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Effect of nitrogen fertilization associated with inoculation of *Azospirillum brasilense* and *Herbaspirillum seropedicae* on corn

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The growing interest in the use of inoculants with diazotrophic bacteria that promote plant growth, providing increase in crop productivity, occurs because of the high costs of chemical fertilizers and the concern about environmental quality. Given the above, this study aimed to evaluate, in field conditions, the effect of nitrogen fertilization and inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* on the productivity, phytotechnical parameters and nutritional state of corn. The experiment was set in a randomized block design, with nine treatments and six replicates: Control without N and without inoculation; *A. brasilense* inoculation without N; *H. seropedicae* inoculation without N; 30 kg ha^{-1} of N at the sowing; *A. brasilense* + 30 kg ha^{-1} of N at the sowing; *H. seropedicae* + 30 kg ha^{-1} of N at the sowing; 30 kg ha^{-1} of N at the sowing + 90 kg ha^{-1} of N in covering; *A. brasilense* + 30 kg ha^{-1} of N at the sowing + 90 kg ha^{-1} of N in covering; and *H. seropedicae* + 30 kg ha^{-1} of N at the sowing + 90 kg ha^{-1} of N in covering. The evaluated parameters were: plant height, stem diameter, ear insertion height, ear weight, ear length, number of grain rows per ear, number of grains per ear, ear base diameter, weight of 1,000 grains, shoot dry weight, yield, chlorophyll content, leaf nutrient content and grain nutrient content. The nitrogen fertilization associated with inoculation of *A. brasilense* and *H. seropedicae* positively influenced ear weight, ear diameter, number of grains per ear, shoot dry weight, yield and chlorophyll content of corn plants. The contents of N, P, K and Zn in corn leaves increased with nitrogen fertilization and inoculation with *A. brasilense* and *H. seropedicae*. The inoculation with *A. brasilense* without nitrogen fertilization promoted higher accumulations of N, K, Ca and Mg in grains compared with the treatments inoculated with *A. brasilense* and *H. seropedicae* and fertilized with 30 and 120 kg ha^{-1} of N. The inoculation with *A. brasilense* or *H. seropedicae* associated with nitrogen fertilization may lead to a reduction in the use of synthetic nitrogen fertilizers in corn cultivation.

Key words: *Zea mays* L., nitrogen, diazotrophic bacteria.

INTRODUCTION

Among mineral nutrients, nitrogen (N) is one of the most important and limiting for corn productivity, and its application is required in large amounts in order to meet crop demand (Dotto et al., 2010). The economic and

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environmental costs related to N fertilization have stimulated the search for alternatives that might reduce the use of these fertilizers, without any decrease in production. One way to make it a possible low-cost production without harming the environment is the use of soil biological resources, like diazotrophic bacteria, which are also considered as growth-promoting bacteria, for their capacity to fix nitrogen (N_2) for the plant and produce growth hormones, such as auxins and gibberellins (Dobbelaere et al., 2002; Radvan et al., 2002).

The Biological Nitrogen Fixation (BNF) process in grass crops is not as efficient as it is in soybean, to which even 94% of the N required by plant can be supplied by BNF (Hungria et al., 2006). In grass crops, the transfer of the fixed N to the plant occurs slowly and only a small amount becomes available to plant; therefore, BNF by these bacteria in association with grass crops is only able to meet plant nitrogen demand partially (Hungria et al., 2011).

Among the diazotrophic microorganisms found in association with grass crops, the species *Azospirillum, Herbaspirillum* and *Burkholderia* are currently the most studied groups. Bacteria capable of fixing atmospheric nitrogen, such as the genera *Azospirillum* and *Herbaspirillum*, have been isolated in rice, wheat, corn and sugarcane plants (Reis Júnior et al., 2004; 2008; Perin et al., 2006; Rodrigues et al., 2006). The results of inoculations with these genera are variable and significant effects on grain production and nutrient absorption have already been reported (Kennedy et al., 2004; Guimarães et al., 2007; Ferreira et al., 2010; Hungria et al., 2010; Braccini et al., 2012). In this context, Alves (2007) verified that inoculation with strains of the genus *Herbaspirillum* contributed with up to 34% of the absorbed N in corn plants. Alves et al. (2011), found that inoculation with the strain BR11417 of *Herbaspirillum seropedicae* contributed in average with 26% of the N necessary for the development of corn. García de Salomone et al. (1996) verified that some corn varieties cultivated in pots fixed around 58% of their N requirement when inoculated with *Azospirillum* sp.

