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RESEARCH ARTICLE

Inoculation methods of *Azospirillum brasilense* associated to the application of soil bioactivator in the maize crop

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ABSTRACT

Seeking greater productivity, cost reduction and greater sustainability, plant growth promoting bacteria and soil bioactivators become a viable alternative for maize producers. The objective was to evaluate the effects of inoculation and foliar application of *Azospirillum brasilense* associated with the application of soil bioactivator on morphometric, physiological and productive characteristics of the maize crop. Conducted in the field, the experimental design adopted was randomized blocks, with 4 repetitions and the following treatments: control; seed inoculation with *Azospirillum brasilense* (100 mL per 60,000 seeds); foliar spraying of *A. brasilense* at the V5 stage of maize; application of soil bioactivator; seed inoculation with *A. brasilense* + foliar spraying of *A. brasilense* at the V5 stage of maize; seed inoculation with *A. brasilense* + application of soil bioactivator; foliar spraying of *A. brasilense* at the V5 stage of maize + application of soil bioactivator; seed inoculation with *A. brasilense* + foliar spraying of *A. brasilense* at the V5 stage of maize + application of soil bioactivator. At the V8, VT and R3 stages morphometric and physiological evaluations were performed. At the VT stage, the inoculation methods of *A. brasilense* and the application of bioactivator were significant only for the dry mass of reproductive structures. In the R3 stage, plants inoculated by foliar spraying of *A. brasilense* together with the application of soil bioactivator showed statistically superior heights. The inoculation methods with *A. brasilense* and the application of soil bioactivator did not influence morphometric attributes throughout the development of maize, not influencing physiological variables. The production components and yield were not affected by the treatments applied.

Highlighted Conclusions

1. The methods of inoculation with *Azospirillum brasilense* and the application of soil bioactivator did not influence morphometric attributes throughout maize development, and did not influence physiological variables.
2. The yield and production components were not affected by inoculation with *A. brasilense* and application of soil bioactivator.

INTRODUCTION

Maize (*Zea mays* L.) is a Poaceae of great importance in the Brazilian and world socioeconomic scenario due to its diversified use. It is estimated that the 2020/2021 crop is expected to reach 105.4 million tons, which represents an increase of 2.9% over the same period last year (CONAB 2021).

Due to technological advances in agriculture, current maize production techniques are modern. These include genetic improvement, appropriate soil use and management, integrated pest, disease, and weed management, and, in addition, alternative managements, such as inoculation with plant growth promoting bacteria (PGPB) and application of nanotechnology products based on organic compounds.

PGPB are free-living organisms that establish a specific symbiotic relationship with plants (Glick 2012). The use of PGPB associated with grasses has as main objective to stimulate the development of the plant through biological nitrogen fixation, synthesis of phytohormones, increased chlorophyll content in the leaves and root absorption area. These characteristics enhance productive parameters and minimize adverse abiotic and biotic

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effects, such as water deficit, long rainy periods, pest and pathogen attacks, common in the growing environment. In other words, it prepares the plant to tolerate these effects, reducing the negative consequences to productivity (Barassi et al. 2008; Bashan and Bashan 2010).

The biological nitrogen fixation promoted by bacteria of the genus *Azospirillum* occurs due to the conversion of atmospheric N_2 into ammonia, through the dinitrogenase complex. However, only part of the fixed nitrogen is excreted into the plant, partially supplying the plant's needs, but not excluding the need for nitrogen fertilization. Bacteria of this genus also have the function of mineralization of soil nutrients, favoring the availability for the plant (Hungary 2011).

Studies prove the efficiency of the use of PGPB associated with maize. Quadros et al. (2014) report stimulation in the development of plants in the vegetative period, standardizing the plant stand, increasing the chlorophyll content and resistance to water stress. Hungria et al. (2010) studying different strains of *Azospirillum brasilense*, found that the contents of macro and micronutrients in leaves and grains, among them, nitrogen (N), phosphorus (P) and potassium (K) increased as a function of inoculation, reducing the need for supplementation with mineral fertilizers in inoculated plants. Araújo et al. (2014) observed in their study that inoculation with *Azospirillum* promotes an increase in productivity and a reduction in the dose of N used.

