *Zea mays*.

Resumo

Dentre as inúmeras operações envolvidas no sistema de produção do milho, a adubação nitrogenada é fundamental para desenvolvimento e produtividade da cultura. No entanto, devido aos custos e impactos ambientais negativos que a adubação química promove, a utilização da bactéria *Azospirillum brasilense* constitui alternativa para diminuir custos e elevar a produtividade. Assim, objetivou-se avaliar o efeito da inoculação da bactéria *Azospirillum brasilense* nos componentes de produção de variedades sintéticas de milho. Os experimentos foram realizados na UNESP – Campus Jaboticabal, SP. O delineamento utilizado foi o de blocos casualizados com duas repetições, utilizando 46 genótipos de milho, sendo 44 genótipos sintéticos e 2 testemunhas. Foram instalados dois experimentos, sendo o primeiro, sem a realização de adubação nitrogenada de cobertura e com inoculação com *Azospirillum brasilense* e o segundo com adubação nitrogenada de cobertura e sem aplicação de *Azospirillum brasilense*. Avaliou-se a produtividade, peso médio de grãos, prolificidade, comprimento de espiga, diâmetro de espiga, número de fileiras, número de grãos por fileira. Foi realizada a análise de variância conjunta dos experimentos, considerando os efeitos de genótipos, experimentos e a interação GenxExp. Utilizando-se as médias obtidas das análises de variância, para os casos onde houve significância do teste F, foi aplicado o teste de médias de Scott-Knott a 5% de probabilidade. Verificou-se que a inoculação da bactéria proporcionou aumento na produtividade, peso médio de grãos, diâmetro de espiga e número de fileiras, enquanto a adubação nitrogenada promoveu melhoria na prolificidade, comprimento de espiga e número de grão por fileira.

Palavras-chave adicionais: adubação nitrogenada; bactérias diazotróficas; fixação biológica de N; *Zea mays*.

Introduction

Maize is one of the main crops produced in Brazil accounting for approximately 30% of the cultivated area in the country, which is only surpassed by soybeans in cultivated area. The national production of the 2018/19 season totaled 99,984.1 thousand tons referring to the first and second crop seasons, which represents an increase of 23.9% in relation to the previous crop season and becoming the second largest crop season in history (CONAB, 2019).

The maize cultivation requires large amounts of nitrogen fertilizers to obtain high yields. Nitrogen (N) is essential for the growth and development of plants, being the nutrient with the most expressive results to increase grain yield (Costa et al., 2015). However, nitrogen fertilizers are obtained from non-renewable sources, besides being one of the most expensive fertilizers in crop production. If used in excess, they may leach, contaminating groundwater and causing environmental problems (Cantarella, 2007). Thus, alternatives are needed to reduce nitrogen fertilization in crops.

Among the least impacting ways of production is the biological nitrogen fixation (BNF) with diazotrophic bacteria like *Azospirillum brasilense*. They are capable of fixing nitrogen mutually with the maize plant, increasing yields with decreased use of nitrogen fertilizer in crops, contributing to reduce production costs and to develop less impacting and less polluting agricultural practices (Milléo & Cristófoli, 2016).

Bacteria of the *Azospirillum* genus have the potential to stimulate plant development by multiple mechanisms, including the synthesis of phytohormones, improvement of nitrogen nutrition, stress mitigation, and biological control of the pathogenic microbiota (Bashan & De-Bashan, 2010).

Hungria (2011) reported several studies using *Azospirillum* spp. That promoted effects on maize plants such as weight gain, increased nitrogen content in leaves, seeds and flowers, early silking, increased number of ears, number of grains, plant height, leaf area, leaf area index, and germination rate.

Kotowski (2015) also reported increased yield in studies with the presence of *Azospirillum brasilense*, although it did not have the capacity to meet all the demand of available N for the maize plant, acting as a complement for better absorption of available N.

Thus, the search for understanding the effects of *Azospirillum brasilense* on the agronomic performance of maize genotypes is growing. Therefore, this study evaluates the effect of *Azospirillum brasilense* on the yield components of synthetic varieties of maize.

