Australian Journal of
Crop Science AJCS

AJCS 10(7):1061-1068 (2016) ISSN:1835-2707
DOI: 10.21475/ajcs.2016.10.07.p7895

Synergistic effects of seed inoculation with *Azospirillum brasilense* and nitrogen sources on double cropped maize production in tropical savanna of Brazil

Flávio Hiroshi Kaneko[1, *], Michelle Traete Sabundjian[2], João Paulo Ferreira[2], Douglas de Castilho Gitti[3], Vagner Nascimento[2], Aguinaldo José Freitas Leal[4], Salatiér Buzetti[2], André Rodrigues dos Reis[2, 5], Orivaldo Arf[2]

[1]Federal Institute of Mato Grosso do Sul (IFMS), Rodovia MS 473, km 23, 79750-000, Nova Andradina-MS, Brazil
[2]São Paulo State University (UNESP), Avenida Brasil, 56, centro, 15385-000, Ilha Solteira-SP, Brazil
[3]Agricultural Research Supporting Foundation of Mato Grosso do Sul (MS Foundation), PO Box 137, 79150-000, Maracaju-MS, Brazil
[4]Federal University of Mato Grosso do Sul (UFMS), PO Box 112, 79560-000, Chapadão do Sul-MS, Brazil
[5]São Paulo State University (UNESP), Rua Domingos da Costa Lopes, 780, 17602-496, Tupã-SP, Brazil

***Corresponding author: fhkaneko@hotmail.com**

Abstract

Nitrogen (N) fertilization on maize crops has been going through modifications due to the use of technologies such as biological N fixation and modified fertilizers. This study aimed to evaluate the effect of seed inoculation with *Azospirillum brasilense* (with and without inoculation), N sources (urea and polymer coated urea) and side-dressed N levels (0; 45; 90; 135 and 180 kg ha^{-1}), on double cropped maize in two regions of Brazilian savanna (Chapadão do Sul and Selvíria, State of Mato Grosso do Sul). The experiment design was arranged in randomized plots at a 2 x 2 x 5 factorial scheme with 4 replications. The following characteristics were evaluated: plant population (plants ha^{-1}), plant dry matter, leaf N concentration, stem diameter, weight of 100 grains and grain yield. Seed inoculation with *A. brasilense* did not increase double cropped maize yield in both environments. The application of coated urea provided higher dry biomass, however, the polymer coated urea application showed no effect on grain yield in comparison to conventional urea. Increasing side-dressed N levels is the main key factor responsible to improve the double cropped maize yield in tropical Brazilian savanna.

Keywords: *Zea mays*; biological N fixation; coated fertilizers; polymers; nitrogen fertilization.

Introduction

Brazil outstands on worldwide maize production as the third biggest producer with 78 millions tons of grains produced in 15 million hectares (USDA, 2015). According to CONAB (2015), the growing season 2014/2015 database showed that 53% of total maize were produced in double crop system. After the establishment of no-tillage crop systems, it became usual to perform seeding of premature soybean in the summer-spring, and immediately after its harvest, to implant in succession double cropped maize.

Among the research topic priorities of Soil Science in the 21st century, Marks et al. (2013) and Adewopo et al. (2014) highlight the search for nutrient supply, in a way that at food production can be kept while promoting a sustainable use of natural resources. In order to increase the N efficiency use by plants, more studies related to biological nitrogen fixation, synthetic fertilizers application are required in several crops and different environments.

Biological nitrogen fixation occurs mainly through microorganisms, especially by associative bacteria, endophytic diazotrophs, or N_2 fixators, which associate with many plant species in different specificity ranges (Hungria and Kaschuk, 2014; Hungria et al., 2015). Regarding grasses, nitrogen fixation has been discussed for years (Bashan and de-Bashan, 2010). In this context, bacteria of genus *Azospirillum* show high capability to growth using atmosphere N as the only N source. Araújo et al. (2015) using isotopic techniques shows that species *A. brasilense* were able to provide only 19.4% of the total N required by maize growing in savanna of Brazil. However, the benefits of *Azospirillum* go beyond biological nitrogen fixation. These bacteria also produce growth promoters such as auxins, gibberellins, cytokinins and ethylene (Cassán et al., 2014, Hungria et al., 2015) besides increasing leaf chlorophyll content (Inagaki et al., 2015). Roesch et al. (2007) observed that *Azospirillum* spp. enhanced the nitrogen fixation range from 15.43 μg to 95.21 μg of N mg protein^{-1} day^{-1} indicating a highly potential to increase crop yield. In addition, Reis et al. (2000), Welbaum et al. (2004), and Moutia et al. (2010) shows the benefits of *Azospirillum* spp. enhancing the yield of several crops.