Soil bioactivators developed through nanotechnology are alternative techniques that have been disseminated in the scientific environment and tend to stimulate and promote the exponential multiplication of beneficial microorganisms to soil microbiota. Thus, there are gains in tolerance to water deficit, improvement of soil physical conditions and greater resistance to pests, diseases and nematodes (Matysiak et al. 2011).

Based on the above, the objective of this study was to verify the effects of the application of soil bioactivator associated with *Azospirillum brasilense* inoculation methods on morphological, physiological, and productive characteristics of the maize crop.

MATERIAL AND METHODS

Characterization of the experimental area. The experiment was conducted in the city of Marechal Cândido Rondon, coordinates 24º 53' 19" S, longitude 54º 01' 73" W and altitude of 420 meters. The climate is Cfa (Peel et al. 2007) with well distributed rainfall throughout the year and hot summers, with temperatures in the coldest quarter being below 18 ºC and the hottest quarter temperatures being above 28 ºC. Environmental conditions are summarized in the Figure 1.

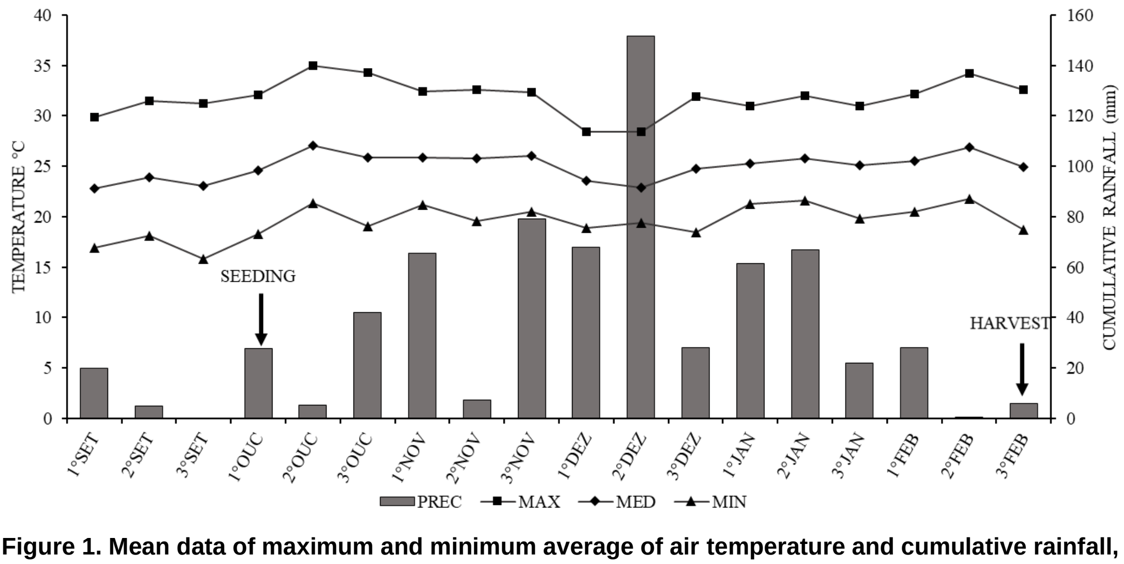

Figure 1. Mean data of maximum and minimum average of air temperature and cumulative rainfall, separated every ten days, from September 2019 to February 2021.

The soil was classified as an Oxisoil (Santos et al. 2018), with the following characteristics in the 0-20 cm layer: CEC of 10.17 $cmol_c$ dm^{-3}, base saturation (V%) of 53.68%, base sum of 5.46 $cmol_c$ dm^{-3}, $CaCl_2$ pH of 5.22 and organic matter content of 20.50 g dm^{-3}, P content of 55.58 mg dm^{-3} and K of 0.35 $cmol_c$ dm^{-3}, Ca^{2+} 2.89 $cmol_c$ dm^{-3} and Mg^{2+} 2.22 $cmol_c$ dm^{-3}.

Experimental design. The experiment was conducted in the field, in a randomized block design, with 8 treatments and 4 repetitions. The treatments were: T1 - control; T2 – seed inoculation with *Azospirillum brasilense* (100 mL per 60,000 seeds); T3 - foliar spraying of *A. brasilense* at the V5 stage of maize (300 mL ha^{-1}); T4 - application of soil bioactivator (15 g a.i. per ha); T5 – seed inoculation with *A. brasilense* + foliar spraying of *A. brasilense* at the V5

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stage of maize; T6 - seed inoculation with *A. brasilense* + application of soil bioactivator; T7 - foliar spraying of *A. brasilense* at the V5 stage of maize + application of soil bioactivator; and T8 - seed inoculation with *A. brasilense* + foliar spraying of *A. brasilense* at the V5 stage of maize + application of soil bioactivator.