Most studies related to this topic address only the isolation of these bacteria in corn plants and their biochemical studies in laboratory; while studies that are more comprehensive, in field conditions, showing the plant-microorganism-environment interaction related to crop development, are scarce (Dotto et al., 2010). Therefore, the knowledge about the potentialities and the use of these bacteria as an alternative for nitrogen nutrition for many economically important Poaceae crops, such as corn, may result in great impact, not only on production volume and size of planted area, but also on their socioeconomic and environmental importance, which makes the study on the agronomic efficiency of these bacteria an essential and economically viable strategy. Given the above, this study aimed to evaluate, in field conditions, the effect of nitrogen fertilization and inoculation with *Azospirillum brasilense* and *H. seropedicae* on the productivity, phytotechnical parameters and nutritional state of corn.

MATERIALS AND METHODS

The experiment was carried out in the experimental field of Embrapa Western Region Agriculture, in Dourados-MS, Brazil, from March to July of 2012. The geographical coordinates are 22° 14' S and 54° 9' W, with average altitude of 450 m. The climate of the region is Cwa, according to Köppen's classification. The soil was classified as distroferric Red Latosol, with very clayey texture (Embrapa, 2013). Average data of temperature and rainfall during the experiment were obtained from the Weather Station of Embrapa Western Region Agriculture, in Dourados-MS, and are shown in Figure 1.

The results of soil chemical analysis, in the layer of 0-20 cm, before the experiment was installed, are as follows: pH ($CaCl_2$): 4.5; O.M.: 31.18 g dm^{-3}; C: 18.13 g dm^{-3}; P (mehlich): 22.07 mg dm^{-3}; K: 6.0 $mmol_c$ dm^{-3}; Ca: 35.4 $mmol_c$ dm^{-3}; Mg: 8.7 $mmol_c$ dm^{-3}; Al: 4.8 $mmol_c$ dm^{-3}; H+Al: 62.1 $mmol_c$ dm^{-3}; SB: 50.1 $mmol_c$ dm^{-3}; CEC: 112.2 $mmol_c$ dm^{-3}; Base saturation: 44.65%; Zn: 1.65 mg dm^{-3}; Cu: 9.27 mg dm^{-3}; Fe: 29.14 mg dm^{-3}; and Mn: 24.06 mg dm^{-3}. Granulometric analysis showed the following values: 215 g kg^{-1} of sand, 115 g kg^{-1} of silt and 670 g $kg^{-1}$of clay. Soil correction was performed one month before sowing, with 1720 kg ha^{-1} of dolomitic limestone (RNV 100%), considering the results of soil chemical analysis, aiming to increase base saturation to 60%. The area was irrigated after crop installation and in periods with high water deficit. The adopted experimental design was randomized blocks, with nine treatments and six replicates: 1) Control without N and without inoculation; 2) *A. brasilense* inoculation without N; 3) *H. seropedicae* inoculation without N; 4) 30 kg ha^{-1} of N at the sowing; 5) *A. brasilense* + 30 kg ha^{-1} of N at the sowing; 6) *H. seropedicae* + 30 kg ha^{-1} of N at the sowing; 7) 30 kg ha^{-1} of N at the sowing + 90 kg ha^{-1} of N in covering; 8) *A. brasilense* + 30 kg ha^{-1} of N at the sowing + 90 kg ha^{-1} of N in covering; 9) and *H. seropedicae* + 30 kg ha^{-1} of N at the sowing + 90 kg ha^{-1} of N in covering.

The used seeds of the simple hybrid P3646H were previously inoculated with a liquid inoculant containing a combination of two strains of *A. brasilense* (Ab-V5 and Ab-V6) and a peat-based inoculant containing the Z-94 strain of *H. seropedicae*, produced by Embrapa Agrobiology, Seropédica-RJ. The applied dose was 150 ml of peat-based inoculant for each 50 kg of corn seeds. For the inoculation with the Z-94 strain of *H. seropedicae*, 60 ml of a sugary solution at 10% (m/v) were added to each 10 kg of seeds, aiming to increase the adhesion of the inoculant to the seeds. Broadcast fertilization was performed at sowing, with later incorporation, by applying 300 kg ha^{-1} of a 0-20-20 formulation in order to supply 60 kg ha^{-1} of P_2O_5 and K_2O, respectively. Sowing was performed manually, with the aid of a hand-held corn planter (known as "matraca"), planting two seeds per hole and six plants per meter were left after thinning. Each experimental unit was composed of five 5-m long rows, spaced 0.90 m apart, with a population of 6000 plants/hectare. The three central rows were considered as the useful plot area, excluding 0.5 m of each side. Nitrogen fertilization was applied using 30 kg ha^{-1} of N in the furrow and 90 kg ha^{-1} of N divided into two applications of 45 kg ha^{-1}, in covering, as urea (45%), in all the plot area, at the development stages of V4 and V7, respectively.