Regarding the synthetic fertilizers, urea is the main source of N on worldwide market. To guarantee that N-urea fertilization benefits crops and the environment, actions based on researches have been taken to improve the N efficiency by plants and at the same time reducing environmental pollution (Wang et al., 2015). There are several types of modified fertilizers such as coated with polymers, urease and/or nitrification inhibitors (Chien et al., 2009; Halvorson and Bartolo, 2014). Synthetic fertilizers show a highly potential for use under different conditions of tropical climates such as savannas of Brazil. Promising results were observed by Kaneko et al. (2013) and Sun et al. 2015. However, Valderrama et al. (2011) did not observe significant differences between polymer coated urea and conventional urea on maize crops.

Recently, recommendations to reduce the application of N fertilizers are being questioned. There is a lack of information about the combination of inoculation with microorganisms technology and synthetic fertilizers. We hypothesize that seed inoculation with *A. brasilense* combined with synthetic N fertilizer shows a synergistic effect on maize plant growth and yield.

This study aimed to evaluate the effect of inoculation with *A. brasilense* and the possible interaction with sidedressed N levels and sources on double cropped maize yield in two different regions of Brazilian savanna.

Results and Discussion

Plant final population and plant dry matter

There was no significant effect ($p \leq 0.05$) of inoculation with *Azospirillum* for the plant final population (Table 3). In addition, the application of sidedressed N levels and sources show no effect of plant population (Table 3) in both enviroments. According to Sangoi et al. (2015), the number of plants per unit of area is the first and most important component which influences on maize grain yield. Regarding plant dry matter (Table 3), maize seed inoculation with *A. brasilense* shows no effect of maize dry matter growing in Chapadão do Sul. However, in the experiment located in Selvíria, there was a higher production of plant dry matter ($p \leq 0.05$) in response to seed inoculation with *Azospirillum* (Table 3). This happened because in Chapadão do Sul, double cropped maize was exposed to long periods of hydric stress by excess of rainfall (Figure 1) during the vegetative development. The excess of rainfall contributed to minimize the effect of seed inoculation with *Azospirillum* on maize dry matter increment.

According to Figure 1, during February and March, there was large volume of precipitation in Chapadão do Sul. The excess of rainfall leads the saturation of soil macropores with water. Hypoxia conditions decrease the oxygen available in soil and affect directly the nitrogenase, which is the main enzyme on biological nitrogen fixation process (Hartmman, 1988; Kennedy and Tchan, 1992). On the other hand, the field experiments in Selvíria was under controlled irrigation systems (Figure 1 and 2) that possibly provided a good establishment of symbiosis between plants and *Azospirillum* - stimulating the production of growth promoters (Bashan e de-Bashan, 2010; Hungria et al., 2015). Therefore, the well-watered environment contributed to plant develops the higher dry matter (Puente et al., 2009). In addition, field experiment conducted in Selviria, we observed the greatest maize root length system as illustrated in Attachment 1.

There was a significant increment ($p \leq 0.05$) on maize plant dry matter in response to polymer coated urea growing in Chapadão do Sul (Table 3). However, for maize growing in Selvíria, no differences between N sources were observed for plant dry matter (Table 3). Levels of N sidedressed affected significantly the maize plant dry matter in both field experiments (Table 3). The highest maize yield was obtained with application of 116 and 122 kg ha^{-1} of N in Chapadão do Sul and Selvíria, respectively. Regarding the field experiment conducted in Chapadão do Sul, there was probably a good incorporation of urea in the soil in response to a rainfall of 22 mm occurred right after N application. Thus, the less N loss via volatilization can explain the superior performance of polymer coated urea to enhance the maize yield.

Therefore, the hypothesis that would best justify the superiority of this N source would be the maintenance of N in the ammonium form in the soil for a longer period, including during the vegetative phase, which would reduce N loss through leaching. Besides, N assimilation in ammonium form by plants require less energy, due to its direct incorporation to the carbon chain (Bittsánszky et al., 2015). The supply in adequate concentrations of N as ammonia, can increase N absorption (Xu et al., 2012) and also enhance the efficiency use of fertilizers (Sarasketa et al., 2014), resulting in higher production of dry matter (Zanin et al., 2015).

N content of leaves and stem diameter

The N concentration in leaves is shown in Table 4. Seed inoculation with *Azospirillum* decreased significantly ($p \leq 0.05$) the N concentration of maize leaves growing in Chapadão do Sul. This happened probably because the environment conditions in Chapadão do Sul were not favorable for biological nitrogen fixation due to excess of rainfall, which leads the inactivation of nitrogenase enzyme by anaerobic conditions (Hartmman 1988, Kennedy and Tchan 1992). In addition, these results suggested that occurred competition between *Azospirillum* and plants by available N in the soil, reducing the N concentration in maize leaves.

Field experiment conducted in Selviria shows no effect of seed inoculation on N concentration in maize leaves (Table 4). The results regarding the lower N concentration in maize leaves from Chapadão do Sul did not corroborate with results found by Gitti et al. (2012). These authors observed a higher N concentration in bean leaves growing in Brazilian savanna under seed inoculation with *A. brasilense*. However, in the presence or absence of inoculation, N concentration values in leaves were considered adequate range indicated for maize cultivation in Brazilian savanna (Sousa and Lobato, 2004).