Implanting the experiment. Seed inoculation was performed in the laboratory. Seeds were placed into plastic bags, at a dose of 100 mL for 60,000 seeds, then they were manually homogenized with the inoculant for three minutes, 30 minutes before sowing. The foliar spraying of *A. brasilense* was performed when maize reached the V5 phenological stage, using a CO_2 backpack sprayer with constant pressure of 40 kgf cm^{-2} and equipped with Magno 11002 ADGA nozzles spaced at 0.5 m, at 6:00 pm and with no wind. The commercial inoculant used for seed inoculation and foliar application was NITRO1000® GRAMINEAS, which contains the AbV5 and AbV6 strains of *A. brasilense*, at a concentration of 2.0 x 10^8 CFU per mL.

The application of soil bioactivator occurred using the commercial product Vitasoil Nano Science®. The mixture was prepared at the recommended dilution of 1g for each 100 mL of non-chlorinated water and then left for a period of 48 hours for total activation of the product, according to the manufacturer's recommendation. The spraying was divided into three applications of 5 g ha^{-1}, the first one right after sowing, the second at the V4 stage of maize, and the third at the V6 stage, thus totalizing a dose of 15 g ha^{-1}. The spraying of bioactivator and *A. brasilense* was always performed at the late afternoon with the aid of a motorized backpack sprayer, with a flow rate of 300 L ha^{-1}, and fan nozzle type under pressure of 30 psi.

The experiment was conducted during the 2019/2020 crop year. The plant material used was the simple hybrid MORGAN 30A91PWU, with a population of 70,000 plants per hectare. The experimental units consisted of 6 meters long with 12 sowing lines, spaced at 0.50 meters between rows, and the useful plot was 12 m^2, discarding three side rows and one meter at each end of the plot.

A precision sowing machine was used for sowing, regulated for the distribution of 300 kg ha^{-1} of 10-15-15 NPK, for base fertilization. Cover fertilization occurred when the crop reached the V4 phenological stage. Urea (45%) N was used at a dose of 180 kg ha^{-1} of N (SBCS 2017).

Morphometric evaluations. Evaluations were made at the V8, VT and R3 stages. Three plants per plot were randomly collected and the plant height (PH), stem diameter (SD) and the ear insertion height (EIH) were determined. After collected, the plants were sectioned in stem + sheath, leaves and other structures, and then packed in Kraft paper bags for drying in a forced air circulation oven at 65 ºC ± 2 ºC, until reaching constant mass. These samples were then weighed on analytical scales to obtain leaf dry mass (LDM), stem + sheath dry mass (SSDM), reproductive structures dry mass (RSDM), thus calculating the aboveground dry mass (ADM) and total dry mass (TDM). The leaf area (LA) was determined by the Benincasa (2003) methodology.

Gas exchanges. The indices of gas exchange were measured in the V8 and R3 stages, in days of full sun between 8:00 and 11:00 am, being the fourth leaf from the inflorescence with direct exposure to light defined as the standard leaf for the V8 stage and the first leaf below and opposite the ear for the R3 stage. Thus, the net CO_2 assimilation rate (A) (µmol CO_2 m^{-2} s^{-1}), leaf transpiration rate (E) (µmol H_2O m^{-2} s^{-1}), stomatal conductance (gs) (mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci) (µmol CO_2 mol^{-1}) were determined using the IRGA (Infra-Red Gas Analyser) model LI-6400XT (Licor Inc. Lincoln, NE). With these values, the following relations were calculated: WUE = A/E, iWUE = A/gs and Fc = A/Ci, where WUE corresponds to water use efficiency, iWUE to intrinsic water use efficiency and Fc to plant carboxylation efficiency.

Production components and yield. At the time of harvest, the plants had the ears of the useful plot manually harvested, and ten random ears were separated from each plot for the evaluation of the production components. The ear length (EL), ear diameter (ED), number of grain rows (NGR) and number of grains per row (NGPR) were evaluated. The harvested ears were manually threshed to determine the mass of one thousand grains and yield in kg ha^{-1}. To determine the mass of one thousand grains a sample was taken, where eight repetitions of 100 grains were counted, weighed and then determined the mass of one thousand grains, according to the Rules for Seed Analysis (Brazil 2009).