At the flowering stage (emergence of feminine inflorescence) of the crop, to 60 days after the emergency of plants, leaf samplings were performed, according to the methodology proposed by Malavolta et al. (1997), in order to determine nutrient content in the

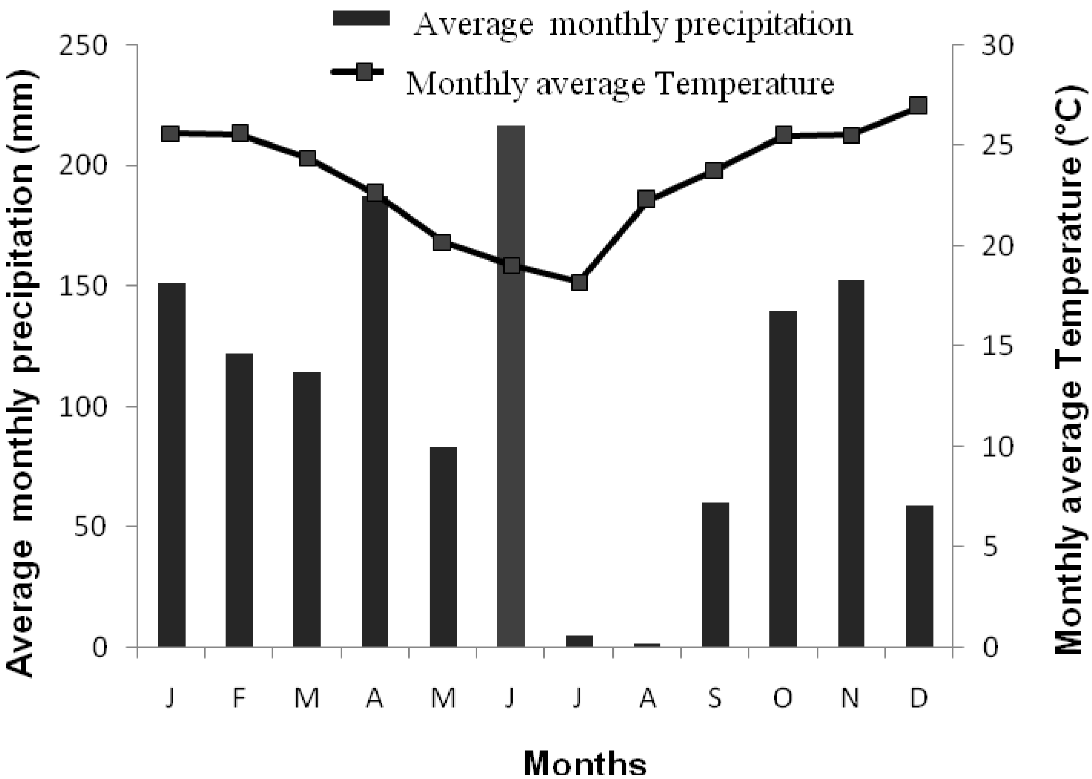

Figure 1. Monthly average rainfall (mm) and temperature (°C), recorded at the Weather Station of Embrapa Western Region Agriculture, in Dourados-MS, Brazil, in 2012.

plant leaf tissue. For this, the middle third, with leaf veins, of the leaf opposite and below the primary ear was collected, totaling 10 leaves per experimental unit and, in the same phenological stage, leaf chlorophyll content was determined with the aid of a chlorophyll meter (model SPAD-502).

All the collected plant material was washed in running water, 0.1 mol L^{-1} HCl solution and deionized water. Samples were accommodated in paper bags, dried in a forced-air oven at 65°C for 72 h and then ground. Ground samples were subjected to sulfuric digestion and nitric-perchloric digestion, followed by the determinations of N, P, K, Ca, Mg, S, Zn, Cu, Fe and Mn leaf contents, using the methodology described by Embrapa (2009). At the harvest, nutrient contents were also determined in corn grains.