Material and methods

The research was carried out in the 2018/2019 summer harvest at the Teaching, Research and Extension Farm of the School of Agricultural and Veterinary Sciences of UNESP - Universidade Estadual Paulista, Jaboticabal Campus, São Paulo State, Brazil (21°15'17" S latitude and 48°19'20" W longitude, altitude of 605 m). The climate corresponds to the Köppen climate classification category Aw, characterized as subtropical with rainy summers, relatively dry winter, and average annual temperature of 23 °C. The soil is classified as Eutroferic Red Latosol.

Two experiments were installed in the same area and under the same conditions, namely: experiment A - without nitrogen topdressing and with *Azospirillum brasilense* inoculation; and experiment N - with nitrogen topdressing and without *Azospirillum brasilense* inoculation.

The experimental design was a randomized block with two replicates, using forty-six maize genotypes consisting of forty-four synthetic genotypes with broad and narrow genetic base from the company Phoenix Agricola Ltd. and two checks, a commercial variety ALBAND (TEST A) and a single-cross hybrid DKB390 (TEST B). The experimental plots consisted of four 4-m long rows, spaced at 0.45 m between rows and 0.30 m between plants. Only the two central rows of each plot were considered usable for evaluation.

The experiments were sown with manual planters on October 18, 2018 and, based on soil analysis and crop requirements, 350 kg ha⁻¹ of 8-28-16 were applied as base fertilizer.

Topdressing was performed on November 12, 2018 using urea as the N source, in the amount necessary to supply 140 kg ha⁻¹ nitrogen. The inoculation of genotypes was performed on November 13, 2018 with the commercial product QualyFix Gramínea® (mixture of Ab-V5 and Ab-V6 strains of *Azospirillum brasilense*) via soil at a dose of 600 mL ha⁻¹, according to the recommendations by the manufacturer. The plants were in the phenological stage V5.

In each experimental plot, the following characteristics were evaluated:

- Grain yield (GY): obtained by threshing the harvested ears and weighing the grains of each plot, correcting the moisture to 13% and converting the values to tons per hectare (t ha⁻¹);

- Ear length (EL): measured with a graduated ruler, using six ears per plot, expressed in cm;

- Ear diameter (ED): measured in the center of the ear with the aid of a digital caliper, using six ears per plot, expressed in mm;

- Number of rows per ear (NR): counting of the number of rows of grains per ear, using a sample of six ears per plot;

- Number of grains per ear row (NGR): counting of the number of grains in the ear row, using a sample of six ears per plot;
- Average weight of 500 grains (AW): weight of 500 grains of each plot, expressed in grams (g);
- Prolificity (PROL): obtained by the ratio of the number of ears harvested in the plot and the number of plants in the plot, carried out before harvest.

Initially, an analysis of variance was performed for each experiment to verify the homogeneity of the residual variance. Subsequently, as the ratio between the mean squares of the two experiments was within the appropriate range, analysis of combined variance was performed considering the effects of genotypes, experiments, and the genotype

x experiment interaction. Using the means from analysis of variance, the Scott-Knott test was applied at 5% probability for the cases in which the F test was significant.

Results and discussion

The genotype variation factor (GEN) showed significant differences at 1% through the F test for all parameters of the yield components. In the case of the environment variation factor (ENV), only the average grain weight (AW) was not significant, with the other components being significant at 1%. There was no genotype x experiment interaction (G*E) (Table 1).

Table 1 - Joint analysis of variance for the grain yield components.

Sources of variation	FD	Mean square						
		GY	AW	PROL	EL	ED	NR	NGR
Genotype (G)	45	1.40**	520.45**	0.06**	3.70**	21.03**	6.95**	32.46**
Experiment (E)	1	2.99**	34.86 ^{ns}	0.21**	20.61**	135.21**	7.78**	236.12**
G*E	45	0.40 ^{ns}	143.79 ^{ns}	0.03 ^{ns}	0.83 ^{ns}	5.62 ^{ns}	0.83 ^{ns}	18.51 ^{ns}
Error	90	0.38	102.69	0.03	0.90	6.32	0.94	16.14
Mean	-	4.89	147.23	1.02	15.13	45.85	14.89	29.64
CV (%)	-	12.68	6.88	16.97	6.30	5.48	6.54	13.55

** Significant by F test ($p < 0.01$), ^{ns} Not significant by F test ($p > 0.05$), CV = Coefficient of variation. FD = Freedom degrees, GY = Grain yield; AW = Average weight; PROL = Prolificacy; EL = Ear length; ED = Ear diameter; NR = Number of rows; NGR = Number of grains per row.

