Seed inoculation with Azospirillum brasilense and N fertilization of corn in the Cerrado biome

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ABSTRACT

There is a great interest to reduce doses and increase efficiency of inputs in agriculture. One alternative to lower doses of N fertilizers in corn is seed inoculation with diazotrophic bacteria, which can fix atmospheric N in soil. This study aimed to evaluate the effect of N doses – in the absence and presence of *Azospirillum brasilense* – on plant nutritional status at different growth stages and on seed yield of corn. The experiment was conducted during the 2011/2012 production season on a typical cerrado soil in Uberlândia, Triângulo Mineiro. The experimental design was randomized blocks with six repetitions. The treatments consisted of five N doses and the absence or presence of *Azospirillum brasilense* (100 mL ha^{-1}) as a seed inoculant. A commercial product with minimum concentration of $2x10^8$ CFU mL^{-1} 100 mL ha^{-1} was used. Inoculation with *Azospirillum brasilense* did not significantly affect corn macronutrient content and yield, except for foliar calcium and potassium at 200 kg N ha^{-1} at the V8 stage. Corn yield increased with N doses up to 150 kg N ha^{-1}.

Key words: *Zea mays* L .; biological nitrogen fixation; diazotrophic bacteria.

RESUMO

Inoculação de sementes de milho com *Azospirillum brasilense* em sementes e fertilização nitrogenada no bioma Cerrado

Existe um grande interesse em práticas que visem à redução na aplicação e aumento de eficiência na utilização de insumos nas áreas de produção agrícola. Uma das alternativas de redução no consumo de fertilizantes nitrogenados na cultura do milho é a inoculação de sementes com bactérias diazotróficas que possuem a capacidade de fixar N atmosférico no solo. Objetivou-se avaliar o efeito de doses de N na ausência e na presença de *Azospirillum brasilense* no teor de macronutrientes em diferentes estádios fenológicos e na produtividade da cultura do milho. O experimento foi instalado durante a safra 2011/2012 em solo característico de cerrado, em Uberlândia, na mesorregião do Triângulo Mineiro. O delineamento foi em blocos casualizados, com 6 repetições. Os tratamentos consistiram de 5 doses de N, na ausência e na presença de *Azospirillum brasilense* (100 mL ha^{-1}), no tratamento de sementes. Foi utilizado produto comercial com concentração mínima de $2x10^8$ UFC mL^{-1} 100 mL ha^{-1}. A inoculação com *Azospirillum brasilense* em milho não promoveu efeitos significativos nos teores de macronutrientes e na produtividade do milho, com exceção dos teores foliares de cálcio e potássio, na dose de 200 kg ha^{-1}, ambos no estádio V8 da cultura. As doses crescentes de N proporcionaram aumento na produtividade do milho, até a dose de 150 kg ha^{-1}.

Palavras-chave: *Zea mays* L.; fixação biológica do nitrogênio; bactéria diazotrófica.

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INTRODUCTION

Corn is one of the most cultivated cereals in the world due to its various forms of use and yield potential. The area planted with corn reached 6.6951 million hectares under the first crop in 2014 – a decrease of 1.3% relative to the previous year. This year adverse weather conditions decreased corn seed output by 6.8%, from 34,576,700 tons to 32,223,500 tons. This demonstrates the need for alternative crop management programs that could guarantee yield and, therefore, profit for the farmer (CONAB, 2014).

One of the main limitations to achieve high corn yields is related to the management of nitrogen fertilization. Another limiting factor responsible for low productivity is the availability of micronutrients. Deficiency of one of them may disrupt metabolic processes, and consequently cause a deficiency of one of the macronutrients, such as N (Coelho *et al.*, 2012).

Nitrogen may become available for plant use by mineralization of organic matter, or be supplied with N fertilizers, which burdens the production system. However, there are currently plant varieties and efficient bacterial strains that can supply more than 50% of N for plant use (Ferreira *et al.*, 2013). There are various non-symbiotic bacterial diazotrophs (NSBD), including *Azospirillum* genus, considered to be facultative diazotrophs, which are able to colonize, internally and externally, non-legume plant roots and perform biological fixation of N. These biotechnological innovations improve plant development and grain yield of corn.