The corn harvest was performed manually, to 150 days after the emergency, collecting all ears of a useful plot area of 9.0 m^2. In order to determine production components, 10 representative ears were sampled per plot, outside the area of grain production. The following parameters were evaluated: ear weight, ear length, number of grain rows per ear, number of grains per ear, ear base diameter and weight of 1,000 grains. For the plant, the following parameters were evaluated: plant height, stem diameter and ear insertion height. In order to determine grain productivity, ears were threshed with the aid of a manual machine and then weighed. The obtained results were converted to kg ha^{-1}, correcting moisture to 13% on a wet basis. Shoot dry weight of plants was estimated by the sampling of three plants in each plot. The determination of shoot dry weight was performed through the drying of plant samples in a forced-air oven at 65°C for 72 h. Shoot dry weight data were expressed in g/plant. The obtained results were subjected to analysis of variance and means were compared by Tukey test at 5% of probability, using the statistical analysis software SISVAR (Ferreira, 2000).

RESULTS AND DISCUSSION

There was significant difference ($p \leq 0.05$) for ear weight, ear diameter, number of grains per ear, shoot dry weight, productivity and chlorophyll content in response to nitrogen fertilization and inoculation with *A. brasilense* and *H. seropedicae* (Tables 1 and 2). Ear weight showed higher values in the treatment corresponding to inoculation with *H. seropedicae* + 120 kg ha^{-1} of N, being higher than the control (without either inoculation or N) and the treatment only inoculated with *H. seropedicae*, and similar to the other evaluated treatments (Table 1). The values ranged from 217.42 g/plant (control) to 249.42 g/plant (*H. seropedicae* + 120 kg ha^{-1} of N), with increases of 14.71% compared with the control (Table 1). The ear diameter and the number of grains per ear were higher in the treatment fertilized with 120 kg ha^{-1} of N, statistically differing ($p \leq 0.05$) from the control, the treatment inoculated with *H. seropedicae* and that inoculated with *A. brasilense* + 30 kg ha^{-1} of N (Table 1). Guimarães et al. (2012) verified that the inoculation of corn seeds with *Azospirillum* spp., in combination with nitrogen fertilization (60 kg ha^{-1} of N), resulted in ears with higher diameter and higher number of grain rows.

It was not found significant effect of nitrogen fertilization associated with inoculation of *A. brasilense* and *H. seropedicae* for plant height, ear insertion height, stem

Table 1. Plant height (PH), ear insertion height (EIH), stem diameter (SD), ear weight (EW), ear length (EL), ear diameter (ED), number of grain rows per ear (NGRE) and number of grains per ear (NGE) of corn plants in response to nitrogen fertilization and inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae*. Dourados-MS, Brazil (2012).

Treatments	PH	EIH	SD	EW	EL	ED	NGRE	NGE
	(cm)		(mm)	(g)	(cm)	(mm)		
1. Control	216.30	122.63	21,32	217.42 b	17.04	51.45 b	15.44	493.16 b
2. *A.brasilense*	218.40	124.00	20.20	220.52 ab	17.03	52.72 ab	15.52	517.16 ab
3. *H. seropedicae*	219.30	122.63	20.31	216.23 b	17.13	51.53 b	15.66	501.00 b
4. 30 kg ha^{-1} N	222.20	126.20	20.51	242.19 ab	18.12	53.12 ab	15.47	543.38 ab
5. *A.brasilense* + 30 kg ha^{-1} N	216.80	122.93	20.30	224.08 ab	17.57	51.43 b	15.66	510.33 ab
6. *H. seropedicae* + 30 kg ha^{-1} N	218.83	124.20	21.48	220.63 ab	17.82	52.59 ab	15.61	544.30 ab
7. 30 kg ha^{-1} N + 90 kg ha^{-1} N	223.36	125.06	21.62	245.97 ab	17.89	53.83 a	16.55	576.66 a
8. *A. brasilense* + 120 kg ha^{-1} N (30+90)	223.66	123.60	21.18	238.82 ab	17.82	53.08 ab	16.00	530.64 ab
9. *H. seropedicae* + 120 kg ha^{-1} N (30+90)	228.86	127.46	22.09	249.42a	17.82	53.34 ab	16.00	554.94 ab
Average	220.85	124.30	21.00	230.59	17.64	52.57	15.73	530.17
Teste F	1.40ns	0.51ns	1.00ns	3.91*	1.63ns	3.41*	0.93ns	2.95*
CV (%)	3.78	4.59	7,98	7.14	5.15	2.26	5.60	7.32

* e ns– significant 5% probability and non-significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between them by Tukey test, the 5% probability. CV: coefficient of variation.