Data Analysis. Data were submitted to analysis of variance (ANOVA) and when significant were compared by Tukey's test at 5% probability. The analysis was performed using the SISVAR software (Ferreira 2014).

RESULTS AND DISCUSSION

There was no statistically significant difference for the morphometric variables evaluated at the V8 stage (Table 1). These results agree with those of Cunha et al. (2014) who, in maize crop, found no effect of *Azospirillum brasilense* on plant height, stem diameter, ear insertion height and leaf area index, under different soil and climate conditions, indicating that the result of using *A. brasilense* is not linked to favorable climate and soil conditions in which the plant is subjected.

Table 1. Averages of plant height (PH), stem diameter (SD), stem + sheath dry mass (SSDM), leaf dry mass (LDM), total dry mass (TDM) and leaf area (LA) in plants of Hybrid maize, Morgan 30A91PWU, submitted to the application of soil bioactivator associated with inoculation methods of *Azospirillum brasilense*, in the phenological stage V8 of development.

Treatment	PH	SD	SSDM	LDM	TDM	LA
	-- cm --	-- mm --		----- g -----		-- cm^2 --
				V8		
Control	96.8^{ns}	26.0^{ns}	63.7^{ns}	88.1^{ns}	151.8^{ns}	2,837.6^{ns}
Azo Seed.	93.7	27.2	65.0	86.8	151.8	2,881.6
Azo Fol.	97.3	25.2	58.7	85.6	144.3	2,739.4
VS	97.6	25.8	63.7	83.0	146.8	2,782.7
Azo Seed. + *Azo* Fol.	98.0	27.7	57.5	80.6	138.1	2,561.4
Azo Seed. + VS	98.7	26.5	68.7	89.3	158.1	2,754.0
Azo Fol. + VS	90.7	26.0	73.7	95.6	169.3	2,858.4
Azo Seed. + *Azo* Fol. + VS	97.1	26.5	55.0	80.5	135.5	2,681.5
CV (%)	4.0	9.2	19.9	14.0	16.2	14.7
LSD	9.14	5.76	29.87	28.70	57.4633	961.369

^{ns}: no statistically significant difference by Tukey test at 5% error probability. VS: Vitasoil Nano Science® soil bioactivator.

To understand the absence of increments in PH, SD, SSDM, LDM, TDM, LA in maize plants inoculated in soil treated with bioactivator, it is observed that these treatments exert low influence when plants are cultivated in high fertility soils and subjected to adequate environmental conditions throughout the crop cycle (Cunha et al. 2014; Rezende et al. 2019). This discussion is confirmed by the fact that the maize plants in the present study were subject to development in a high fertility soil and suitable environmental conditions (Figure 1).

At the VT stage (Table 2), the inoculation methods of *A. brasilense* and the application of bioactivator had little influence, acting in a punctual manner for dry mass of reproductive structures. The isolated application of bioactivator resulted in higher averages than seed treatment with *A. brasilense* and their association.

Table 2. Averages of plant height (PH), stem diameter (SD), stem + sheath dry mass (SSDM), leaf dry mass (LDM), reproductive dry mass (RSDM), total dry mass (TDM) and leaf area (LA) in plants of Hybrid maize, Morgan 30A91PWU, submitted to the application of soil bioactivator associated with inoculation methods of *Azospirillum brasilense*, in the phenological stage VT of development.

Treatment	PH	SD	SSDM	LDM	RSDM		TDM	LA
	-- cm --	-- mm --		----- g -----				-- cm^2 --
				VT				
Control	242.7^{ns}	29.2^{ns}	313.5^{ns}	176.8^{ns}	155.4	ab*	655.7^{ns}	4,614.6^{ns}
Azo Seed.	238.7	27.2	247.1	186.9	136.6	b	549.1	4,204.9
Azo Fol.	245.0	27.8	277.3	179.3	171.1	ab	635.4	4,649.4
VS	235.5	27.7	227.5	186.8	284.6	a	689.5	4,390.3
Azo Seed. + *Azo* Fol.	243.7	28.3	232.2	165.4	174.0	ab	585.5	4,279.7
Azo Seed. + VS	245.0	27.9	263.8	177.5	143.1	b	581.8	4,590.0
Azo Fol. + VS	240.2	27.9	281.6	174.9	211.2	ab	680.4	4,461.7
Azo Seed. + *Azo* Fol. + VS	242.0	27.7	287.9	187.6	156.0	ab	620.7	4,691.5
CV (%)	3.5	6.3	19.4	11.5	31.8		16.2	12.5
LSD	0.20	4.21	122.37	49.15	135.25		239.65	1,326.36