Therefore, the objective of this study was to evaluate the effect of various N doses – in the absence and presence of *Azospirillum brasilense* – on grain yield and nutritional parameters of corn in the Cerrado biome.

MATERIAL AND METHODS

The experiment was conducted during the 2011/12 production season on two adjacent plots (both in the absence and presence of *Azospirillum brasilense*) on Fazenda Experimental Capim Branco (18°55’23"S, 48°17’19"W and 872 m altitude), which belongs to the Universidade Federal de Uberlândia (UFU), MG.

According to Köppen classification (1928), the climate is classified as Aw: tropical with dry winter. The average annual rainfall in the region is 1500 mm. Rainfall data collected during the experimental period are shown in Figure 1.

The soil in the experimental area was classified as Dark Red Dystrophic Latosol (Embrapa, 2013) with clayey texture (580 g clay kg^{-1}).

Soil samples were taken from 0–20 cm before the experiment, according to CFSEMG (1999). Soil chemical analysis was done at the Soil Analysis Laboratory, Universidade Federal de Uberlândia, according to Embrapa (2011). Soil chemical characteristics were: pH (H_2O) 4.7; 2,8 dag kg^{-1} organic matter (OM); 51 mg dm^{-3} phosphorus (P) (Mehlich); 0.52 $cmol_c$ dm^{-3} of potassium (K); 1.0 $cmol_c$ dm^{-3} of calcium (Ca); 0.5 $cmol_c$ dm^{-3} of magnesium (Mg); 4.40 $cmol_c$ dm^{-3} (H + Al); 0.2 $cmol_c$ dm^{-3} of aluminum (Al); 2.02 $cmol_c$ dm^{-3} sum of bases (SB); 6.42 $cmol_c$ dm^{-3} of cation exchange capacity (CEC); and base saturation V% = 32%.

The experimental design was two randomized blocks with six replications. The treatments consisted of seeds inoculated – or not – with N-fixing bacteria *Azospirillum brasilense* (100 mL ha^{-1}) and five doses of N: 0, 50, 100, 150 and 200 kg ha^{-1} (urea) applied at the V4 stage.

Inoculation with *A. brasiliense* was done using a commercial product with AbV5 AbV6 strains and minimum concentration $2x10^8$ cells mL^{-1}.

Each plot consisted of ten six-meter-long rows spaced 0.5 m apart. The harvest was carried out on four central rows, discarding 1 meter at each end.

A simple corn hybrid DKB 390 VTPRO was used. This hybrid is tolerant to sugarcane borer, fall armyworm and corn earworm. It is also characterized by early cycle with high grain production in the Cerrado biome.

Seedbed preparation in the experimental area consisted of: (i) disk harrowing, (ii) application of 1 t ha^{-1} of phosphogypsum incorporated with another heavy disk, (iii) light disking. Dolomitic limestone (1 t ha^{-1}) containing 40.2% CaO and 14% MgO, PRNT 100%, was applied via broadcast, according to recommendation of CFSEMG (1999). Planting furrows were drawn with a ripper. Sowing – 3.5 seeds per meter or 70,000 per hectare – was done manually on December 14, 2011.

The following fertilizers were applied at planting: 120 kg ha^{-1} P_2O_5 (triple superphosphate); 50 kg ha^{-1} K (potassium chloride) and 50 kg ha^{-1} N (urea), except for the control treatments with zero N. Topdressing with 100 kg ha^{-1} K_2O (potassium chloride) and N rate corresponding to each treatment was done at the V4 stage of crop development.

Weed control was performed at the V6 stage using a manual knapsack sprayer with a volume of 200 L ha^{-1}. The following herbicides were used: atrazine (400 g L^{-1}), at a dose of 4.0 L h^{-1} and tembotrione (420 g L^{-1}) at a dose of 0.25 L h^{-1}.