Table 2. Shoot dry weight (SDW), productivity (PRO), weight of 1,000 grains (W1000) and chlorophyll content (CLO) of corn plants in response to nitrogen fertilization and inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae*. Dourados-MS, Brazil (2012).

Treatments	SDW	PRO	W1000	CLO
	(g)	(kg ha^{-1})	(g)	(SPAD)
1. Control	246.63 b	9231.71 b	358.67	61.58 b
2. *A.brasilense*	257.26 ab	9078.41 b	352.04	65.18 abc
3. *H. seropedicae*	258.50 ab	9023.88 b	351.03	61.85 c
4. 30 kg ha^{-1} N	271.03 ab	9302.05 b	350.76	61.60 c
5. *A.brasilense* + 30 kg ha^{-1} N	274.40 ab	9531.75 ab	351.55	63.45 abc
6. *H. seropedicae* + 30 kg ha^{-1} N	293.96 ab	9133.32 b	357.62	62.51bc
7. 30 kg ha^{-1} N + 90 kg ha^{-1} N	276.68 ab	10146.52 a	356.35	69.06a
8. *A. brasilense* + 120 kg ha^{-1} N (30+90)	283.83 ab	9861.37 ab	362,27	69.06 a
9. *H. seropedicae* + 120 kg ha^{-1} N (30+90)	314.80 a	9858.23 ab	359.43	68.18 ab
Average	275.23	9463.00	355.52	64.72
Teste F	2.27*	4.76*	0.57ns	5.92*
CV (%)	12.21	4.81	3.90	5.04

* e ns– significant 5% probability and non-significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between them by Tukey test, the 5% probability. CV: coefficient of variation.

diameter, ear length, number of grain rows per ear and weight of 1,000 grains (Tables 1 and 2). Similar results were obtained, also in field conditions, by Lana et al. (2012), who did not observe positive response of the nitrogen fertilization and inoculation with *Azospirillum* sp. for plant height and ear insertion height in corn plants; by Braccini et al. (2012), who did not verify effect of inoculation with *A. brasilense* on the weight of 1,000 grains of corn; and by Dotto et al. (2010), who did not find significant effect of inoculation with *H. seropedicae* on stem diameter, ear insertion height, ear weight, cob weight, ear length and weight of 1.000 grains of corn.

The shoot dry weight yield ranged from 246.63 g/plant in the control treatment to 314.80 g/plant in the treatment inoculated with *H. seropedicae* + 120 kg ha^{-1} of N. It should be pointed out that this last treatment proved to be superior ($p \leq 0.05$) to the control and similar to the other ones (Table 2). The increase in shoot dry weight was of 27.64% in relation to the control (without either fertilization or inoculation). It is worth noticing that this higher shoot dry weight production of plants inoculated with *H. seropedicae* and supplied with 120 kg ha^{-1} of N may have been favored by the production of growth-promoting substances by bacteria. Reis Junior et al. (2008) observed increase in dry weight of corn plants inoculated with *Azospirillum* spp. Lana et al. (2012) verified that inoculation with *Azospirillum* spp. without nitrogen fertilization increased dry weight of corn plants in 7.2%. Braccini et al. (2012) found relative increase in dry weight production with the inoculation of corn seeds with *A.brasilense*. Similar results were also found by Quadros (2009), with inoculation of *Azospirillum* spp. in corn and by Ferreira et al. (2010) and Guimarães et al. (2010) in rice plants inoculated with *H. seropedicae.*

The highest corn grain yields were obtained in the treatment corresponding to fertilization with 120 kg ha^{-1} of N, which was not statistically different from the treatment inoculated with *H. seropedicae* + 120 kg ha^{-1} of N and from that inoculated with *A. brasilense* + 30 and 120 kg ha^{-1} of N (Chart 2). It is noteworthy that the treatment corresponding to the fertilization with 120 kg ha^{-1} of N promoted an increase in grain yield of about 10%, compared with the control, which did not have either inoculation or nitrogen fertilization. As for the treatments with inoculation of *H. seropedicae* + 120 kg ha^{-1} of N and those inoculated with *A. brasilense* + 30 and 120 kg ha^{-1} of N promoted increases in grain yield of about 6.78, 6.82 and 3.25%, respectively, compared with the control. In average, these treatments promoted an increase of 518.74 kg ha^{-1} of corn grains compared with the control, which represents a gain of 8.64 sacks per hectare, suggesting the applicability of inoculation associated with nitrogen fertilization in corn cultivation.