Similarly, N sources also showed no effect on N concentration in maize leaves. However, levels of N applied promoted a linear increase on N concentration in maize leaves growing in both enviroments. Linquist et al. (2013), observed in several countries the increments of 8% on N accumulation by rice plants using high efficiency N fertilizers. These authors state that most benefits of these fertilizers are observed in alkaline soils. However, this study was carried out in a different environmental condition showing acid soils, which is the main characteristic of Brazilian savanna soils.

The seed inoculation and levels of N-sidedressed showed a significant interaction ($p \leq 0.05$) on stem diameter (Table 4 and 5) in the experiment performed in Chapadão do Sul. Treatments without N-sidedressed and with 45 kg ha^{-1} of N, the presence of *Azospirillum* influenced negatively, causing a reduction on stem diameter. At the same time, there was a positive effect of N sidedressing increments only for the plots with inoculation. For the experiment in Chapadão do Sul, the smaller stem diameter was verified on the treatments without N and with 45 kg ha^{-1} of sidedressed N, which can be justified by the possible production of auxins by *A. brasilense* (Kuss et al., 2007), which influence the apical dominance of

Table 1. Chemical characteristics (0-0.20 m) of the experimental areas.

Area	O.M.	pH ($CaCl_2$)	P (Resin)	S	K	Ca	Mg	H+Al	N total
	g dm^{-3}		------mg dm^{-3}-------		-----------------mmol$_c$ dm^{-3}---------------------				g dm^{-3}
1	31	4.7	39	23	2.3	34	11	48	2.24
2	28	4.9	58	14	7.0	31	21	50	1.62

[1] Chapadão do Sul ; [2] Selvíria.* Abbreviations: O.M. – organic matter, P – phosphorus, S – sulfur, K – potassium, Ca – calcium, Mg – magnesium, H + Al - potential acidity, N – nitrogen. Methods of extraction: O.M – sodium dichromate; pH- $CaCl_2$; P – Resin; S – ammonium acetate; K – Melich 1 ; Ca e Mg – KCl 1 N, H+Al – SMP; N total – Kjeldhal.

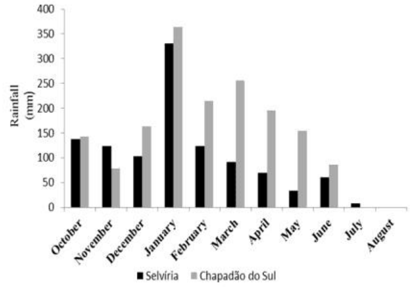

Fig 1. General rainfall data for the experimental areas of Chapadão do Sul and Selvíria-MS, Brazil, 2011/12 growing season.

Table 2. Rainfall close to nitrogen sidedressing for maize cultivated in Chapadão do Sul and Selvíria-MS, 2011/12 growing season.

Place	Last rainfall Previous period	Nitrogen fertilization Date	Rainfall Monitoring period
Chapadão do Sul	03/18/2012 (16 mm)	22/03/2012	22/03/2012 (22 mm) 25/03/2012 (20 mm) 26/03/2012 (13 mm) 31/03/2012 (05 mm)
Selvíria[1]	04/22/2012 (14 mm)	26/04/2012	27/04/2012 (18 mm) 30/04/2012 (11 mm) 01/05/2012 (07 mm)

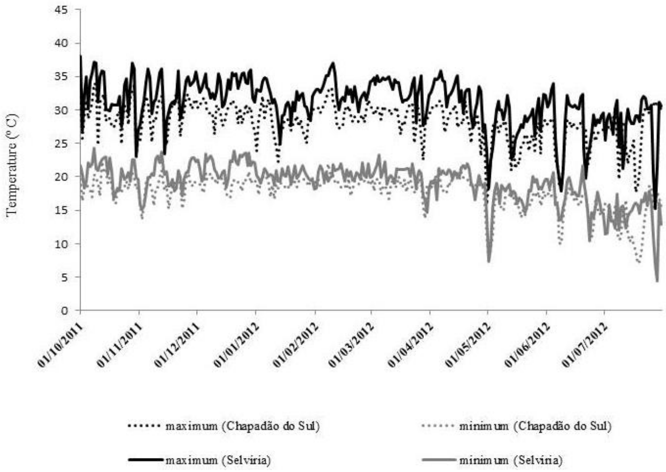

Fig 2. Maximum and minimum air temperatures observed for the experimental areas of Chapadão do Sul and Selvíria-MS, 2011/12 growing season.

the plant, making the lower parts like the stem base, disadvantaged during development when compared to apical regions.

Weight of 100 grains and grain yield

The Table 6 shows the values related to weight of 100 grains and yield. Seed inoculation with *Azospirillum* caused a negative influence on the weight of grains ($p \leq 0.05$) in Chapadão do Sul. However, for maize cultivated in Selvíria, no effect of inoculation on the weight of 100 grains was observed (Table 6). The reduced weight of grains in the experiment of Chapadão do Sul can be explained by the reduced N supply to plants due to the lower leaf N concentration on inoculation treatments (Table 3). Gitti et al. (2012) also observed a reduced weight of grains in the

Table 3. Final plant population and plant dry matter, for second crop maize cultivated in Chapadão do Sul and Selvíria, 2012.

