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Effect of nitrogen fertilization associated with diazotrophic bacteria inoculation on nitrogen use efficiency and its biological fixation by corn determined using ^{15}N

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The use of plant growth-promoting diazotrophic bacteria as an alternative to increase nitrogen (N) availability and contribute to its use by corn can be a less costly and ecologically more viable option. Thus, this study aimed to evaluate the effects of the inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* in association with N fertilization on the N use efficiency and its biological fixation by corn, in field conditions, using the ^{15}N-isotope technique. A randomized-block design was used, with nine treatments and six replicates. The following parameters were evaluated: grain productivity, dry matter production, accumulated N in plant, percentage of N from the fertilizer, total N from the fertilizer, use efficiency of N applied as fertilizer and percentage of biological N fixation. Results show that N fertilization associated with *A. brasilense* and *H. seropedicae* inoculations influences positively grain productivity in corn. The inoculation with *A. brasilense* and *H. seropedicae* combined with 30 and 120 kg ha^{-1} of N promotes a reduction of N percentages in corn grains and shoot. The increase in N dose associated with the inoculation of *A. brasilense* and *H. seropedicae* promotes an increase in the N from the fertilizer in corn grains and shoot and reduces the N use efficiency by the crop. Using the ^{15}N natural abundance technique, it was found that the inoculation with *A. brasilense* and *H. seropedicae* contributed, respectively, with in average 19.40% and 9.49% of the N required for the development of corn plants.

Key words: *Zea mays* L., *Azospirillum brasilense, Herbaspirillum seropedicae*, ^{15}N, biological nitrogen fixation.

INTRODUCTION

Corn cultivation has a high demand for nitrogen fertilizers, making necessary the application of this nutrient to obtain high productivities (Bastos et al., 2008; Fidelis et al., 2007). Due to the high economic and environmental costs of the industrial process for nitrogen fixation, combined with the increase in food demand, there arises the need to incorporate new technologies into agricultural activities, aiming to rationalize the use of nitrogen fertilizers. One option is using the benefits provided by the association between diazotrophic bacteria and crops of great economic interest, since these microorganisms are capable of promoting plant growth and increasing plant development and productivity (Baldani et al., 1997).

According to Hungria (2011), diazotrophic bacteria associated with grasses can stimulate plant growth in different ways. In addition to their capacity for biological nitrogen fixation (BNF) (Han et al., 2005; Huergo et al., 2008), they can act in the increase of nitrate reductase activity, when they grow endophytically (Cássan et al., 2008); in the production of plant hormones, such as auxins, gibberellins, ethylene etc. (Khaliq et al., 2004; Donate-Correa et al., 2004; Radwan et al., 2004; Creus et al., 2004; Dobbelaere et al., 2003); in the solubilization of zinc phosphates and oxides (Rodriguez et al., 2004; Baldotto et al., 2010); and in the biological control of pathogens (Mariano et al., 2004).

The genera *Azospirillum* and *Herbaspirillum* include a group of plant growth-promoting bacteria with a good capacity to associate with corn plants. These bacteria have nitrogenase enzymatic complex and are able to break the triple bond connecting the two nitrogen atoms and reduce N_2 to ammonia. However, N_2 fixation efficiency has not proved to be enough to meet corn demand (Hungria, 2011), thus complementary nitrogen fertilization is required in addition to the inoculation.

In general, nearly 70% of field experiments with diazotrophic bacteria inoculation, using various crops and under different soil and climate conditions, showed increases in productivity of up to 30% (Okon and Labandera-González, 1994), and this contribution is higher when plants receive variable doses of nitrogen fertilizers (Dobbelaere et al., 2003).

Currently, the ^{15}N isotope technique is the most used method to determine N use efficiency (NUE), when N is applied as a fertilizer in corn cultivation. Among the various results obtained, it is verified great variation of NUE in corn, with values ranging from 10 to 65% of the applied N (Lara Cabezas et al., 2000; Cantarella et al., 2003; Silva et al., 2003; Gava et al., 2006; Gava et al., 2010). BNF contribution to corn can also be quantified through the ^{15}N isotope technique, based on its natural abundance.

The use of plant growth-promoting bacteria (PGPB) as an alternative to increase N availability and contribute to its use by corn can be a less costly and ecologically more viable option. Given the aforementioned, this study aimed to evaluate the effects of *Azospirillum brasilense* and *Herbaspirillum seropedicae* inoculations associated with N fertilization on the N_2 use efficiency and its biological fixation by corn, in field conditions, using the ^{15}N-isotope technique.