* similar lowercase letters in the column did not differ by Tukey's test at 5% probability. ^{ns} there was no significant statistical difference by Tukey's test at 5% error probability. VS: Vitasoil Nano Science® soil bioactivator.

Jakienė et al. (2009) reports the synergistic effect of the use of bioactivators in the soil-plant relationship, activating the cells that participate in the metabolism process and the plants begins to assimilate a greater amount of available nutrients, consequently resulting in gains in morphological and physiological attributes.

Therefore, this study indicates a positive influence of bioactivator application on the dry mass of reproductive structures (Table 2), providing a gain of 83% when compared to the control and 108% when compared to seed inoculation with *A. brasilense*. According to Castro et al. (2008), bioactivators may have various compositions, positively influencing metabolic processes and plant physiology, providing greater water and nutrient uptake, resistance to biotic and abiotic factors, increased synthesis of chlorophyll and photosynthesis and elongation of cell division.

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In view of the positive trends of bioactivator use, higher and significant statistical means can be verified (Table 3), for plant height and dry matter of stem + sheath. A study indicates that the use of bioactivators in plants can result in increased root length, stem diameter and dry mass accumulation in different plant organs, due to the stimulation of secondary metabolism, promoting increased protein content, affecting nitrogen metabolism, photosynthetic pigments and plant defense enzymes (Matysiak et al. 2011). These effects do not always cause significant changes in the plant, but some studies indicate an increase in the health of maize (Battistus et al. 2013).

Table 3. Averages of plant height (PH), stem diameter (SD), stem + sheath dry mass (SSDM), leaf dry mass (LDM), reproductive structure dry mass (RSDM), total dry mass (TDM), ear insertion height (EIH) and leaf area (LA) in plants of Hybrid maize, Morgan 30A91PWU, submitted to the application of soil bioactivator associated with inoculation methods of *Azospirillum brasilense*, in the phenological stage R3 of development.

Treatmemt	**PH**		**SD**	**SSDM**		**LDM**	**RSDM**	**TDM**	**EIH**	**LA**
	-- m --		**-mm-**			**----- g -----**			**-- cm --**	**-- cm^2 --**
						R3				
Control	2.3	d*	28.5 ^{ns}	333.5	ab*	175.9 ^{ns}	437.4 ^{ns}	946.8 ^{ns}	109.5 ^{ns}	4,336.6 ^{ns}
Azo Seed.	2.4	abcd	26.8	334.9	ab	186.4	378.4	899.7	121.7	4,776.4
Azo Fol.	2.4	bcd	29.2	344.4	ab	179.2	391.7	915.2	121	4,458.4
VS	2.5	ab	27.5	398.5	a	180.8	452.6	1031.9	116.2	4,470.6
Azo Seed. + *Azo* Fol.	2.4	cd	26.5	323.8	b	171.1	387.4	882.3	112	4,102.3
Azo Seed. + VS	2.5	abc	25.7	335.3	ab	167.2	395.8	898.3	114.5	4,379.5
Azo Fol. + VS	2.5	a	27.9	374.1	ab	184.2	443.5	1001.8	116.2	4,398.5
Azo Seed. + *Azo* Fol. +VS	2.5	ab	29.7	358.3	ab	182.6	445	985.9	121.5	4,858
CV (%)	1.8		6.3	8.1		8.7	26	13.6	8.3	11
LSD	0.10		4.13	67.19		36.66	256.50	304.68	0.23	1,168.14

* similar lowercase letters in the column did not differ by Tukey's test at 5% probability. ^{ns} there was no significant statistical difference by Tukey's test at 5% error probability. VS: Vitasoil Nano Science® soil bioactivator.