The following foliar applications were carried out at the V8 stage: molybdenum (Mo) and cobalt (Co) (22.5 g L^{-1} Co and 225 g L^{-1} Mo, density = 1,58 g L^{-1}) at a dose of 40.0 g ha^{-1} Mo and 4.0 g ha^{-1} Co; manganese (Mn) and sulfur (S) (67.0 g L^{-1} S and 135 g L^{-1} Mn, density = 1,35 g L^{-1}) at 300 g ha^{-1} Mn and 147.4 g ha^{-1} S. In addition, two micronutrients were applied to the soil: boron (B – 130 g L^{-1}, density = 2.0 g L^{-1}) at the dose of 400 g ha^{-1} B and zinc (Zn - 1.000 g L^{-1}, density = 1.3 g L^{-1}) at a dose 2 kg ha^{-1} Zn.

Harvest was carried out manually in May 2012. Grain moisture was adjusted to 13% for yield assessment.

The following traits were analyzed: (i) foliar nutrient content according to Embrapa (2011) at stages V8 (number of kernel rows are defined), R1 and R3 (ear leaf in the reproductive phase) using the last fully developed leaf; and (ii) grain yield after adjusting for moisture to 13%.

The data were subjected to statistical analysis using the Sisvar and SigmaPlot software. The results for the quantitative factors were submitted to polynomial regression analysis (Vieira, 2008) due to different rates of N in the presence and absence of *A.brasilense*.

RESULTS AND DISCUSSION

Statistical analysis of foliar macronutrient levels at V8, R1 and R3 indicate positive response to inoculation of corn plants (Tables 1, 2, 3, 4).

According to Malavolta (2006), foliar N levels were suitable for corn (Tables 1, 2, 3), except for: (i) the control treatments with and without *A. brasilense* at R1 and R3 (tables 2, 3), and (ii) the 50 kg ha^{-1} N treatment without *A. brasilense* at R1 (Table 2).

Foliar P levels at the V8 stage were below sufficient (Table 1). Although this stage is characterized by rapid P uptake, its accumulation in plant vegetative parts is low (between 20 and 30%), as about 80 to 90% of absorbed P is translocated to kernels (Coelho *et al.*, 2010). As for the R1 and R3 stages (Table 2), except for the control, P levels (Table 3) were considered appropriate in the absence and in the presence of the inoculant (Malavolta, 2006).

According to Malavolta (2006), K levels were above appropriate only at the V8 stage (Table 1). At this stage K absorption by corn plants is rapid, higher than N and P, creating high demand for K (Coelho *et al.*, 2010). Potassium levels at other plant development stages were adequate (Tables 2 and 3).

The uptake of K is accelerated during the vegetative growth with high accumulation rates during the first 30–40 days of plant development. Corn demonstrates two periods of maximum absorption of N and P: during the vegetative stages and during the reproductive stage or ear formation. Low uptake occurs between tasseling and the beginning of ear formation (Olness & Benoit, 1992).

Both Mg and S levels were sufficient at all development stages, according to Malavolta 2006 (Tables 1, 2, 3). On the other hand, according to the same author, Ca levels were below sufficient for corn at all stages (Tables 1, 2, 3).

There was no positive correlation between N doses, the presence or absence of *A. brasilense* and foliar N, P, Mg and S status. The levels of K and Ca showed significant correlation with the presence of *A. brasilense* at the V8 stage (Table 1).

Araújo *et al.* (2013), evaluating nutritional status of corn under various N applications and inoculation with diazotrophic bacteria, noted that N and P levels in leaves were positively affected by nitrogen fertilization and seed inoculation, contrary to the results obtained in this study.

There was a significant correlation between N doses, diazotrophic bacteria and K content. Significant response was obtained in the treatment with *A. brasilense* and 200 kg ha^{-1} N (Table 1).

As Figure 2b shows, K content increased with N doses up to 111 kg ha^{-1} N at the V8 stage in the absence of *A. brasilense*, whereat K content was 38 g kg^{-1}. From there, K content decreases as N doses increased. Similar results were also obtained by Andrade *et al.* (2000), studying the content of K in leaves of elephant grass (*Pennisetum*