MATERIALS AND METHODS

The experiment was carried out at the experimental field of Embrapa Western Region Agriculture, in Dourados-MS, from March to July 2012. Geographical coordinates of the area are 22° 14' S and 54° 9' W, with average altitude of 450 m. The climate of the region is classified as Cwa, according to Köppen's classification system. 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 at Embrapa Western Region Agriculture, in Dourados-MS (Figure 1).

Results of the soil chemical analysis in the layer of 0-20 cm, before the experiment installation, were, pH ($CaCl_2$), 4.5; Organic matter, 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 215 g kg^{-1} of sand, 115 g kg^{-1} of silt and 670 g kg^{-1} of clay. Soil pH correction was performed a month before sowing, with 1720 kg ha^{-1} of dolomitic limestone (RNV 100%), considering soil analysis results, in order to increase base saturation to 60%. The area was irrigated after crop implementation and in periods with higher water deficit.

A randomized block design was used, with nine treatments and six replicates, as follows: 1) Control without N and without inoculation; 2) Inoculation with *A. brasilense*, without N; 3) Inoculation with *H. seropedicae*, without N; 4) 30 kg ha^{-1} of N at sowing; 5) *A. brasilense* + 30 kg ha^{-1} of N at sowing; 6) *H. seropedicae* + 30 kg ha^{-1} of N at sowing; 7) 30 kg ha^{-1} of N at sowing + 90 kg ha^{-1} of N in covering; 8) *Azospirillum brasilense* + 30 kg ha^{-1} of N at sowing + 90 kg ha^{-1} of N in covering; and 9) *H. seropedicae* + 30 kg ha^{-1} of N at sowing + 90 kg ha^{-1} of N in covering.

The seeds of the simple hybrid P3646H used in the study had been previously inoculated with a combination of two strains of *A. brasilense* (Ab-V5 and Ab-V6) (inoculant cell concentration of about 10^8), using a liquid inoculant, and the Z-94 strain of *H. seropedicae* (inoculant cell concentration of about 10^9), using a peat-based inoculant produced by Embrapa Agrobiology, Seropédica-RJ. The applied doses were 150 mL of the liquid inoculant for each 50 kg of corn seeds, and 250 g of the peat-based inoculant for each 10 kg of corn seeds. For the inoculation with the Z-94 strain of *H. seropedicae*, 60 mL of a 10% sugar solution (m/v) were added in order to increase the adhesion of the inoculant to the seeds.

Base fertilization was applied by broadcasting at sowing, with later incorporation, using 300 kg ha^{-1} of a 0-20-20 formulation to supply 60 kg ha^{-1} of P_2O_5 and K_2O, respectively. Planting was performed manually, with the aid of a hand-held corn planter (known as "matraca"), placing two seeds per hole and leaving six plants per linear meter after thinning.

Each experimental unit was composed of five 5-m rows, spaced 0.90 m apart. The three central rows were considered as the useful plot area, excluding the last 0.5 m of each row. A microplot was

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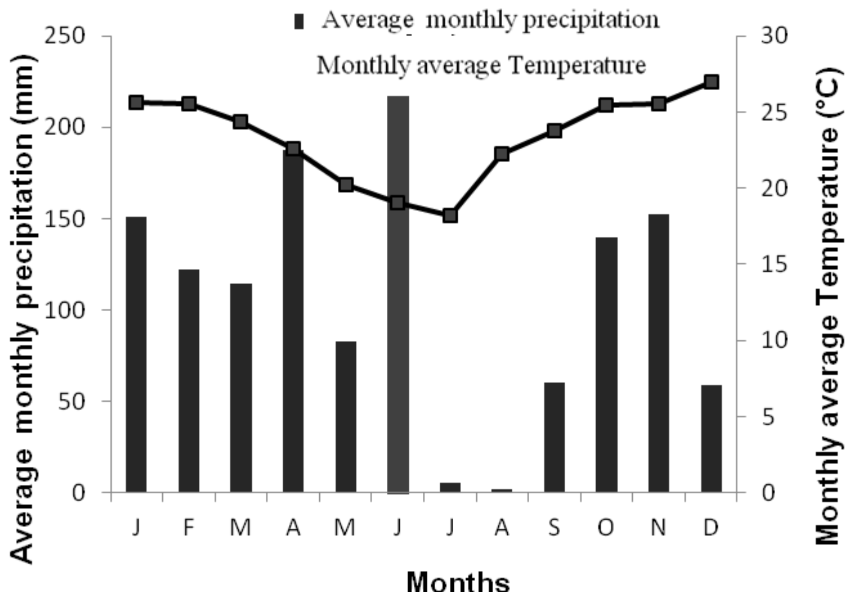