Treatments	Plant final population (plants ha^{-1})		Plant dry matter (g plant^{-1})	
	Chapadão do Sul	Selvíria	Chapadão do Sul	Selvíria
Inoculation				
Without	66.250	66.055	93.62	93.59 b
With	63.750	65.444	90.09	98.50 a
Sources				
Urea	65.278	65.944	86.62 b	96.88
Coated urea	64.722	65.555	98.11 a	95.22
N levels (kg ha^{-1})				
0	63.958	65.555	80.26[1]	85.47[2]
45	65.694	65.139	91.44	97.42
90	65.995	66.667	100.18	98.52
135	64.305	66.111	92.13	99.45
180	65.347	66.278	95.29	99.37
F Test				
Inoculation (I)	2.24	0.17	1.46	21.93 ($p \leq 0.01$)
Source (S)	0.11	0.1	8.18 ($p \leq 0.01$)	0.01
Levels (L)	0.19	0.2	3.88 ($p \leq 0.05$)	8.08 ($p \leq 0.01$)
I x S	0.03	0.05	0.35	0.97
I x L	1.72	0.95	2.51	1.84
S x L	0.13	0.19	0.34	0.35
I x S x L	1.94	0.09	1.17	0.33
LSD (I)	5.316	4.964	6.04	3.33
LSD (S)	3.351	2.533	8.11	3.77
CV (%)	11.49	9.31	12.75	6.82

Abbreviations: LSD - Least Significant Difference. Means followed by different letters differ from each other at a 5% probability according to the Tukey test. [1]$y = 81{,}020 + 0{,}2768\ x - 0{,}0012\ x^2$ ($R^2 = 0{,}79$); [2] $y = 86{,}6183 + 0{,}22\ x - 0{,}0008\ x^2$ ($R^2 = 0{,}92$).

Table 4. Leaf N concentration and stem diameter of double cropped maize growing in Chapadão do Sul and Selvíria, 2012.

Treatments	N content of leaves (g kg^{-1})		Stem diameter (mm)	
	Chapadão do Sul	Selvíria	Chapadão do Sul	Selvíria
Inoculation				
Without	29.59 a	26.60	21.27	23.53
With	27.68 b	26.59	20.50	24.26
Sources				
Urea	29.20	26.71	20.94	23.94
Coated urea	28.74	27.13	20.83	23.86
N levels (kg ha^{-1})				
0	26.03[1]	25.32[2]	20.43	22.07[3]
45	28.08	26.16	20.85	23.79
90	28.37	26.48	20.79	24.82
135	29.71	27.44	21.38	24.60
180	29.70	27.59	20.98	24.21
F Test				
Inoculation (I)	38.91 ($p \leq 0.01$)	0.03	3.74	2.47
Source (S)	0.75	0.76	0.13	0.04
Levels (L)	10.07 ($p \leq 0.01$)	3.18 ($p \leq 0.05$)	1.01	6.63 ($p \leq 0.01$)
I x L	1.03	0.05	0.28	0.03
I x L	0.99	1.31	3.19 ($p \leq 0.05$)	1.45
S x L	0.39	0.8	1.13	1.28
I x S x L	0.08	0.59	0.19	0.47
LSD (I)	1.48	0.51	1.28	2.00
LSD (S)	0.89	0.98	1.76	0.77
CV (%)	4.62	4.73	7.55	6.75

Abbreviations: LSD - Least Significant Difference. Means followed by different letters differ from each other at a 5% probability according to the Tukey test. [1]$y = 26{,}583 + 0{,}020\ x$ ($R^2 = 0{,}88$); [2]$y = 25{,}60 + 0{,}0117\ x$ ($R^2 = 0{,}93$); [3]$y = 22{,}1031 + 0{,}004598\ x - 0{,}000193\ x^2$ ($R^2 = 0{,}98$).

Table 5. Development of the significant interaction between inoculation with *A. brasilense* and N-sidedressed levels, for stem diameter (mm) double cropped maize in Chapadão do Sul, 2012.

Inoculation	N-sidedressed levels (kg ha^{-1})				
	0	45	90	135	180
Without	21.63 a	21.72 a	20.93	21.20	20.83
With^1	19.24 b	19.99 b	20.64	21.55	21.06
LSD			1.37		

Abbreviations: LSD - Least Significant Difference. Means followed by different letters in the column differ from each other at a 5% probability according to the Tukey test. ^{1}y = 19,454 + 0,01158 x (R^2 = 0,82).

Table 6. Weight of 100 grains and yield of double cropped maize cultivated in Chapadão do Sul and Selvíria, 2012.