Various studies have so far reported beneficial effect of inoculation with *Azospirillum* or *Herbaspirillum* on corn. Kappes et al. (2013) found increases of 9.4% in corn grain yield when seeds were inoculated with *A. brasilense*. Alves (2007) observed percent increases of 24 and 34% in corn yield with the use of *H. seropedicae* in second and first corn cropping seasons, respectively, and that inoculation can supply up to 40 kg ha^{-1} of N. Hungria et al. (2011) and Lana et al. (2012) verified that inoculation with *A. brasilense* in corn promoted increase in grain yield of 26 and 15.4%, respectively. In studies with wheat and rice, Dalla Santa et al. (2008) and Pedraza et al. (2009) also reported that inoculation with *Azospirillum* spp. increased grain yield compared with the control (without either N or inoculant).

Increases in shoot dry weight and grain yield of corn in response to inoculation can be attributed to the stimulus that diazotrophic bacteria provide to the development of the root system, with increase in root hair density, length, volume and number of lateral roots, resulting in higher capacity to absorb and use water and nutrients, as reported by Hungria et al. (2011) and Huergo et al. (2008). Based on the results already shown, it is important to point out that most treatments with diazotrophic bacteria inoculation combined with 30 kg ha^{-1} of N showed results similar to those from the treatment with the highest N dose (120 kg ha^{-1} of N) regarding ear weight, ear diameter, number of grains per ear, shoot dry weight production and grain yield (Tables 1 and 2). It allows one to suggest that the application of 30 kg ha^{-1} of N is less costly than the application of 120 kg ha^{-1} of N, which in turn can lead to a reduction in the use of synthetic nitrogen fertilizers and, consequently, reduction in production costs.

The highest chlorophyll contents were verified in the treatments with inoculation of *A. brasilense* combined with 120 kg ha^{-1} of N or only fertilized with 120 kg ha^{-1} of N, which did not differ statistically ($p \geq 0.05$) from the treatments with only the inoculation of *A. brasilense* or also supplied with 30 kg ha^{-1} of N, and from the treatment with inoculation of *H. seropedicae* + 120 kg ha^{-1} of N (Table 2). It is observed that leaf chlorophyll content is positively correlated with N content in the plant (BOOIJ et al., 2000). Many studies have demonstrated that the addition of N in corn has a direct effect on root exudation, increasing the supply of carbon sources to bacteria, stimulating their colonization and the effectuation of the inoculation (Kolb and Martin, 1987), as well as it can benefit biological nitrogen fixation (Dalla Santa et al., 2004; Alves, 2007; Ferreira et al., 2011). It occurs because, under N deficiency conditions, the plant cannot either excrete, deposit or exudate sufficient organic compounds and/or root exudates to emit signals to microorganisms. Thus, it is essential a nitrogen supplementation that allows good development of plant, without harming BNF, since the movement of microorganisms towards the roots occurs when there is biochemical recognition (chemotaxis), that is, the emission of signals by plants to microorganisms. Canellas et al. (2013), also observed increase in chlorophyll content of corn plants when inoculated

Table 3. Effect of nitrogen fertilization and inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* on the nutrient content (g kg^{-1} for macronutrients and mg g^{-1} for micronutrients) of corn leaves during flowering stage. Dourados-MS, Brazil (2012).