Figure 1. Monthly average rainfall (mm) and temperature (°C), recorded at the Weather Station of Embrapa Western Region Agriculture, in Dourados-MS, Brazil in 2012.

installed in each plot and urea marked with ^{15}N was applied. Each microplot had one 2-m row, and the sampling area was restricted to 1 m in the center of the line.

As for N fertilization, 30 kg ha^{-1} of N were applied in the planting furrow and 90 kg ha^{-1} of N were divided into two applications of 45 kg ha^{-1} and applied in covering as urea (45%) in the whole plot, except for the microplots.

In the treatments 4, 5 and 6, N fertilization was applied as urea enriched with 2.6% of ^{15}N atoms in excess. For these treatments, 12 g of marked urea were applied in each 2-m microplot.

In the treatments 7, 8 and 9,fi N fertilization was applied as urea enriched with 0.7% of ^{15}N atoms in excess, totaling 120 kg ha^{-1} of N, with 30 kg ha^{-1} of N applied at sowing and 90 kg ha^{-1} of N applied in covering, which were divided into two times of 45 kg ha^{-1}. In these treatments, 12 g of urea enriched with 2.6% of ^{15}N atoms in excess were applied at sowing and 36 g of urea enriched with 0.7% of ^{15}N atoms in excess, divided into two times of 18 g, were applied in covering in order to meet the 90 kg ha^{-1} of N. The first N application in covering was performed during the V4 development stage, which corresponds to 4 leaves fully expanded, and the second application during the V7 development stage, corresponding to 7 leaves fully expanded.

Corn harvest was performed manually, collecting all plant ears from the plot useful area (9.0 m²). In order to determine grain productivity, ears were threshed with the aid of a manual device and then weighed. The obtained results were converted to kg ha^{-1}, correcting the humidity to 13% on a wet basis. Plant dry matter (straw) was estimated through the sampling of three plants inside each microplot. Dry matter was determined by drying the samples in a forced-air oven at constant temperature of 65ºC for 72 h. Dry matter data were expressed in kg ha^{-1}.

At the harvest, plants were divided into shoot (straw) and grains. All collected plant material was washed in a detergent solution (3 mL L^{-1}), running water, 0.1 mol L^{-1} solution of HCl and deionized water, respectively. Then, samples were accommodated in paper bags and dried in a forced-air oven at 65ºC for 72 h. After drying, all the plant material was weighed and ground in a Wiley-type mill for the analyses of Total N and N isotopic composition.

Total N in different plant parts (straw and grains) was determined through the Kjeldahl method, according to the methodology described in Embrapa (2009). As for the ^{15}N isotopic composition analyses, samples were processed according to the methodology in Rittenberg (1946); using the final distillate obtained in the Total N% analysis, the extracts were again acidified with 0.5 mol L^{-1} H_2SO_4 and concentrated through evaporation, and the N-NH_4^+ was converted to N_2 through oxidation with lithium hypobromite (LiOBr) (Porter and O'deen, 1977). ^{15}N isotopic composition analyses were performed in a Delta Plus mass spectrophotometer, from the John M. Day Stable Isotope Laboratory at Embrapa Agrobiology. With the results of nitrogen isotopic composition (% of ^{15}N atoms) in the samples, the following parameters were calculated:

Total N content accumulated in the plant (TN, mg/plant):

$$TN = \frac{DMY \times N}{100}$$

Where, DMY is the dry matter yield and N is the N content in the plant (g kg^{-1}).

b) Percentage of N in the plant coming from the fertilizer (%PNF):

$$\%PNF = \left(\frac{\%\ \text{in 15N atoms excess plant test}}{\%\ \text{in 15N excess fertilizer}}\right) x100$$

c) N in the plant coming from the fertilizer (PNF):

$$PNF = \frac{\%PNF \times DMY}{100}$$

d) Use efficiency of N applied as a fertilizer as a function of the

Table 1. Productivity (PRO), total dry matter (MST), grain N percentage (%NG), shoot N percentage (%NPA), total grain N (NTG) and total shoot nitrogen (NTPA) of corn plants in response to nitrogen fertilization and inoculation with *Azospirillumbrasilense* and *Herbaspirillum seropedicae*. Dourados-MS, 2012.