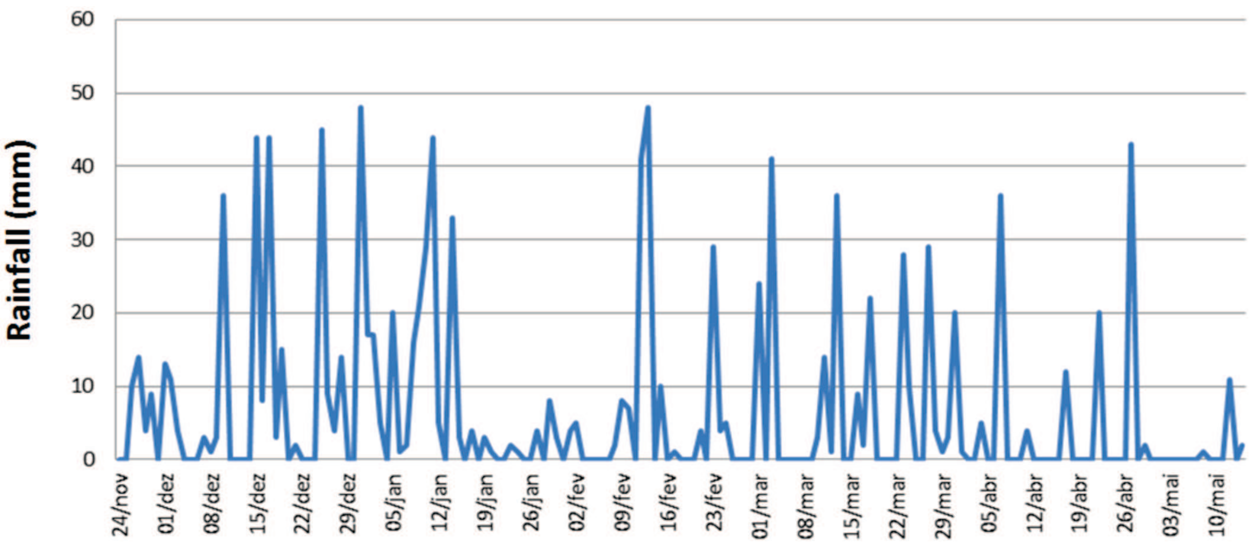

Figure 1: Rainfall data for the trial period. INMET, Fazenda Capim Branco, Uberlândia, MG, 2011/2012.

purpureum) in combination with N and K doses, reported reduced foliar K content due to higher N doses. The effect of N sources and doses on nutritional status of dry matter of marandu grass was studied by Primavesi *et al.* (2006) found an increase in K content, ranging from 21 to 35 g kg^{-1} with higher N doses.

Potassium levels increased at doses between 50 and 200 kg N ha^{-1} in the presence of the inoculant (Figure 2b); however, a significant increase in K content was observed in the presence of *A. brasilense* at the dose of 200 kg ha^{-1} N (Table 1).

The increase of K levels in the presence of the inoculant (Figure 2b) may be related to synthesis of phytohormones by these bacteria, which stimulate the growth of fine roots of the plants, and consequently increase water and nutrient absorption capacity (Oliveira *et al.*, 2008).

The levels of K – in the absence and presence of *A. brasilense* – were similar at 21 kg ha^{-1} N and 178 kg ha^{-1} N: 34.66 g kg^{-1} K and 36.18 g kg^{-1} K, respectively (Figure 2b).

There was a significant correlation between *A. brasilense* and Ca assimilated by corn, regardless of N doses (Table 1).

According to data presented in tables 2 and 3, macronutrient content showed no significant correlation between N doses and *Azospirillum brasilense* at R1 and R3, respectively. In the same way, Francisco *et al.* (2012) also did not find correlation between foliar nutritional status, N doses and inoculation with *Azospirillum* in corn. However, in a study carried out by Batista & Monteiro (2010), N fertilization positively affected the proportion of Ca and Mg and negatively the proportion of K in the shoots of marandu grass.

Regarding N fertilization, a significant correlation was observed between N doses and levels of: N (at V8, R1 and R3), Ca and Mg (at R1 and R3) and P (at R3).