Plants that received the treatments application of bioactivator, foliar spraying of *A. brasilense* + application of bioactivator and seed inoculation with *A. brasilense* + foliar spraying of *A. brasilense* + application of bioactivator showed statistically superior height measurements at the R3 stage (Table 3) compared to the control and seed inoculation with *A. brasilense* + foliar spraying of *A. brasilense*. Conceição et al. (2008) mention that inoculation with PGPB stimulated the development of the aerial part of maize plants, relating the increase in growth to the action of bacteria in the production of plant hormones (Glick 2012), causing cell elongation, by vacuolar turgescence (Guimarães et al. 2017).

The use of a soil microbiota activator may also have contributed to the results, because the product used in question presents in its composition seaweed extract, which contains several growth regulators such as cytokinins, auxins and gibberellins (Durand et al. 2003). The positive effects on growth, development (Zhang and Schmidt, 2000; Arthur et al. 2003; Mansy et al. 2004; Payan and Stall 2004) and yield (Ferrazza and Simonetti 2010) of foliar products containing seaweed extracts in their composition are demonstrated in different cultivated species.

For leaf area (LA), there was no statistically significant difference between treatments at any phenological stage of the crop. Mógor et al (2008), evaluating the effect of foliar application of seaweed extract on the development of bean, 49 days after emergence observed an 85% increase in leaf area of plants that received the seaweed extract when compared to control plants.

Therefore, plants grown in a favorable environment for their development, can make the effects of products that have biostimulant action less pronounced, so the visualization and identification of these effects is easier when there is occurrence of stress situations (Galindo et al. 2019). It is worth noting, that this may explain the results found, since the plants did not pass through any environmental stress conditions along its cycle.

As for the gas exchange measurements at the phenological stages V8 and R1 (Table 4), the values of net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), leaf transpiration rate (*E*), water use efficiency (*WUE*), intrinsic water use efficiency (*iWUE*), and carboxylation efficiency (*Fc*), did not show significant variation by Tukey's test ($p \leq 0.05$).

In the absence of statistical significance, the high values of net CO_2 assimilation rate (*A*) obtained in the present study are due to the photosynthetic efficiency of *Zea mays* L., a plant that presents a C4 metabolism, with high photosynthetic capacity and a great response to light when under adequate environmental conditions.

Table 4. Averages of gas exchange indexes, net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), leaf transpiration rate (*E*), water use efficiency (*WUE*), intrinsic water use efficiency (*iWUE*) and carboxylation efficiency (*Fc*), in plants of Hybrid maize, Morgan 30A91PWU, submitted to the application of soil bioactivator associated with inoculation methods of *Azospirillum brasilense* analyzed in the phenological stage V8 and R1 of development.

PHENOLOGICAL STAGE V8							
	A	***gs***	***Ci***	***E***	***WUE* (*A/E*)**	***iWUE* (*A/gs*)**	***ACi* (*A/Ci*)**
Treatments	µmol CO^2 m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	µmol CO^2 mol^{-1}	µmol H_2O m^{-2} s^{-1}	µmol CO^2 (µmol H_2O) m^{-2} s^{-1}	µmol CO_2 (µmol H_2O) m^{-2} s^{-1}	(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$
Control	33.77 ^{ns}	0.34 ^{ns}	144.07 ^{ns}	5.80 ^{ns}	5.91^{ns}	99.95 ^{ns}	0.25 ^{ns}
Azo Seed.	32.17	0.39	159.45	5.69	6.10	91.08	0.21
Azo Fol.	23.89	0.21	131.18	4.03	6.13	116.14	0.18
VS	29.82	0.28	136.95	4.91	6.22	112.19	0.22
Azo Seed. + *Azo* Fol.	33.03	0.34	144.59	5.58	6.11	101.53	0.23
Azo Seed. + VS	31.91	0.33	144.07	5.43	6.03	101.43	0.23
Azo Fol. + VS	27.18	0.26	127.35	4.57	6.53	119.68	0.24
Azo Seed. + *Azo* Fol. + VS	28.72	0.29	136.73	4.72	6.18	111.18	0.22
LSD	13.08	0.22	55.71	2.40	1.61	41.41	0.14
CV (%)	18.35	30.68	16.71	19.91	11.07	16.37	26.97
PHENOLOGICAL STAGE R1							
	A	***gs***	***Ci***	***E***	***WUE* (*A/E*)**	***iWUE* (*A/gs*)**	***ACi* (*A/Ci*)**
Treatments	µmol CO^2 m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	µmol CO^2 mol^{-1}	µmol H_2O m^{-2} s^{-1}	µmol CO^2 (µmol H_2O) m^{-2} s^{-1}	µmol CO_2 (µmol H_2O) m^{-2} s^{-1}	(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$
Control	28.27 ^{ns}	0.38 ^{ns}	174.61 ^{ns}	5.55 ^{ns}	5.41^{ns}	91.37 ^{ns}	0.16 ^{ns}
Azo Seed.	25.32	0.36	212.09	5.09	5.18	71.84	0.13
Azo Fol.	23.96	0.29	175.70	4.61	5.74	96.81	0.14
VS	24.06	0.29	162.00	4.57	5.78	104.56	0.18
Azo Seed. + *Azo* Fol.	23.99	0.28	188.08	4.55	5.34	90.29	0.13
Azo Seed. + VS	31.37	0.47	198.08	6.30	5.14	73.28	0.16
Azo Fol. + VS	24.64	0.28	166.85	4.60	6.07	102.08	0.16
Azo Seed. + *Azo* Fol. + VS	25.86	0.29	169.15	4.68	5.87	103.41	0.15
LSD	12.3600	0.28	76.8200	2.8500	2.3200	54.3900	0.10
CV (%)	20.10	36.32	17.91	24.11	17.58	25.00	28.89