Treatments	N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn
	(g kg^{-1})						mg g^{-1}			
1. Control	32.20 ^{b}	3.23 ^{c}	17.98 e	1.90	0.56	1.34	13.34 ^{ab}	8.99	296.67	40.67
2. *A.*^{b}*rasilense*	40.44 ^{ab}	3.48 ^{bc}	21.20 ^{c}de	2.02	0.65	1.15	13.20 ^{ab}	9.24	292.34	42.19
3. *H. seropedicae*	39.69 ^{ab}	3.92 ^{ab}	20.71 de	2.00	0.61	1.29	13.03 ^{ab}	9.27	255.87	39.09
4. 30 kg ha^{-1} N	39.94 ^{ab}	4.02 ^{ab}	25.91 ^{ab}	2.10	0.64	1.40	12.85 ^{ab}	9.77	215.41	49.65
5. *A.*^{b}*rasilense* + 30 kg ha^{-1} N	40.69 ^{ab}	3.52 ^{bc}	24.51 ^{bc}d	2.21	0.62	1.33	11.09 ^{b}	8.60	289.84	46.41
6. *H. seropedicae* + 30 kg ha^{-1} N	37.69 ^{ab}	4.24 ^{a}	25.75 ^{ab}	2.17	0.71	1.26	12.31 ^{ab}	8.57	202.62	45.93
7. 30 kg ha^{-1} N + 90 kg ha^{-1} N	45.43 ^{ab}	4.21 ^{a}	26.24 ^{ab}	1.97	0.64	1.14	13.45 ^{ab}	9.64	266.28	53.05
8. *A.* ^{b}*rasilense* + 120 kg ha^{-1} N	49.18 ^{a}	3.91 ^{ab}	25.33 ^{abc}	1.67	0.53	1.34	14.24 ^{a}	8.98	263.43	47.87
9. *H. seropedicae* + 120 kg ha^{-1} N	42.94 ^{ab}	4.20 ^{a}	29.72 ^{a}	1.89	0.63	1.44	15.14 ^{a}	9.40	285.45	47.98
Average	40.91	3.86	24.15	1.99	0.62	1.30	13.18	9.16	274.21	45.87
Teste F	2.19*	6.64*	13.34*	2.11^{ns}	1.18^{ns}	1.94^{ns}	2.86*	0.78^{ns}	1.50^{ns}	1.79^{ns}
CV (%)	19.28	9.04	9.87	13.83	18.89	13.67	12.46	12.66	13.82	17.88

*e ns– significant 5% probability and non-significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between them by Tukey test, the 5% probability. CV: coefficient of variation.

with *H. seropedicae* in association with humic substances.

The contents of N, P, K and Zn in leaves and N, K, Ca and Mg in grains of corn were positively influenced by nitrogen fertilization and inoculation with diazotrophic bacteria, whereas the contents of Ca, Mg, S, Cu, Fe and Mn in leaves and P, S, Zn, Cu and Fe in grains did not respond to the treatments (Tables 3 and 4). The N contents in corn leaves ranged from 32.20 g kg^{-1} in the control treatment to 49.18 g kg^{-1} in the treatment inoculated with *A. brasilense* + 120 kg ha^{-1} of N, evidencing that the inoculation coupled with 30 kg ha^{-1} of N at sowing + 90 kg ha^{-1} of N in covering increased N content in corn leaves in about 52.73%, compared with the control (without either inoculation or N) (Table 3). The contents of P, K and Zn in the leaves, unlike N content, were higher in the treatment inoculated with *H. seropedicae* + 120 kg ha^{-1} of N. There were increases of 30%, 64.29 and 13.49% in the contents of P, K and Zn, respectively, compared with the control (Table 3). These results suggest that inoculation with these diazotrophic bacteria associated with nitrogen fertilization increases N, P, K and Zn contents in corn leaves and that inoculation can contribute to improving the use of N-fertilizers by the plant (Dobbelaere et al., 2003).

Dobbelaere et al. (2001) observed increase in N, P, and K contents of corn leaves when working with bacteria from the genus *Azospirillum.* Francisco et al. (2012) found increase in Zn concentrations of corn leaves when inoculated with *A. brasilense* and supplied with 30 kg ha^{-1} of N. Hungria et al. (2010) observed higher leaf contents of N, P, Zn and Cu in corn plants inoculated with *Azospirillum* spp. Bashan et al. (2004) and Alves (2007) also reported increase in nutrient contents of plants inoculated with diazotrophic bacteria.

This higher absorption of nutrients N, P, K and Zn, by roots can occur as a result of the production of growth-promoting substances by bacteria (Baldani and Baldani, 2005), or changes in root architecture (Dobbelaere et al., 1999), which allows better exploration of soil and increases plant's capacity to absorb nutrients (Creus et al., 2004). Besides, it can be attributed to nitrogen fertilization and to solubilization of zinc phosphates and oxides by bacteria (Baldotto et al., 2010).

Regarding the contents of N and K in corn grains, the inoculation with *A. brasilense* without nitrogen fertilization promoted higher accumulation in grains, statistically differing from the treatments with inoculation coupled with nitrogen fertilization (Table 4). The inoculation with *A. brasilense* promoted average increases in N and K grain contents of approximately 25.47 and 72.35%, respectively, compared with the

Table 4. Effect of nitrogen fertilization and inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* on the nutrient contents (g kg^{-1} for macronutrients and mg g^{-1} for micronutrients) of corn grains. Dourados-MS, Brazil (2012).