Regression analysis demonstrates linear increase of N levels as a function of N doses at V8 and R1 (Figure 2a). The uptake of N increases at the V8 stage due to high

Table 1: Foliar macronutrient content (g kg^{-1}) at V8 in corn as a function of various N doses and presence or absence of *A. brasilense*

Azospirillum brasilense	**Doses of N (kg ha^{-1})**					
	0	**50**	**100**	**150**	**200**	**Mean**
			N			
Absence	31	32	34	35	35	33ns
Presence	32	32	34	34	34	33 ns
		DMS$_{Azospirillum}$ = 1.074		CV= 6.20%		
			P			
Absence	2.05	2.17	2.08	2.08	2.17	2.11 ns
Presence	2.17	2.27	2.30	2.15	2.27	2.23 ns
		DMS$_{Azospirillum}$ = 0.209		CV = 18.53%		
			K			
Absence	33.42 a	35.83 a	36.83 a	39.08 a	33.75 b	35.78
Presence	36.25 a	32.50 a	34.33 a	36.92 a	38.83 a	35.77
		DMS$_{Azospirillum}$ = 4.059		CV = 9.76%		
			Ca			
Absence	8.25	8.62	8.38	8.22	8.40	8.37 B
Presence	8.37	8.68	8.55	8.47	8.67	8.55 A
		DMS$_{Azospirillum}$ = 0.139		CV = 3.16%		
			Mg			
Absence	3.70	3.72	3.32	2.70	2.68	3.22 ns
Presence	3.95	3.97	3.23	2.87	2.92	3.39 ns
		DMS$_{Azospirillum}$ = 1.694		CV = 17.93%		
			S			
Absence	2.60	2.55	2.60	2.58	2.53	2.57 ns
Presence	2.47	2.57	2.65	2.55	2.52	2.55 ns
		DMS$_{Azospirillum}$ = 0.143		CV = 10.75%		

Means followed by different letters in the column differ by Tukey test at 5% significance.
ns = not significant

growth of the root system (Fancelli & Dourado Neto, 2004). For every kg of applied N ha^{-1}, there was an increase of 0.0171 g kg^{-1} N at the V8 stage, with 88.57% of positive predictive value of the model. The maximum dose – 200 kg ha^{-1} N – resulted in 31 g kg^{-1} N at R1 stage (Figure 2a); thus, each kg of applied N increased N content at the R1 stage by 0.0163 g kg^{-1}, with 91.62% of positive predictive value of the model.

Weather conditions during the experiment may have affected these results. Rainfall data during the experiment (Figure 1) recorded by an automatic weather station located next to the experimental site show a good distribution of rain. Mass flow is an important process for the uptake of N as the amount of N that reaches the plant depends on transpiration conditions, concentration of the nutrient in the soil solution and water content in soil. During drought N uptake is limited by the amount of N that reaches the roots, which may not be enough to meet the demand of the plant even under high N concentration in the soil (Ernani, 2003).

Aratani *et al.* (2006), testing doses of N in corn under no-tillage, also found linear correlation between foliar N content and increasing N doses. However, Gitti *et al.*, (2013) evaluating the effect of inoculation with *A. brasilense* and various N doses, did not find significant differences regarding N levels, contrary to the results obtained in this experiment. This fact demonstrates the importance of evaluation of meteorological data to better understanding the results since proper water balance plays an important role in a given region with respect to better absorption of nutrients, especially N, which occur almost entirety by mass flow.

As for the R3 stage (Figure 2a), there was a quadratic increase in N content with N doses up to 129 kg ha^{-1} N, whereat N content was 29 g kg^{-1}. Goes *et al.* (2013) also found a quadratic response of N content to increasing urea doses in corn.

Regression analysis demonstrates Ca increase with N doses at R1 stage, up to 73 kg N ha^{-1}, reaching 7.47 g kg^{-1} of foliar Ca. Thereafter, higher doses reduced Ca content

Table 2: Foliar micronutrient content (g kg^{-1}) at R1 in corn as a function of various N doses and absence or presence of *A. brasilense*