^{ns} no significant statistical difference by Tukey test at 5% error probability. VS: Vitasoil Nano Science® soil bioactivator.

Even with high values of the net CO_2 assimilation rate (*A*), this study provides opposite results to other studies that report that the inoculation of maize and brachiaria hybrids with PGPB provides greater acclimation of the plants, an increase in the efficiency of nitrogen uptake and utilization, and consequently an improvement in the photosynthetic energy flow, probably due to a mechanism that aims to maintain photosynthesis by reducing chlorophyll degradation, reflecting in a higher net CO_2 assimilation rate (*A*) (Barassi et al. 2008; Tikkanen and Aro 2014; Cunha et al. 2016; Bulegon et al. 2017; Calzavara et al. 2018).

In general, the analysis of water use efficiency (*WUE*), intrinsic water use efficiency (*iWUE*) and carboxylation efficiency (*Fc*), in both phenological stages, did not show statistical differences. The *WUE* and *iWUE*, are important for quantifying the adaptation of plants to the growing environment in which they were subjected, demonstrating how well the plant is able to use the absorbed water and convert it into plant biomass (Bulegon et al. 2016a). Thus, the results contrast with research with *Urochloa ruziziensis*, which prove that the use of *A. brasilense* increases the efficiency of water use in plants under severe water deficit, surpassing in some moments plants that had constant irrigation (Bulegon et al. 2017).

In relation to stomatal conductance (*gs*), another point to be highlighted is that the opening and closing of stomata are closely linked to environmental factors, being influenced by various factors such as temperature, relative humidity of the air, incident light. When using growth promoting microorganisms such as *A. brasilense*, it is not known what is directly linked to stomatal regulation, however, it is speculated that it is linked to the hormonal balance of the plant, especially hormones that modulate root growth, due to the ability of the bacterium to provide plant hormones such as auxins, cytokinins and gibberellins. (Perrig et al. 2007; Glick 2014; Bulegon 2016b; Taiz et al. 2017).

The inoculation and foliar application with *A. brasilense*, as well as the application of soil bioactivator, did not show increases in production and yield components (Table 5).

Table 5. Averages of the production components ear diameter (ED), ear length (EL), number of grain rows (NGR), number of grains per row (NGPR), mass of 1000 grains (M1000) and yield (YIELD) of Morgan hybrid maize plants 30A91PWU submitted to the application of soil bioactivator associated with inoculation methods of *Azospirillum brasilense* analyzed in phenological stages V8, VT and R3 of development.