A 5% increase in GY with the inoculation of *Azospirillum* was observed when compared with nitrogen application. In the evaluated genotypes, 65% had a better response when inoculated with the bacteria, and genotypes one and twenty stood out, demonstrating that the N fixation response may be related to the genotype used. The yield increase when inoculated with *Azospirillum* is probably due to the increase in ear diameter and number of rows per ear, which can be explained by the increased biological fixation that the bacterium offers to the plant, favoring the development of roots, growth, and structure in the plant, besides favoring the photosynthetic process, increasing yield. According to Bashan et al. (2004), these bacteria can fix N₂ for the plant and produce growth hormones, such as auxins and gibberellins, which stimulate plant growth, mainly of roots, by increasing the absorption of nutrients and water.

Cunha et al. (2014) proved that maize inoculated with the bacterium produced 5.5 more bags (0.33 t ha⁻¹) than without inoculation while evaluating the effect of inoculation of the bacterium *Azospirillum brasilense* on maize genotypes, stating

the bacterium efficiency to increase the yield of this crop.

There was a 2% increase in AW with application of *Azospirillum* when compared to that of nitrogen. Of the evaluated genotypes, 54% showed better response with bacterial inoculation.

Inoculation with *Azospirillum* resulted in a 4% increase in ED when compared to chemical fertilization. Among genotypes, 74% performed better in the treatment with inoculation. Different results were obtained by Cunha et al. (2014), who did not observe significant differences in ear diameter (ED) of plants inoculated with *Azospirillum brasilense*.

The NR increased by 3% in the treatment with *Azospirillum* inoculation, and 65% of the genotypes performed better with bacterial inoculation. Genotypes fourteen and twenty performed better in the treatment with *Azospirillum brasilense*.

Table 2 - Averages values of grain yield components in maize. Jaboticabal-SP. Agricultural year 2018/2019.

GEN	GY		AW		PROL		EL		ED		NR		NGR	
	AZOS	N	AZOS	N	AZOS	N	AZOS	N	AZOS	N	AZOS	N	AZOS	N
1	5.66*	4.39	140.95b	129.35b	0.96a	1.03a	15.80 ^a	16.15a	49.91a	45.26a	16.40a	16.50a	29.40a	32.60a