Azospirillum brasilense	**Doses of N (kg ha^{-1})**					
	0	**50**	**100**	**150**	**200**	**Mean**
			N			
Absence	27	27	30	29	30	29 ns
Presence	27	29	29	30	31	29 ns
		DMS$_{Azospirillum}$ = 0.817		CV= 5.43%		
			P			
Absence	3.17	3.25	3.10	3.15	3.13	3.16 ns
Presence	3.20	2.95	2.83	2.88	3.30	3.03 ns
		DMS$_{Azospirillum}$ = 0.255		CV= 15.84%		
			K			
Absence	23.92	25.25	23.42	24.17	24.83	24.12 ns
Presence	25.67	25.17	23.08	25.00	25.08	24.80 ns
		DMS$_{Azospirillum}$ = 1.461		CV= 11.48%		
			Ca			
Absence	6.67	7.23	7.05	7.10	5.52	6.71 ns
Presence	7.42	7.27	7.32	7.07	5.37	6.89 ns
		DMS$_{Azospirillum}$ = 0.749		CV= 21.17%		
			Mg			
Absence	3.98	4.23	4.22	3.85	3.30	3.92 ns
Presence	4.32	4.43	4.33	3.83	3.45	4.07 ns
		DMS$_{Azospirillum}$ = 0.178		CV= 10.39%		
			S			
Absence	1.83	1.95	1.62	1.55	1.58	1.71 ns
Presence	1.75	1.63	1.63	1.55	1.67	1.65 ns
		DMS$_{Azospirillum}$ = 0.175		CV= 20.10%		

ns = not significant ($p \geq 0,05$)

(Figure 2c). In contrast, Costa *et al.* (2008) observed that Ca content in 'xaraés grass' did not change as a function of higher N doses.

Likewise, Ca content increased with N doses at the R3 stage (Figure 2c), up to 6.65 g kg^{-1} Ca at 96 kg ha^{-1} N. After this point, the increase of N doses decreased Ca levels. According to Malavolta (2006), the additions of N usually elevates Ca content in leaves, unless there is a sharp increase of this element in dry matter, which causes an apparent decrease of Ca, as found in citrus. Still, according to the same author, apple trees fertilized with monoammonium phosphate demonstrate lower foliar Ca content relative to plants fertilized with ammonium sulfate. This effect is partly due to pH changes of the substrate. Thus, foliar Ca decreases in the presence of ammonia nitrogen, as observed in this study, because urea is biologically converted to ammoniated form (Kamowaga *et al.*, 2000).

Teixeira *et al.* (2010), evaluating the effect of N doses and millet mulch on bean crop, found the highest Ca content for the highest N dose applied at planting, (60 kg ha^{-1}). These results contradict the results obtained in our study, which may be due to accelerated mineralization of organic matter caused by high N levels in the planting row.

Regression analysis (Figure 2d) at R1 shows that the maximum level of Mg was 4.61 g kg^{-1} at 55 kg ha^{-1} N. After this point, Mg content decreased to 3.37 g kg^{-1} at 200 kg ha^{-1} N. Studying 'marandú grass', Batista & Monteiro (2010) found the highest Mg content of 3.4 g kg^{-1} in the shoots after the second cutting at 266 mg dm^{-3} N. In contrast, Costa *et al.* (2008) studying N and P fertilization on 'xaraés grass', did not observe significant effects of N doses on Mg content in the shoots in all cuts.

At R3 (Figure 2d), an increase of Mg content was observed as a result of higher N doses, up to 4.32 g kg^{-1} Mg at 74 kg ha^{-1} N. Above that, higher N doses decreased Mg levels. In contrast, Teixeira *et al.*, (2010) found a linear response of Mg content to increasing N doses.

Furthermore, Figure 2d shows the same 4.30 g kg^{-1} Mg content at 55.7 kg ha^{-1} N at both reproductive stages.

Table 3: Foliar macronutrient content (g kg^{-1}) at R3 in corn as a function of various N doses and absence or presence of *A. brasilense*