Treatments	Weight of 100 grains (g)		Yield (kg ha^{-1})	
	Chapadão do Sul	Selvíria	Chapadão do sul	Selvíria
Inoculation				
Without	21.30 a	33.76	5.120	7.612
With	20.57 b	33.91	4.902	7.403
Sources				
Urea	20.41	34.09	4.954	7.782
Polymer coated urea	21.34	34.64	5.205	7.838
N levels (kg ha^{-1})				
0	21.17	31.59^1	4.746^2	6.299^3
45	20.67	32.99	4.830	7.216
90	20.91	34.68	4.802	8.099
135	21.52	34.67	5.312	7.883
180	20.40	35.23	5.364	8.042
F test				
Inoculation (I)	12.60 ($p \leq 0.05$)	0.07	1.22	1.71
Source (S)	1.81	1.68	3.08	0.01
Levels (L)	0.48	13.19 ($p \leq 0.01$)	13.69 ($p \leq 0.01$)	11.57 ($p \leq 0.01$)
I x L	0.14	1.93	0.14	1.05
I x L	0.27	1.91	0.27	1.68
S x L	1.37	0.44	2.23	0.44
I x S x L	0.54	0.29	0.54	2.01
LSD (I)	0.65	1.93	625.5	296.99
LSD (S)	1.01	0.92	288.98	335.66
CV(%)	6.25	7.34	14.64	10.95

Abbreviations: LSD - Least Significant Difference. Means followed by different letters differ from each other at a 5% probability according to the Tukey test.^{1} y= 32,04 + 0,019 x (R^2 = 0,88); ^{2} y = 4667,04 + 3,821 x (R^2 = 0,85); ^{3} y = 6677,49 + 9,22 x (R^2 = 0,74).

presence of *Azospirillum* for rice crops cultivated in succession to millet (yield with herbicide on the occasion of blooming). The authors attribute among other factors, the greater need of N by microorganisms to decompose the remains of the millet. It is valid to point out that the experiment in Chapadão do Sul was implanted in succession to soybean, however, in rotation with millet for grain production, whose C/N rate is higher when compared to the same crop yield in the occasion of blooming.

In the same way, N sources showed no significant effect on weight of 100 grains (Table 6) in both experiment environments, while sidedressed N levels increased the weight of 100 grains in Selvíria plots. The absence of response to sidedressed N levels for maize cultivated in Chapadão do Sul is associated to hydric stress, mainly during the cultivation period. As Selvíria was under irrigated system, there was no effect due to the lack of water, justifying the positive effect on weight of 100 grains, when compared to the non-irrigated system in Chapadão do Sul.

Regarding the double cropped maize yield (Table 6), there was no significant influence ($p \leq 0.05$) of inoculation with *A. brasilense* on neither experiments. The absence of response to inoculation opposes the results obtained by Swedrzynska e Sawicka (2000) in Poland, and Puente et al. (2009) in one of the field experiments implanted in Argentina, the second with application of 90 kg ha^{-1} of sidedressed N. Under Brazilian conditions, Hungria et al. (2010) observed increases on yield due to inoculation with *A. brasilense* on maize and wheat cultivated in the South of Brazil. Increases of 27 and 31% for maize and wheat were observed, respectively, without N sidedressing. However, Repke et al. (2013), while evaluating the efficiency on inoculation with *A. brasilense*, combined with levels of N, also did not observe benefits of inoculation with these microorganisms on maize yield. These authors concluded that this N management practice does not replace N fertilizers. Also, Cavallet et al. (2000) reassure that the effects of this technology are very variable, making precise recommendations on general use of *Azospirillum* on maize crops is difficult. One of the effects of inoculation on maize is a better rooting (Bashan e de-Bashan, 2010). Despite not influencing yield, under irrigated conditions, better rooting of maize plants on phase V_8, was verified as illustrated in Fig 1.

The use of polymer coated urea showed no effect on maize yield. However, the increment on sidedressed N levels (Table 6) promoted linear increase ($p \leq 0.05$) on maize yield in both environments. Regarding the use of polymer coated urea, no advantages compared to conventional urea were observed on maize yield. Similar results were obtained by Queiroz et al. (2012) who did not observed increase on maize yield between the use of conventional urea and polymer coated urea. In addition, Valderrama et al. (2011) and Zavaschi et al. (2014) compared the use of conventional urea and soluble polymer coated urea on maize plants and found no difference between the sources on maize yield.

In this study, it is interesting to note that the maize was implanted in succession to soybean (Chapadão do Sul) and to millet yield during blooming phase (Selvíria). Both environment situations could supply part of the N required by maize, reducing the chances of possible differences between urea and polymer coated urea (Halvorson and Bartolo, 2014). In addition, Deng et al. (2014) and Wang et al. (2015) highlight that the use of coated urea demonstrated better N use efficiency, and the adequate levels of applied N was an important key factor to increase rice yield. This meets the results obtained in this study, where increasing levels of N was the most decisive key factor to enhance double cropped maize yield. Even in less favorable environmental conditions such as hydric stress occurred in Chapadão do Sul, the N management was the main factor driving high maize yield.