Treatment	PRO	MST	NG	NPA	NTG	NTPA
	kg ha^{-1}		%		kg ha^{-1}	
1. Control	9231.71b	9977.91	1.66ab	0.86ab	154.46ab	83.61
2. *A.brasilense*	9078.41b	9802.84	1.82ab	0.90a	165.67ab	89.85
3. *H. seropedicae*	9023.88b	10098.58	1.99a	0.79abc	180.83a	80.02
4. 30 kg ha^{-1} N	9302.05b	10633.24	1.29cd	0.62bc	121.33c	67.64
5. *A.brasilense* + 30 kg ha^{-1} N	9531.75ab	11587.76	1.23d	0.58c	117.58c	68.76
6. *H. seropedicae* + 30 kg ha^{-1} N	9133.32b	9531.11	1.25d	0.66bc	114.92c	63.74
7. 30 kg ha^{-1} N + 90 kg ha^{-1} N	10146.52a	10198.58	1.26cd	0.85ab	128.31bc	87.46
8. *A. brasilense* + 120 kg ha^{-1} N	9861.37ab	11197.74	1.35cd	0.75abc	134.02bc	84.37
9. *H. seropedicae* + 120 kg ha^{-1} N	9858.23ab	11867.71	1.47bcd	0.76abc	144.98bc	92.88
Média	9463.03	10543.89	1.48	0.75	140.23	79.81
Teste F	4.76*	1.55^{ns}	10.02*	4.52*	6.39*	1.81^{ns}
CV (%)	4.81	15.42	14.50	16.95	15.85	24.05

*and ^{ns}significant 5% probability and non-significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between themselves by Tukey test, the 5% probability.

applied quantity (NAQ):

$$NUE = \frac{PNF}{NAQ} x\ 100$$

BNF contribution to corn was quantified through the ^{15}N-isotope dilution technique, based on the natural abundance of ^{15}N (Shearer and Kohl, 1986). In addition to the corn plant samples obtained at the R6 stage, non-leguminous spontaneous species were collected to serve as a reference for the natural abundance of ^{15}N available in soil. Thus, three plants of each control treatment replicate were collected as a reference, which were used to estimate BNF: *Commelia benghalenis* L., *Digitaria insularis* and *Cenchrus echinatus*. The collected material was dried in an oven at 65ºC, ground and analyzed for ^{15}N natural abundance (Okito et al., 2004).

BNF estimate through the ^{15}N natural abundance was calculated using the equation:

$$\%FBN = (1 - \frac{\%\ \text{atoms of 15N in excess in plant test}}{\%\ \text{atomos of 15N in excess control}})\ x100$$

Results were subjected to variance analysis and means were compared using Tukey test at 5% of probability, using the statistical software SISVAR (Ferreira, 2000).

RESULTS

The highest corn grain productivities were obtained in the treatment fertilized with 120 kg ha^{-1} of N, which did not differ statistically from that inoculated with *H. seropedicae* and supplied with 120 kg ha^{-1} of N and that inoculated with *A. brasilense* and supplied with 30 and 120 kg ha^{-1} of N (Table 1). It should be pointed out that the treatment fertilized with 120 kg ha^{-1} of N promoted an increase of about 10% in grain productivity compared to the control, without either N fertilization or inoculation. The treatment inoculated with *H. seropedicae* and supplied with 120 kg ha^{-1} of N and that inoculated with *A. brasilense* and supplied with 30 and 120 kg ha^{-1} of N promoted increases in grain productivity of about 6.78, 6.82 and 3.25%, respectively, although they had not shown any significant difference compared to the control. In average, these treatments promoted an increase of 518.74 kg ha^{-1} in corn grains compared to the control, which represents a gain of 8.64 bags per hectare, suggesting the applicability of inoculation associated with nitrogen fertilization for corn cultivation.