Azospirillum brasilense	**Doses of N (kg ha^{-1})**					
	0	**50**	**100**	**150**	**200**	**Mean**
			N			
Absence	26	28	29	31	30	29 ns
Presence	26	29	30	30	31	30 ns
		$DMS_{Azospirillum}$ = 0.662		CV= 4.40%		
			P			
Absence	2.35	2.58	2.55	2.72	2.20	2.58 ns
Presence	2.37	2.37	2.65	2.78	2.75	2.58 ns
		$DMS_{Azospirillum}$ = 0.125		CV= 9.33%		
			K			
Absence	18.67	19.58	18.75	19.75	19.58	19.27 ns
Presence	19.17	18.17	18.92	20.25	18.75	19.05 ns
		$DMS_{Azospirillum}$ = 0.483		CV= 5.85%		
			Ca			
Absence	6.20	6.78	6.42	6.65	6.38	6.49 ns
Presence	6.28	6.58	6.90	6.52	6.47	6.55 ns
		$DMS_{Azospirillum}$ = 0.208		CV= 6.13%		
			Mg			
Absence	6.20	6.78	6.42	6.65	6.38	6.49 ns
Presence	6.28	6.58	6.90	6.52	6.47	6.55 ns
		$DMS_{Azospirillum}$ = 0.208		CV= 6.13%		
			S			
Absence	1.72	1.88	1.72	1.72	1.62	1.73 ns
Presence	1.65	1.75	1.73	1.85	1.60	1.72 ns
		$DMS_{Azospirillum}$ = 0.118		CV= 13.18%		

ns = not significant ($p \geq 0,05$)

Regression analysis of P levels (Figure 2e) shows a linear response to N doses, from 2.37 g kg^{-1} P at 0 kg ha^{-1} N, to 2.75 g kg^{-1} P at 200 kg ha^{-1} N. There was an increase by 0.002 g kg^{-1} P for each kg ha^{-1} N, with 91.36% of positive predictive value of the model. Similarly, Casagrande & Fornasiere Filho (2002), evaluating nitrogen fertilization in the second corn crop, found higher P content due to increasing N doses.

Corn production has gotten more technologically savvy in recent years with high response in productivity. However, yields are still considered low due to technological advances are not occurring uniformly in all regions (Peixoto, 2014). Nevertheless, there are regions where state-of-the-art technology is used, including efficient varieties of plants and bacterial strains. According to results obtained by Kappes *et al*. (2013), higher productivity is obtained when corn seeds are inoculated with *A. brasilense*, with a 9.4% increase in crop yield.

Cavallet *et al.* (2000) observed an increase of 17% in the average grain yield, from 5211 kg ha^{-1} to 6067 kg ha^{-1}, and an increase of 6% in the mean ear length, from 13.6 cm to 14 4 cm, using *Azospirillum* inoculation. Morais (2012) also observed an increase from 4% to 7% in productivity with yields from 7.6 to 11.2 t ha^{-1}.

However, in this study no significant differences in productivity due to inoculation with *A. brasilense* were