Materials and Methods

Sites description and field management

Field experiments were carried out in Chapadão do Sul and Selvíria, State of Mato Grosso do Sul. In Chapadão do Sul, the experiment was performed at the Experimental Station of the Chapadão Foundation, located at latitude -18°41’33’’ S, longitude -52°40’45” W at a height of 810 m. In Selvíria, the experiment was located at the Experimental Research Farm of UNESP *Campus* of Ilha Solteira, latitude -20º 22’ S, longitude -51º 22’ W and height of 335 m (low altitude cerrado).

Chapadão do Sul and Selviría environments were select to perform the field experiments due to high representative edaphoclimatic conditions for Brazilian savanna. In both environments the soil was classified as clayey dystrophic red latosol (Santos et al., 2013). Results of chemical analysis are shown in Table 1. For both environments the climate of region is Aw according to Koppen’s classification. In Selvíria, the average annual temperature is 23.5 ºC, and relative humidity around 70 and 80%. In Chapadão do Sul, the average annual temperature is around 21 ^{o}C and relative humidity between 50 and 70%. Figure 2, shows maximum and minimum temperatures (ºC) observed during the conduction of the experiment in each environment.

General rainfall data for Selvíria and Chapadão do Sul are shown in Figure 1. Table 2 shows specific rainfall data for periods close to N-sidedressing, also considering supplemental sprinkler irrigation performed in Selvíria according to Fancelli and Dourado Neto (2000) recommendation for maize crop, while in Chapadão do Sul the maize plants were cultivated under a non-irrigated system.

Experimental design

The experiments were arranged in a randomized block design with factorial scheme of 2x2x5, and four replications. Treatments consisted in inoculation of maize seeds with *A. brasilense* AbV$_5$ AbV$_6$ (with and without inoculation), N sources (urea and polymer coated urea) and sidedressed N levels (0, 45, 90, 135 and 180 kg ha^{-1}). The polymer used is commercially named Policote®, anionic and soluble in water. Urea coated with this product is commercially available in the Latin American market of fertilizers.

The experimental plots consisted in 7 rows of 11 and 6 m lengths for Chapadão do Sul and Selvíria, respectively, with a space of 0.45 m between rows. The 5 central rows of 5 m length were considered useful areas. The seeding at Chapadão do Sul and Selvíria occurred in 21/02/2012 and 05/04/2012 using Pioneer hybrids seeds 30 S 31 Hx and P 3646 Hx, respectively. Important to note that each hybrid was more adapted to each growing environment. In Chapadão do Sul seeding was made on the remains of soybean and in Selvíria on the remains of millet for biomass production.

Fertilizer management

The NPK fertilization consisted in 300 kg ha^{-1} of 08-24-12 and 08-28-16, respectively, for Chapadão do Sul and Selvíria, respectively. For the seed treatment with insecticide, 25 g i.a ha^{-1} of fipronil was used, for both environments. The inoculation of plots with *A. brasilense* was made manually, after completely drying of treated seeds with 200 g of peat inoculums for 25 kg of seeds.

Nitrogen sidedressing was made in both environments on maize physiological phase V$_{5,}$ according to the treatments, manually, in strips. Dates corresponded to 22/03/2012 and 26/04/2012, respectively, for Chapadão do Sul and Selvíria, respectively. It is important to note that for the experiment in Chapadão do Sul, in the same day, hours after N-sidedressing, there was a rainfall of 22 mm, while in Selvíria such fact occurred on the following day, with a rainfall of 18 mm (Table 2). Feminine blooming occurred in 05/07/2012 and 06/11/2012, for Chapadão do Sul and Selvíria, respectively, and the harvesting of the experiments, occurred in 25/07/2012 and 21/08/2012 for both places.

Plant sampling and harvesting

The following evaluations were made: plant final population – at the time of harvest, plants were counted on two central lines of 5 m in length, then the data were expressed in plants per hectare; plant dry matter – on the occasion of plant blooming, 4 consecutive plants were collected from each plot; stem diameter - measured in millimeters with a caliper rule 20 cm from the soil on consecutive plants; weight of 100 grains (g) - after to mechanical trail, were counted 100 grains by plot, then, weighed and the humidity adjusted to 13% in humid base; grain productivity (kg ha^{-1}) – the ears harvested from plants in the 2 central lines of 5 m, were submitted to mechanic trail, and in sequence the grain weight was determined, and the data was expressed in kg ha^{-1}.

Leaf N analysis

The leaf samples were collected and dried in a forced-air oven at 65 °C during 48 h and ground. Thereafter, the samples were digested and chemically analyzed. Total-N was determined by the Kjeldahl method, which consist in the sulfuric digestion of the plant material to decompose the organic-N into ammonium, which is then converted to ammonia, distilled, and the amount of ammonia was determined by back-titration (Reis et al., 2015).