The percentage of N in grains (%NG) and the total N in grains (NTG) of corn plants showed significant differences between treatments inoculated with diazotrophic bacteria and supplied with 30 and 120 kg ha^{-1} of N compared to the control (without either N fertilization or inoculation) (Table 1). The treatments inoculated with *A. brasilense* and *H. seropedicae* supplied with 30 and 120 kg ha^{-1} of N showed reduction in %NG and, as a consequence, in NTG. As for the N shoot percentage (%NPA), the inoculation with *A. brasilense* promoted the highest increase, not differing from the control and the treatments inoculated

Table 2. Percentage of N in grains from the fertilizer (%NGPF), percentage of N in the shoot from the fertilizer (%NPAPF), total grain N from the fertilizer (QNGPF), total shoot N from the fertilizer (QNPADF), N use efficiency in grains (EUNG), N use efficiency in the shoot (EUNPA) and N use efficiency by corn plants (EUNPL) in response to nitrogen fertilization and inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae*. Dourados-MS, 2012.

Treatment	NGPF	NPAPF	QNGPF	QNPAPF	EUNG	EUNPA	EUNPL
	%		kg ha^{-1}			%	
4. 30 kg ha^{-1} N	11.70b	17.66b	14.33b	11.77b	47.76	39.26	87.03a
5. A.brasilense + 30 kg ha^{-1} N	11.58b	17.73b	13.70b	12.22b	45.68	40.73	86.44a
6. H. seropedicae + 30 kg ha^{-1} N	11.63b	17.30b	13.26b	10.90b	44.22	36.34	80.57a
7. 30 kg ha^{-1} N + 90 kg ha^{-1} N	38.74a	43.83a	49.33a	38.36a	36.54	28.42	64.96b
8. A. brasilense + 120 kg ha^{-1} N	36.86a	39.69a	49.15a	33.61a	36.40	24.89	61.30b
9. H. seropedicae + 120 kg ha^{-1} N	37.27a	40.19a	52.84a	38.48a	39.14	28.50	67.64b
Average	24.63	29.40	32.10	24.22	41.63	33.79	74.66
Test F	45.28*	37.63*	76.41*	14.22*	2.08 ^{ns}	2.09 ^{ns}	3.31*
CV (%)	21.05	17.72	17.54	37.29	19.99	21.37	21.31

* and ^{ns}significant 5% probability and non-significant, respectively. Medium followed by the same letter in the columns, do not differ statistically between themselves by Tukey test, the 5% probability.

and fertilized with 120 kg ha^{-1} of N (Table 1).

In average, the percentage of N in grains from the fertilizer (%NGPF) was equal to 11.63% in the treatment fertilized with 30 kg ha^{-1} of N and in the treatments with *A. brasilense* and *H. seropedicae* supplied with 30 kg ha^{-1} of N, not differing statistically (Table 2). However, when supplied with 120 kg ha^{-1} of N in the absence and presence of inoculation with *A. brasilense* and *H. seropedicae*, %NGPF was in average 37.62%, statistically differing ($p \leq 0.05$) from the treatments inoculated and supplied with 30 kg ha^{-1} of N (Table 2). The percentage of shoot N from the fertilizer (%NPAPF) was equal to 17.56% in the treatments fertilized with 30 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae*, while %NPAPF was equal to 41.23% when there was the addition of 120 kg ha^{-1} of N. However, there was an average increase higher than 100% with the addition of 120 kg ha^{-1} of N compared to the addition of 30 kg ha^{-1} of N (Table 2).

The total N in grains from the fertilizer (QNGPF) and the shoot N from the fertilizer (QNPAPF) followed the same trend observed for %NGPF and %NPAPF. There were average increases of 266 and 216 kg ha^{-1} of N in corn grains and shoot, respectively, when fertilized with 120 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae* compared to the treatments fertilized with 30 kg ha^{-1} of N and inoculated (Table 2).

However, N use efficiency by the plant (EUNPL) was contrary to the previous parameters. Corn plants fertilized with 30 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae* showed the highest percentages of N use efficiency. In average, EUNPL in these treatments was equal to 84.65% compared to 64.63% of the ones fertilized with 120 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae* (Table 2). It is verified that N use efficiency was not calculated for treatments without N application, since it takes into account the N applied to soil, which did not happen in these cases.

Results of N use efficiency for the grains (EUNG) and for shoot (EUNPA) were not significantly different ($p \geq 0.05$). However, EUNG and EUNPA had average values of 45.88 and 38.77%, respectively, in the treatments fertilized with 30 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae,* corroborating the results found for EUNPL (Table 2).