Treatments	N	P	K	C^a	Mg	S	Zn	Cu	Fe	Mn
	(g kg^{-1})						(mg g^{-1})			
1. Control	18.72 ^{ab}	5.48	4.34 ^{ab}	0.32 ^{ab}	1.14 ^{a}	0.69	13.71	1.50	51.53	1.71
2. *A.brasilense*	19.99 ^{a}	4.88	4.92 ^{a}	0.34 ^{a}	1.01 ^{a}	0.63	13.26	0.49	41.90	1.68
3. *H. seropedicae*	15.47 ^{bc}	4.86	3.76 ^{ab}	0.24 ^{bc}	0.93 ^{a}	0.61	13.30	0.72	41.96	1.67
4. 30 kg ha^{-1} N	15.97 ^{bc}	4.90	4.26 ^{ab}	0.17 ^{c}	0.54 ^{b}	0.68	14.90	0.78	39.21	2.06
5. *A.brasilense* + 30 kg ha^{-1} N	12.48 ^{c}	4.52	4.01 ^{ab}	0.21 ^{c}	0.48 ^{b}	0.76	13.99	0.83	54.50	1.79
6. *H. seropedicae* + 30 kg ha^{-1} N	14.48 ^{bc}	5.07	3.76 ^{ab}	0.18 ^{c}	0.53 ^{b}	0.78	12.22	1.46	30.62	1.71
7. 30 kg ha^{-1} N + 90 kg ha^{-1} N	15.47 ^{bc}	5.26	3.51 ^{b}	0.20 ^{c}	0.60 ^{b}	0.72	13.54	2.06	22.48	1.98
8. *A. brasilense* + 120 kg ha^{-1} N (30+90)	15.47 ^{bc}	5.40	3.59 ^{b}	0.35 ^{a}	1.06 ^{a}	0.73	10.17	2.24	42.16	2.02
9. *H. seropedicae* + 120 kg ha^{-1} N (30+90)	14.98 ^{bc}	5.56	3.59 ^{b}	0.36 ^{a}	1.10 ^{a}	0.76	10.02	2,34	47.62	2.05
Average	16.36	5.10	3.97	0.26	0.82	0.70	12.74	1.38	41.33	1.85
Teste F	7.94*	1.56^{ns}	3.12*	15.17*	17.37*	8.96^{ns}	11.15^{ns}	8.01^{ns}	1.87^{ns}	8.95^{ns}
CV (%)	17.84	13.21	16.05	17.80	19.64	8.27	13.02	79.17	43.03	20.40

* e ns– significant 5% probability and non-significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between them by Tukey test, the 5% probability. CV: coefficient of variation.

treatments inoculated and fertilized with 30 and 120 kg ha^{-1} of N, not differing statistically from the control. These results corroborate those found by Rodrigues et al. (2006) and Pedraza et al. (2009), who verified significant increase in N content of wheat and rice grains with the inoculation of A*zospirillum* spp., without N addition. Guimarães (2006) observed increases in N accumulation in grains of 64% of rice plants (variety IR 42) inoculated with the ZAE 94 strain and fertilized with 50 kg ha^{-1} of N, compared with the control treatment, with no inoculation or fertilization.

The contents of Ca, Mg and Mn were higher when plants were inoculated with *A. brasilense* and *H. seropedicae*, both without nitrogen fertilization and in combination with the highest N dose (120 kg ha^{-1} of N), not differing statistically from each other. The inoculation with diazotrophic bacteria and nitrogen fertilization promoted an improvement in the quality of corn grains for trading, with an increase in protein content, which allows obtaining better grains from a nutritional perspective. Results found by Hungria et al. (2010) suggest that inoculation with *Azospirillum* spp. promotes small increases in the contents of P, K, Mg, S, Zn, Mn and Cu of corn grains, which often may not differ from the control treatment.

Conclusions

Nitrogen fertilization associated with inoculation of *A. brasilense* and *H. seropedicae* positively influenced ear weight, ear diameter, number of grains per ear, shoot dry weight, productivity and chlorophyll content of corn plants. N, P, K and Zn leaf contents increased with nitrogen fertilization and the inoculation with *A. brasilense* and *H. seropedicae*. The inoculation with *A. brasilense* without nitrogen fertilization promoted higher accumulations of N, K, Ca and Mg in the grains compared with the treatments inoculated with *A. brasilense* and *H. seropedicae* and fertilized with 30 and 120 kg ha^{-1} of N. The inoculation with *A. brasilense* or *H. seropedicae* associated with nitrogen fertilization can lead to a reduction in the use of synthetic nitrogen fertilizers in corn cultivations.

Conflict of Interest

The authors have not declared any conflict of interest.

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