Statistical analysis

The data was submitted to analysis of variance using the SISVAR 5.0 program of statistics (Ferreira, 2011). According to the F test significance level for the treatments (N levels, sources and inoculation with *Azospirillum*), the data was submitted to linear and quadratic regression analysis using the GLM (general linear model) and the Tukey test ($p \leq 0.05$) for mean comparisons.

Conclusion

Leaf nitrogen concentration, plant dry matter, and yield of double cropped maize were not influenced by seed inoculation with *Azospirillum brasilense.*

The application of coated urea provided higher dry biomass, however, the polymer coated urea application showed no effect on grain yield in comparison to conventional urea.

Increasing side-dressed N levels is the main key factor responsible to improve the yield of double cropped maize growing in tropical Brazilian savanna.

Acknowledgements

The authors thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - Brazil) for fellowship. We also thanks Chapadão Foundation (Fundação de Apoio à Pesquisa Agropecuária de Chapadão) and PET group of Federal University of Mato Grosso do Sul for help to perform field experiment in Chapadão do Sul.

References

Adewopo JB, Vanzomeren CV, Bhomia RK, Almaraz M, Bacon AR, Eggleston E, Mooberg C, Snyder EH, Tiedeman M (2014) Top-ranked priority research questions for soil science in the 21st century. Soil Sci Soc Am J. 78: 337-347.

Araújo EO, Martins MR, Vitorino ACT, Mercante FM, Urquiaga SS (2015) Effect of nitrogen fertilization associated with diazotrophic bacteria inoculation on nitrogen use efficiency and its biological fixation by corn determined using ^{15}N. Afr J Microbiol Res. 9: 643-650.

Bashan Y, de-Bashan LE (2010) How the plant growth-promoting bacteria *Azospirillum* promotes plant growth – a critical assessment. Adv Agron. 108: 77–136.

Bittsánszky A, Pilinszk K, Gyulai G, Komives T (2015) Overcoming ammonium toxicity. Plant Sci. 231: 184-190.

Cassán FD, Vanderleyden J, Spaepen S (2014) Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus Azospirillum. J Plant Growth Regul. 33: 440–459.

Cavallet LE, Pessoa ACS, Helmich JJ, Helmich PR, Ost CF (2000) Corn productivity in response to nitrogen application and seed inoculation with *Azospirillum spp*. R Bras Eng Agrí Ambient. 4: 129-132.

Chien SH, Prochnow LI, Cantarella H (2009) Recent developments of fertilizer production and use to improve nutrient efficiency and minimize environmental impacts. Adv Agr. 102: 267-322.

CONAB (2015) - Companhia Nacional De Abastecimento. *Acompanhamento Da Safra Brasileira: Grãos, Sexto Levantamento, Março De 2015*. Available at <www.conab.gov.br>. (accessed 30.04.2015).

Deng F, Wang L, Ren WJ, Mei XF, Li SX (2014) Optimized nitrogen managments and polyaspartic acid urea improved dry matter production and yield of indica hybrid rice. Soil Till Res. 145: 01-09.

Fancelli AL, Dourado Neto D (2000) Maize production. Guaíba: Agropecuária. 360 p.

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Ciê Agrotec. 35:1039-1042.

Gitti DC, Arf O, Portugal JR, Corsini DCDC, Rodrigues RAF, Kaneko FH (2012) Cover crops, nitrogen rates and seeds inoculation with *Azospirillum brasilense* in upland rice under no-tillage. Bragantia 71:509-517.

Halvorson AD, Bartolo ME (2014) Nitrogen source and rate effects on irrigated corn yields and nitrogen-use efficiency. Agron J. 106: 681-693.

Hartmann A (1988) Ecophysiological aspects of growth and nitrogen fixation in Azospirillum spp. Plant Soil. 110: 222-238.

Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010) Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331: 413-425.

Hungria M, Kaschuk G (2014) Regulation of N_2 fixation and NO_3^-/NH_4^+ assimilation in nodulated and N-fertilized *Phaseolus vulgaris* L. exposed to high temperature stress. Environ Exp Bot. 98: 32-39.

Hungria M, Nogueira MA, Araujo RS (2015) Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. Am J Plant Sci. 6: 811-817.

Inagaki AM, Guimarães VF, Lana MC, Klein J, Costa ACR, Rodrigues LFOS, Rampim L (2015) Maize initial growth with the inoculation of plant growth-promoting bacteria (PGPB) under different soil acidity levels. Aust J Crop Sci. 9:271-280.

Kaneko FH, Leal AJF, Anselmo JL, Buzetti S, Tosta FS (2013) Sources and managements of nitrogen fertilization in cotton plants. Pesq Agropec Trop. 43: 57-63.

Kennedy IR, Tchan YT (1992) Biological nitrogen fixation in non-leguminous field crops: recent advances. Plant Soil. 141: 93-118.

Kuss AV, Kuss VV, Lovato T, Flôres ML (2007) Nitrogen fixation and in vitro production of indolacetic acid by endophytic diazotrophic. Pesq Agropec Bras. 42:1459-1465.