2	5.77a	5.61a	143.50b	143.30b	0.86a	1.09a	13.90	15.85*	48.80a	48.11a	15.80a	14.60b	28.70a	31.50a
3	4.12b	4.92b	125.95b	135.95b	1.04a	0.93a	14.80 ^a	15.40a	44.05b	40.43b	14.20b	14.60b	32.40a	29.90a
4	4.66b	4.77b	144.00b	131.45b	0.96a	0.89a	14.25b	15.00b	42.48b	40.07b	12.75b	13.37b	26.00a	33.25a
5	4.67b	4.56b	146.40b	145.85b	0.93a	0.89a	15.47 ^a	15.55a	45.23b	42.26b	14.90b	14.80b	30.55a	30.60a
6	6.15a	5.28a	161.75a	145.25b	0.99a	1.22a	13.80b	13.60b	48.98a	45.64a	15.50a	16.40a	27.80a	24.80a
7	4.66b	4.81b	165.20a	169.45a	0.99a	1.09a	15.65 ^a	16.30a	43.32b	42.94b	13.70b	12.40b	26.90a	30.70a
8	4.11b	4.63b	138.30b	143.45b	0.80a	1.01a	13.85	15.85*	45.59b	47.75a	14.20b	15.20a	23.00a	33.00*
9	3.91b	3.83b	161.55a	141.05b	1.11a	1.52*	11.35	13.60*	44.80b	44.84a	13.10b	12.40b	20.90a	26.40a
10	4.63b	5.52a	142.85b	139.15b	1.08a	1.00a	15.55 ^a	16.65a	48.61a	47.10a	16.60a	15.30a	29.00a	28.60a
11	5.15b	5.11a	148.50b	131.35b	0.94a	1.00a	15.80 ^a	15.60a	46.57b	46.23a	15.60a	16.20a	28.60a	28.70a
12	6.14a	5.46a	154.60a	160.75a	0.99a	1.18a	16.45 ^a	18.00a	49.33a	50.88a	16.30a	17.80a	32.00a	34.00a
13	5.86a	5.70a	137.10b	139.00b	1.09a	0.91a	16.05 ^a	16.10a	47.86*	41.3	16.30a	16.60a	30.00a	33.20a
14	5.61a	5.85a	126.80b	144.25b	1.05a	0.87a	16.35 ^a	16.75a	46.19b	47.67a	18.20*	16.00	30.80a	31.00a
15	4.95b	5.71a	136.95	158.15*	0.80a	0.84a	14.37b	15.05b	46.92a	47.96a	17.00a	16.80a	27.90a	31.60a
16	4.35b	4.93b	130.65b	150.10a	1.36a	1.24a	14.15b	14.30b	46.76a	46.88a	15.20a	13.90b	25.80a	32.50a
17	4.20b	3.98b	157.40*	122.45	1.17a	1.10a	14.80 ^a	15.01b	44.91*	39.53	14.30b	13.05b	25.80a	28.12a
18	4.67b	3.55b	158.95a	152.65a	0.55a	1.03*	14.70 ^a	14.16b	43.81b	42.07b	14.20b	12.27b	26.50a	25.97a
19	4.72b	4.91b	164.05a	162.05a	0.82a	1.10a	13.20	15.40*	46.60b	46.06a	14.20b	14.80b	24.30a	27.60a
20	6.82*	5.26	147.55b	146.30b	1.03a	1.05a	14.55b	14.45b	49.56a	45.54a	15.80*	13.40	28.80a	29.50a
21	4.20b	3.82b	147.95b	153.80a	0.89a	0.90a	13.30b	14.80b	45.46b	46.31a	13.40b	13.60b	26.80a	32.40a
22	3.98b	4.56b	145.35b	133.95b	1.08a	0.93a	13.20b	14.15b	45.85b	42.20b	13.80b	13.20b	28.70a	29.20a
23	5.39a	4.41b	146.55b	141.20b	0.95a	1.06a	14.40b	14.45b	48.56a	45.27a	15.20a	13.30b	30.80a	29.20a
24	5.01b	4.61b	131.10b	134.85b	1.23a	1.28a	14.05b	15.30a	48.94*	43.95	16.70a	15.80a	25.90a	35.30*
25	5.79a	4.84b	154.00a	138.05b	0.87a	1.15a	16.00a	14.25b	48.54a	45.51a	15.20a	14.60b	32.50a	28.50a