The evaluation of BNF quantification (Tables 3 and 4) shows the capacity of the P3646H corn genotype to obtain N from BNF for its development. For these evaluations, *Commelia benghalenis* L., *Digitaria insularis* and *Cenchrus echinatus* were used as reference plants (RP) to calculate BNF, along with plots without the addition of mineral N.

Table 3 shows the results of RP used as a control in the determination of BNF contributions. It was observed that BNF values for the P3646H corn hybrid inoculated with both *A. brasilense* and *H. seropedicae* were not significant. RP values for ^{15}N natural abundance were lower than the ones for corn plants, which did not allow the application of the ^{15}N technique described in Shearer and Kohl (1986) to estimate BNF contribution for corn.

Table 4 shows the results for non-inoculated plants that were used as a control in the determination of BNF contributions. For that, it was assumed that the BNF contributions to these plants through the association with native diazotrophic microbes (not inoculated) occurred uniformly for all tested treatments. Therefore, the values refer to the BNF gains obtained with the inoculation of the selected strains of *A. brasilense* and *H. seropedicae*. It was verified that the BNF rate for the P3646H corn hybrid inoculated with *A.brasilense* in these evaluations was significantly higher ($p \leq 0.05$) than that inoculated with

Table 3. Mean values of δ ^{15}N of inoculated and non-inoculated treatments, estimated by the analysis of ^{15}N natural abundance for corn plants in the second cropping season.

Treatment	δ ^{15}N	δ ^{15}N PR1	δ ^{15}N PR2	δ ^{15}N PR3	δ ^{15}N media
1. Control	8.0 ± 0.56				
2. *A.brasilense*	6.7 ± 0.32	6.4 ± 0.04	6.26 ± 0.06	6.60 ± 0.35	6.45 ± 0.12
3. *H. seropedicae*	7.3 ± 0.74				

Averages of four repetitions, using plants as reference control for BNF. PR1, *Commelia benghalenis L.*; PR2, *Digitaria insularis*; PR3, *Cenchrus echinatus*.

Table 4. Mean values of δ ^{15}N and BNF of inoculated treatments, estimated by the analysis of ^{15}N natural abundance for corn plants in the second cropping season.

Treatment	δ ^{15}N inoculado	δ ^{15}N não inoculado	FBN (%)
1. *A.brasilense*	6.7 ± 0.32	8.0 ± 0.56	19.40
2. *H. seropedicae*	7.3 ± 0.74		9.49b

Averages of six repetitions, using plants inoculated as control to BNF.

H. seropedicae. The inoculation with *A. brasilense* contributed with 19.40% of the N from BNF, while the inoculation with *H. seropedicae* contributed with 9.49% of the N from BNF (Table 4). These results confirm corn potential for BNF, corroborating results from other studies, claiming that BNF is responsible for meeting part of the crop N demand (Riggs et al., 2001; Dobbelaere et al., 2002).

DISCUSSION

In a study conducted by Neto (2008), the inoculation of a product based on *A. brasilense* provided significant increase in grain productivity, from 9021 to 9814 kg ha^{-1}, that is an average increase of 9%. Alves et al. (2014) observed increases of 34 and 24% in corn productivity with the use of *H. seropedicae* in the first and second cropping seasons, and found that the inoculation can supply up to 40 kg ha^{-1} of N. Dalla Santa et al. (2004), studying inoculations of the RAM-7 and RAM-5 strains of *Azospirillum* sp., observed a reduction of 40% in the amount of nitrogen fertilizer required for corn. Hungria et al. (2010) and Lana et al. (2012) verified that the inoculation with *A. brasilense* promoted increases of 26 and 15.4%, respectively, in corn grain productivity. In 2013, Canellas and co-workers observed increase of 65% in corn grain productivity, compared to the control, when inoculated with *H. seropedicae* in combination with humic substances.

According to Huergo et al. (2008) and Hungria et al. (2011), increases in corn dry matter production and grain productivity in response to inoculation can be attributed to the stimulus that diazotrophic bacteria provide to root system development, increasing root hair density, length, volume and number of lateral roots, resulting in higher capacity to absorb and use water and nutrients.