Linquist BA, Liu L, Kessel CV, Groenigen KJV (2013) Enhanced efficiency nitrogen fertilizers for rice systems: Meta-analysis of yield and nitrogen uptake. Field Crop Res. 154: 246-254.

Marks BB, Megías M, Nogueira MA, Hungria M (2013) Biotechnological potential of rhizobial metabolites to enhance the performance of *Bradyrhizobium* spp. and *Azospirillum brasilense* inoculants with soybean and maize. AMB Express. 3:01-21.

Moutia JFY, Saumtally S, Spaepen S, Vanderleyden J (2010) Plant growth by Azospirullum sp. in sugarcane is influenced by genotype and drought stress. Plant Soil 337: 233-242.

Puente ML, García JE, Alejandro P (2009) Effect of the bacterial concentration of *Azospirillum brasilense* in the inoculum and its plant growth regulator compounds on crop yield of corn (*Zea Mays* L.) in the field. World J Agri Sci. 5: 604-608.

Queiroz AM, Souza CHE, Machado VJ, Lana RMQ, Korndorfer GH, Silva AA (2012) Evaluation of different sources and rates of nitrogen fertilization in maize (*Zea mays* L.). Rev Bras Milho Sorgo. 10: 257-266.

Reis VM, Baldani JI, Baldani VLD, Dobereiner J (2000) Biological dinitrogen fixation in gramineae and palm trees. Crit Rev Plant Sci. 19:227–247

Reis AR, Favarin JL, Gratão PL, Capaldi FR, Azevedo RA (2015) Antioxidant metabolism in coffee (Coffea arábica L.) plants in response to nitrogen supply. Theor Exp Plant Physiol. 1: 1-11.

Repket RA, Cruz SJS, Silva CJ, Figueiredo PG, Bicudo SJ (2013) *Azospirillum brasilense* efficiency in combination with doses of nitrogen in the development of maize. Rev Bras Milho Sorgo. 12: 214-223.

Roesch LFW, Quadros PD, Camargo FAO, Triplett, EW. (2007) Screening of diazotrophic bactéria Azospirillum spp. for nitrogen fixation and auxin production in multipled field sites in Southern Brazil. World J Microbiol Biotechnol. 23: 1377-1383.

Sangoi L, Silva LMM, Mota MR, Panison F, Schmitt A, Souza NM, Giordani W, Schenatto DE (2015) Maize agronomic performance as affected by seed treatment with Azospirillum sp and mineral nitrogen rates. Rev Bras Ciê Solo. 39: 1-10.

Santos HG, Jacomine, PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Cunha TJF, Oliveira JB (2013). Sistema Brasileiro de Classificação de Solos. Embrapa, Brasília. 353 p.

Sarasketa A, González-Moro MB, González-Murua C, Marino D (2014) Exploring ammonium tolerance in a large panel of *Arabidopsis thaliana* natural accessions. J Exp Bot. 1: 1-11.

Sun H, Zhang H, Powlson D, Min J, Shi W (2015) Rice production, nitrous oxide emission, and ammonia volatilization as impacted by nitrification inhibitor 2-chloro-6-(Trichloromethyl)-pyridine. Field Crop Res. 173:01-07.

Sousa DMG, Lobato E (2004). Cerrado: correção do solo e adubação, 2nd edn. Embrapa, Brasília. 416p.

Swedrzynska D, Sawicka A (2000) Effect of inoculation with *Azospirullum brasiliense* on development and yielding of maize (Zea mays ssp. Saccharata L.) under different cultivation conditions. Pol J Environ Stud. 9: 505-509.

USDA (2015) United States Departament of Agriculture. Grain: world markets and trade. Available at <http://www.fas.usda.gov/data/grain-world-markets-and-trade>. (accessed 15.06.2015).

Valderrama M, Buzetti S, Benett CGS, Teixeira Filho MCM (2011) NPK sources and doses on irrigated corn under no-till system. Pesq Agropec Trop. 41: 254-263.

Xu GH, Fan XR, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Ann Rev Plant Biol. 63:153-182.

Wang S, Zhao X, Xing G, Yang Y, Zhang M, Chen H (2015) Improving grain yield and reducing N loss using polymer-coated urea in southeast China. Agron Sustain Dev. 35:1103-1115.

Zanin RL, Zamboni A, Monte R, Tomasi N, Varanini Z, Cesco S, Pinton R (2015) Transcriptomic analysis highlights reciprocal interactions of urea and nitrate for nitrogen acquisition by maize. Plant Cell Physiol. 56:532-548.

Zavaschi E, Faria LA, Vitti GC, Nascimento CAC, Moura TA, Vale DW, Mendes L, Kamogawa, MY (2014) Ammonia volatilization and yield components after application of polymer-coated urea to maize. Rev Bras Ciên Solo 38: 1200-1206.

Welbaum GE, Sturz AV, Dong ZM, Nowak J (2004) Managing soil microorganisms to improve productivity of agro-ecosystems. Crit Rev Plant Sci. 23:175–193.