Table 2 – Cont....

GEN	GY		AW		PROL		EL		ED		NR		NGR	
	AZOS	N	AZOS	N	AZOS	N	AZOS	N	AZOS	N	AZOS	N	AZOS	N
26	5.13b	4.26b	139.15b	127.85b	0.92a	0.99a	12.75	15.45*	48.04a	43.40b	15.80a	15.70a	30.60a	30.00a
27	5.28a	4.86b	129.95b	133.30b	0.83a	0.92a	14.05b	14.60b	47.54a	45.97a	15.60a	15.30a	30.20a	33.90a
28	4.33b	5.15a	149.45a	137.20b	1.24a	1.28a	13.85b	15.55a	47.24a	49.47a	15.50a	16.80a	26.20a	32.40a
29	4.32b	4.09b	142.15b	147.55b	0.77a	1.03a	15.15 ^a	15.65a	45.61b	42.62b	15.30a	14.20b	28.70a	30.80a
30	5.07b	4.38b	133.40b	132.10b	0.96a	1.25a	17.25 ^a	16.60a	45.74b	42.10b	14.70b	13.80b	30.10a	37.10a
31	5.06b	4.56b	154.70a	148.60a	1.07a	1.13a	15.55 ^a	15.92a	46.05b	43.81b	13.60b	13.55b	30.70a	29.55a
32	5.44a	4.74b	132.05b	134.20b	1.05a	1.05a	15.07 ^a	15.15b	47.15a	43.43b	16.00a	15.60a	37.20a	35.30a
33	5.95a	5.08a	154.35a	147.15b	0.93a	0.86a	16.20 ^a	16.20a	50.42a	48.00a	15.50a	14.70b	31.10a	29.50a
34	4.65b	5.05a	133.80b	150.20a	1.07a	0.90a	13.00b	14.15b	47.61a	47.46a	16.60a	15.60a	25.50a	28.20a
35	5.65a	4.89b	154.80a	158.00a	0.95a	1.02a	15.00a	13.90b	50.00a	45.77a	15.80a	16.10a	27.90a	33.70a
36	5.04b	3.92b	165.20a	152.25a	0.86a	1.02a	14.40b	15.65a	42.09b	44.29b	13.50b	13.60b	24.80a	30.90a
37	4.89b	4.20b	150.10a	166.50a	0.97a	1.14a	14.80 ^a	15.80a	44.94b	42.27b	14.80b	13.80b	24.90a	28.70a
38	4.80b	3.95b	177.40a	166.15a	0.89a	1.30*	15.30 ^a	14.70b	45.26b	41.81b	12.80b	11.90b	24.60a	29.90a
39	4.31b	4.46b	147.50b	161.00a	1.18a	1.08a	14.70	16.60*	41.58b	43.72b	13.80b	14.00b	27.20a	32.40a
40	5.20b	4.26b	154.20a	153.05a	1.00a	1.12a	15.00a	16.40a	42.71b	43.95b	14.30b	13.60b	27.50a	31.20a
41	4.37b	3.89b	156.20a	151.95a	0.97a	0.97a	14.80 ^a	15.60a	44.72b	43.41b	14.50b	14.40b	25.20a	30.40a
42	5.12b	4.20b	135.80	157.25*	1.09a	0.87a	15.60 ^a	16.25a	48.62a	46.86a	15.40a	14.98a	28.81a	29.86a
43	5.05b	4.84b	120.55b	135.10b	0.94a	0.90a	14.75 ^a	15.30a	49.58a	45.64a	17.60a	16.70a	30.50a	30.20a
Check A	5.33a	6.02a	178.60a	172.80a	1.37a	1.36a	16.25 ^a	17.25a	48.63a	48.79a	14.20b	14.80b	29.07a	32.30a
Check B	6.41a	6.78a	160.30a	162.20a	0.99a	1.13a	16.15 ^a	16.40a	53.51a	49.39a	18.00a	16.50a	29.10a	31.70a
46	4.44b	4.66b	165.15a	165.15a	0.92a	1.02a	15.25 ^a	16.60a	44.01b	45.88a	12.60b	13.00b	26.70a	29.50a
Mean	5.02	4.77	150.06	146.79	0.99	1.06	14.80	15.47	46.71	45.00	15.1	14.68	28.13	30.78

GEN = Genotype; AZOS = *Azospirillum*; N = Nitrogen. Means values followed by the same lowercase letter in the column do not differ significantly from each other, using the Scott-Knott test ($p > 0.05$); * significant difference between the experiments by the Scott-Knott test ($p < 0.05$); GY = Grain yield; AW = Mean grain weight; PROL = prolificacy; EL = Ear length; ED = ear diameter; NR = Number of rows; NGR = Number of grains per row.

Nitrogen fertilization increased the EL by 4%, and 83% of the genotypes performed better with nitrogen. The genotypes that stood out were two, eight, nine, nineteen, twenty-six, and thirty-nine. Different results were obtained by Cavalletti et al. (2000), who observed an increase of 17% in the mean length of the ears, from 13.6 to 14.4 cm, with the inoculation of *Azospirillum*. However, these authors did not observe the effect of inoculation on the number of rows per ear.

The results of PROL and NGR increased in the treatment with nitrogen, 7% and 9%, respectively. Of the genotypes used, 67% performed better using chemical fertilization when evaluating PROL and 78% of the genotypes had better responses regarding NGR in the treatment with nitrogen. Cadore et al. (2016) observed that inoculation did not increase the variables analyzed, such as ear length, grains per row, and grain yield while studying the effect of inoculation with *Azospirillum brasilense* on hybrid maize under different nitrogen doses. According to Leben et al. (1987), the inconsistency of positive results is frequent with rhizobacteria that promote plant growth, especially in field conditions. According to Antoun et al. (1998), the possible causes include the complexity of the interactions involved between plants, the bacteria introduced, and other components of the rhizospheric microbiota, among other factors.

The inoculation of the bacteria *Azospirillum brasilense* resulted in increased yield (YIELD), average grain weight (AW), ear diameter (ED), and number of rows (NR), while prolificacy (PROL), ear length (EL), and the number of grains per row (NGR) had better responses with nitrogen application.

Conclusions

It is possible to replace the chemical application of nitrogen in topdressing by bacterial inoculation of *Azospirillum brasilense* in synthetic varieties of maize.

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