Treatment	ED	EL	NGR	NGPR	M1000	YIELD
	-- mm --	-- cm --			-- g --	-- kg ha^{-1} --
Control	46.3 ^{ns}	19.8 ^{ns}	16.9 ^{ns}	38.2 ^{ns}	298.5 ^{ns}	11,031.3 ^{ns}
Azo Seed.	46.2	19.2	16.5	37.1	290.8	11,031.6
Azo Fol.	47.9	19.3	16.9	38.0	305.5	11,985.9
VS	48.5	19.1	16.8	38.0	285.7	11,402.8
Azo Seed. + *Azo* Fol.	48.3	19.9	16.6	38.5	306.5	11,481.8
Azo Seed. + VS	48.7	19.5	16.6	37.5	222.7	10,366.9
Azo Fol. + VS	47.6	20.3	17.0	37.7	283.7	11,208.0
Azo Seed. + *Azo* Fol. + VS	47.7	19.4	17.0	38.8	306.8	11,092.3
LSD	3.38	3.42	0.69	3.75	130.76	2,213.08
CV (%)	3.0	6.9	1.7	3.1	19.2	8.3

^{ns} no significant statistical difference by Tukey test at 5% error probability. VS: Vitasoil Nano Science® soil bioactivator.

Similar to this study, Mumbach et al. (2017) evaluating the effects of *Azospirillum brasilense* associated with nitrogen fertilization on the production components and productivity of maize and wheat, observed that there were no significant gains with inoculation, and the crops showed a strong dependence on the supply of nitrogen fertilization. Bartchechen et al. (2010) who worked with nitrogen fertilization associated with inoculation of *A. brasilense* in maize plants also did not find higher yields compared to non-inoculated plants.

Even in the absence of differences between the treatments tested, studies have indicated positive responses in the production components and productivity when soil bioactivator is used. Alovisi et al. (2017), found increases in productivity in maize plants when a bioactivator was added to the soil. Igna and Marchioro (2010), evaluating the effects of seed treatment and foliar spraying of a bioactivator based on algae extract of *Ascophyllum nodosum*, on production components and productivity of wheat, concluded that the extract of this alga provides significant gains in the number of ears per area and consequently in grain yield.

Cunha et al. (2014), evaluating the efficiency of *Azospirillum spp*. in maize crop observed an increase of 5.5 more bags per hectare compared to non-inoculated plants. This result is attributed to the better use of nutrients by the plant, corroborating for an improvement in the photosynthetic process, due to the increase in the content of chlorophylls, which resulted in greener plants. In their studies, Hungary et al. (2010) verified increases of 24 to 30% in grain yield in maize plants inoculated with different strains of *A. brasilense* and *A. lipoferum* compared to non-inoculated plants.

In the present study, no significant variation was observed in the production components and yield, Grohs et al. (2012), studying the effects of biostimulants based on algae extract in rice culture, observed the stimulation in tillering and increase in the number of panicles per square meter of rice cultivars, however, even with these effects, no influence on grain yield of rice was observed. Similarly, foliar application of biostimulants based on algae extracts of *Egeria densa* and *Ascophyllum nodosum*, did not promote significant increases in plant height and ear insertion height, as well as in production components of irrigated maize (Galindo et al. 2015).

Thus, the grain production process for maize crops is directly related to a series of characteristics, which are called production components. The production components consist of ear length, ear diameter, number of grains per row, number of grain rows and mass of one thousand grains (Lopes et al. 2007). These components, together with plant genotype, nutrient availability and climatic conditions during the grain filling stages, determine the productivity of the crop (Ohland et al. 2005; Pereira et al. 2009).

Grain production is highly influenced by genetics, soil and climate factors; therefore, the use of inoculation with bacteria of the genus *Azospirillum* via seed and foliar has its importance increased, as they contribute to mitigate water stress (Bulegon et al. 2016b), tolerance to crop stress in saline soil (Slama et al. 2015), increase in root system (Dartora et al. 2013) and crop productivity (Morais et al. 2016).

Considering that the soil conditions were adequate for the development of the crop, among other soil and climatic factors, it is possible that these factors were sufficient for the development and maintenance of productivity, minimizing the effect of the treatments and without significant increases in production components.

Therefore, the lack of patterns in responses to *Azospirillum* inoculation and foliar spraying may be linked to the fact that the plant-bacterium interaction is associative and, although not clearly evidenced, there may be affinity between bacteria and cultivar (Hungary 2011), thus presenting great versatility and low specificity (Moreira et al. 2010), making this relationship sensitive to the most diverse variations in climate, soil, plant and bacteria (Quadros et al. 2014).

Thus, due to the scarcity of relevant information about the use of soil bioactivators and their effects on crop development, further studies are important to understand their real effect. Studies with growth promoting bacteria are necessary to support technical positions, to obtain higher yields and consequent profitability.

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