It should be pointed out that the treatments with inoculation of diazotrophic bacteria and addition of 30 kg ha^{-1} of N show similar results to the treatment with the highest N dose (120 kg ha^{-1} of N) for grain productivity (Table 1), which suggests that the application of 30 kg ha^{-1} of N is less costly compared to the dose of 120 kg ha^{-1} of N. Therefore, the tendency to adopt this technology in corn cultivations can promote a reduction in the use of synthetic nitrogen fertilizers and, consequently, a reduction in production costs.

Rodrigues et al. (2006) and Pedraza et al. (2009), verified significant increase in % N in wheat and rice grains with the inoculation of A*zospirillum* spp., without N addition. Guimarães (2006) observed increases of 64% in the N accumulation in the grains of rice plants (IR 42 variety) inoculated with the ZAE-94 strain and fertilized with 50 kg ha^{-1} of N, compared to the control, without either N fertilization or inoculation.

The results reveal that both the percentage and the total N from the fertilizer were proportional to the applied N doses and the inoculations with *A.brasilense* and *H. seropedicae.*

Studies on N use efficiency in production systems are essential, because as the applied quantity exceeds plant's capacity to absorb it for production, nitrogen can be leached or accumulated in plant tissues, reducing its use efficiency. In the present study, the highest N use efficiency values were obtained for plants under 30 kg ha^{-1} of N and inoculated with *A. brasilense* and *H. seropedicae*, which did not differ statistically. These results reveal that corn plants did not have the potential to use N more efficiently in doses higher than 30 kg ha^{-1} of N in field conditions and inoculated with diazotrophic bacteria. Reduction of N use by corn plants as N doses increase has also been reported by other authors (Fernandes et al., 2005; SILVA et al., 2009). Alves et al. (2006), evaluating N fertilization in corn in a Distroferric Red Latosol, found EUNPL values of 18% for the dose of 25 kg ha^{-1} of N applied at sowing; while even higher EUNPL values, 62% for corn, were found for the dose of 45 kg ha^{-1} of N when applied 26 days after emergence.

Many studies on corn, using ^{15}N-isotope methods in a Red Latosol, have shown differences in the efficiency to recover N from the fertilizer: 26 to 49% (Figueiredo et al., 2005); 40 to 50% (Silva et al., 2006); 45% (Gava et al., 2006) and 39% (Duete et al., 2008). The variation in the use of N from mineral fertilizers by corn is due to many factors, especially to soil and climate conditions, type of fertilizer, fertilization management (dose, time and way of application) and cultivation system (direct seeding or conventional tillage) (Lara-Cabezas et al., 2004; Duete et al., 2008). N losses by leaching, volatilization, denitrification, erosion and the microbial immobilization of N also influence the use of N from mineral sources (Lara-Cabezas et al., 2004; Figueiredo et al., 2005).

Many diazotrophs have been identified in corn (Baldani et al., 1980, 1986; Chelius and Triplett, 2000, 2001; Perin et al., 2006) and described as plant growth-promoting bacteria, but the BNF contribution to corn has not been clearly documented (Chelius and Triplett, 2000; Gutiérrez-Zamora and Martínez-Romero, 2001). García de Salamone et al. (1996) verified through the ^{15}N-isotope technique that *Azospirillum* spp. inoculation contributed significant BNF levels depending on the corn genotype. Alves et al. (2006), using the ^{15}N natural abundance technique, found that the SHS 5050 corn hybrid had N contributions of 45 and 36% from BNF in experiments in the first and second cropping seasons, respectively, when inoculated with the BR 11417 strain of *H. seropedicae*. Montanez et al. (2009) observed a BNF variation of 12 to 33% in a series of commercial corn cultivars in Uruguay using the ^{15}N-isotope dilution method.

Conclusions

Nitrogen fertilization associated with the inoculation of *A. brasilense* and *H. seropedicae* influences positively corn grain productivity.

Inoculation with *A. brasilense* and *H. seropedicae* combined with 30 and 120 kg ha^{-1} of N promotes a reduction in the percentage of N in corn grains and shoot.

The increase of the N dose associated with the inoculation of *A. brasilense* and *H. seropedicae* causes increase in the total N from the fertilizer in corn grains and shoot, and reduces N use efficiency by the crop.

Using the ^{15}N natural abundance technique, the inoculation of *A. brasilense* and *H. seropedicae* contributed, respectively, with an average of 19.40 and 9.49% of the N needed for corn crop development.

Conflict of interests

The authors did not declared any conflict of interests.

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