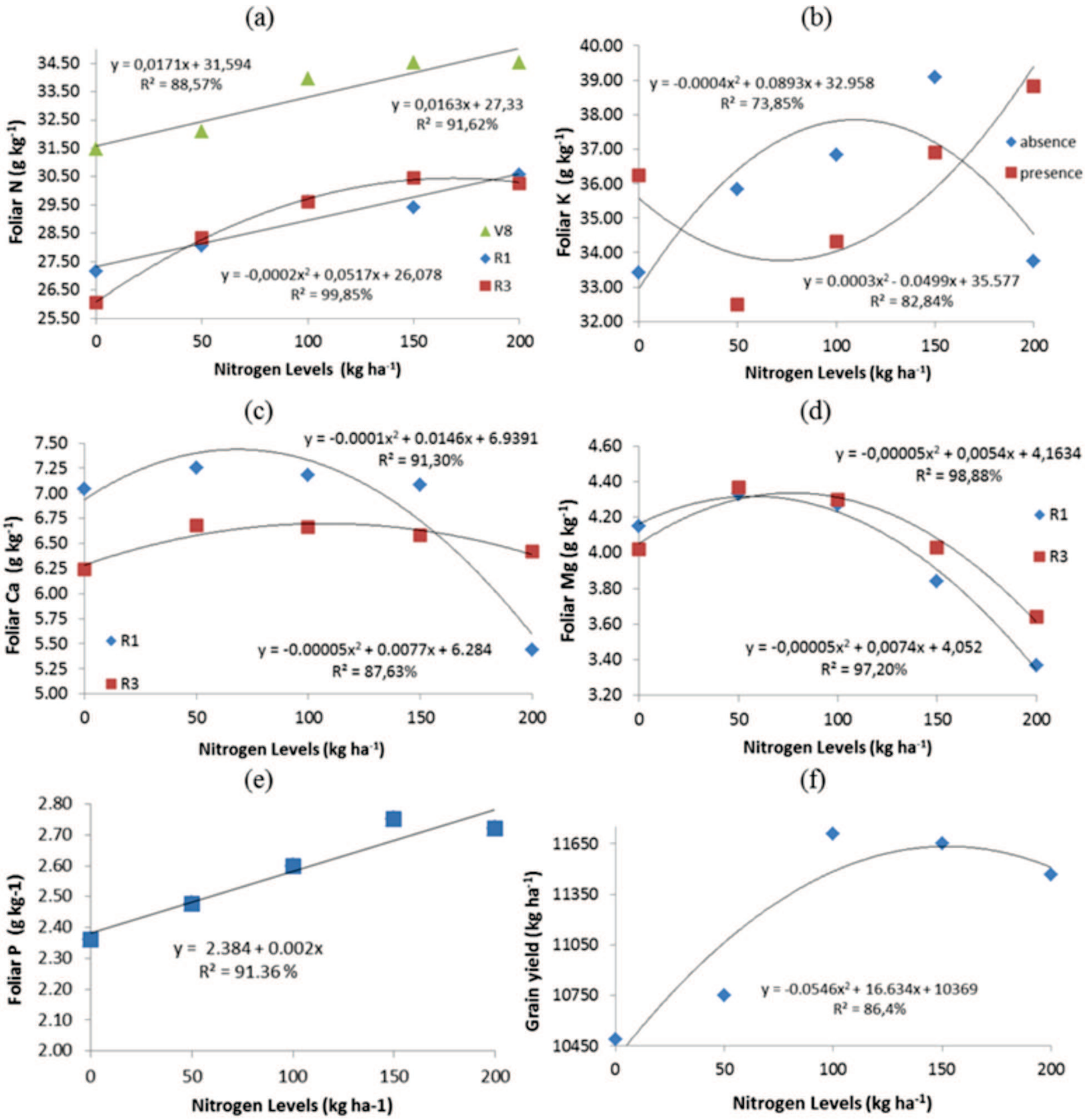

Figure 2: Regressions for N doses: (a) Foliar N at V8, R1 and R3; (b) Foliar K, in the absence and presence of *A. brasilense* at V8; (c) Foliar Ca at R1 and R3; (d) Foliar Mg at R1 and R3; (e) Foliar P at R3; (f) Grain yield.

observed. An average grain yield was 11,130 and 11,300 kg ha^{-1} in the absence and presence of inoculant, respectively. Similarly, Kaminski *et al.* (2011) found no significant effect of inoculation on corn yield. The fact that inoculation with *A. brasilense* did not produce significant differences for most traits may be related to factors that influence the process of interaction between the plants and the bacteria, among which, according to Quadros (2009), are: choice of strain, nutritional status of the plant and the bacteria, plant genotype, environmental conditions and competition with other microorganisms. Furthermore, there is a strong correlation between genotype and bacteria, which can be positive, null or even negative depending on the hybrid.

Regression analysis (Figure 2F) shows that increasing N doses – up to 152.33 kg ha^{-1} – increased productivity of grain, reaching maximum yield 11,635 kg ha^{-1}. Above this dose, productivity declined. Nitrogen plays an important part in corn reproduction by establishing the number and size of ear kernels, which in turn is strongly related to corn yield (Below, 2002). Therefore, corn productivity is highly influenced by the availability of N in soil (Schroder *et al.* 2000).

Morais (2012) reports quadratic response of grain yield to N doses, with maximum yield 10,140 kg ha^{-1} achieved with the application of 260 kg ha^{-1} N. Sichocki *et al.* (2014), evaluating maximum economic efficiency of five N doses in winter corn, also noted that corn yield increased with higher N rates, corroborating the results found in this study.

CONCLUSIONS

The application of diazotrophic bacteria *Azospirillum brasilense* via seed treatment increased Ca levels, regardless of N dose, and K levels at 200 kg ha^{-1} N at the V8 stage.

There was no positive correlation between inoculation with *Azospirillum brasilense* and corn yield.

Levels of: N at V8, R1–R3; Ca and Mg at R1–R3; and P at R3 increased with N doses.

Growing N doses – up to 152 kg ha^{-1} N – increased yield.

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