

## Full Length Research Paper

# Corn agronomic evaluation under different doses of nitrogen and seed inoculation in savanna

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The agronomic potential of corn is below the genetic potential of high technology hybrid available in Brazil due to the inadequate management of soil fertility, especially of nitrogen fertilizer, essential photosynthetic activity and determining the crop yield. This study evaluated the agronomic performance and yield of maize culture grown with increasing doses of nitrogen (N), in the absence and presence of *Azospirillum brasilense* in Brazilian Savanna. The design used was the one of randomized blocks in factorial scheme, with five doses of N in corn (0, 50, 100, 150 and 200 kg ha<sup>-1</sup>), with and without inoculation of the bacterium, with six replications. It analyzed the N content and leaf chlorophyll was measured plant height, stem diameter, insertion spike length and diameter thousand grain weight, grain rot and productivity. The addition of N fertilizers promotes better agronomic performance of maize plants, being this effect greater in the presence of *Azospirillum*. The productivity of corn responds the dose of 200 kg ha<sup>-1</sup> N in the presence of *Azospirillum*. In 2012/13 crop corn inoculation with *Azospirillum brasilense* did not cause differences in productivity, plant height, diameter stem, weight of one thousand grains, number of ears and content of chlorophyll A, B and total.

**Key words:** Fertility, nitrogen fertilization, inoculation, productivity.

## INTRODUCTION

The Corn (*Zea mays L.*) is one of the main cereals produced in the world and has as its main producer the United States with approximately 37% of the total, followed by China (22%), Brazil (7.5%), European Economic Community (6.8%) and Argentina (2.8%) (Emygdio et al., 2013). Even occupying the third position in this scenario, with a planted area estimated at 14.2 million hectares and total production of 75.2 million

megagrams (Mg) in 2013/2014 season, the national average yield was only of 5.3 Mg ha<sup>-1</sup>. Those values are lower when compared to the 2012/13 harvest, when the average yield was of 5.4 Mg ha<sup>-1</sup> and the planted area decreased by around 6.1%, depending on weather conditions and lower use of production technology packages (Conab, 2014), besides the absence of economically viable information that support cultures

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management in tropical conditions (Kappes et al., 2013).

In many regions of Brazil corn production is below the genetic potential of high-tech hybrids, which can reach values ranging from 8.0 to 14.0 Mg ha<sup>-1</sup>. This low performance has been attributed, among other factors, to the inadequate management of soil fertility through acidity correction, fertilization at planting and coverage, as nitrogen (N) is the nutrient that most limits production because it is essential for plants metabolism. It is also crucial in crop yield, is mainly associated to growth and development of reproductive drains, and participates of the chlorophyll molecule as well. The chlorophyll molecule is essential for the maintenance of photosynthetic activity of plants (Cross et al., 2011). Cantarella (2007) points out that to produce 1.0 Mg of grains, considering a productivity of 12 Mg ha<sup>-1</sup> and 7 kg ha<sup>-1</sup> of N are needed.

The macro and micronutrients are usually provided via mineral fertilizer of high solubility, however, the intensive use of inorganic fertilizers can cause nutritional imbalance of plants and influence on the quality of the final product. As for N, one of the alternatives used to reduce consumption of these fertilizers is the biological N fixation (BNF) held by N-fixing bacteria in association with the plant. That is already being successfully used in corn (Lana et al., 2012; Sabundjian et al., 2013), rice (Puente et al., 2013) and cane sugar (Pereira et al., 2013). Specifically for corn, *Azospirillum brasilense* is a bacterium that has provided positive results, once besides producing hormones for the plants, it is also capable to fix atmospheric N. When inoculated in corn it becomes a promising technique for the plants development and increased productivity.

Currently there are technological packages using varieties of plants and efficient bacterial strains. They can supply more than 50% of the N required for the plant (Ferreira et al., 2013). Yet the growth stage of the plant is a determining factor for coverage with N, once it is recommended to apply the remaining amount of the nutrient in whole or split dose what must be made between stages (V4) and (V8) of fully expanded leaves (Fancelli, 2010).

The definition of the number of rows and the size of the ear, which are components of the corn grain yield, is between stages V4 and V12. These stages occur between 40 and 60 days after emergence, a period in which there is the most intense absorption of the nutrient, which is the ideal time to assess the nutritional status of the plant. Rambo et al. (2004) emphasize that the analysis of chlorophyll content is an important parameter for the assessment of plants development, widely used to differentiate plants with (in) adequate levels of N.

The inoculation of maize seed with *Azospirillum* bacteria has proven to have high potential for reducing N fertilization. It improves the physiological characteristics of the plant and can provide increased crop productivity as well (Goês et al., 2013). However, the influence of that

association on the macro and micronutrients accumulation in the plant in stages that precede the definition of the crop yield need better assessment, as they have been provided for the most part in the planting via mineral fertilizer. Given this context, this study evaluated the agronomic performance and corn yield grown with increasing doses of N, in the absence and presence of *Azospirillum brasilense* in Brazilian Savanna.

## MATERIALS AND METHODS

The study was conducted in the area of the Experimental Farm Capim Branco, between geographic coordinates 18° 55'23" S and 48° 17'19" W, at an altitude of 872 m, during the two crops cycles (2011/12 and 2012/13). The area belongs to the Federal University of Uberlândia (UFU), in Uberlândia-Brazil.

The climate is classified as Aw, tropical with a dry season in winter, according to the Köppen classification. Annual average precipitation in the region is up to 1500 mm. The soil was classified as Red Latosol, clayey in texture, containing na arable layer (0 to 0.20 m), 580 g kg<sup>-1</sup> clay, pH H<sub>2</sub>O = 5.5; 2.9 mg dm<sup>-3</sup> P; 101.0 mg dm<sup>-3</sup> K; 12.0 mg dm<sup>-3</sup> S; 1.0 cmol<sub>c</sub> dm<sup>-3</sup> Ca; 0.5 cmol<sub>c</sub> dm<sup>-3</sup> Mg; 0.0 cmol<sub>c</sub> dm<sup>-3</sup> Al; 0.11 mg dm<sup>-3</sup> B; 2.6 mg dm<sup>-3</sup> Cu; 9.0 mg dm<sup>-3</sup> Fe; 0.7 mg dm<sup>-3</sup> Mn; 0.3 mg dm<sup>-3</sup> Zn; 21 g kg<sup>-1</sup> organic carbon; 4.86 cmol<sub>c</sub> cm<sup>-3</sup> cation exchange capacity at pH 7.0 and 36% bases saturation.

The experimental design was a randomized block in a factorial 5 × 2, with five N in corn (0, 50, 100, 150 and 200 kg ha<sup>-1</sup>), two forms of inoculation of the bacterium: (1) Inoculated with *Azospirillum brasilense* with a dose of 100 mL ha<sup>-1</sup>, with a minimum concentration of 2×10<sup>8</sup> cells ml<sup>-1</sup>. (2) No inoculation, with six replications.

The area was prepared for planting with broadcast application of 1.0 Mg ha<sup>-1</sup> of dolomitic limestone, 40.2% CaO and 14% MgO, relative neutralization total power (PRNT) 100% built with heavy harrowing, followed by the application of 1,0 Mg ha<sup>-1</sup> of gypsum built with heavy harrowing. Then we used leveling grating and scarifier to open the grooves, with seeding being done manually on 14/12/2011 and 23/11/2012, using 3.5 seeds per meter, to obtain the stand of 70.000 plants per hectare.

The corn hybrid used was DKB 390 VTPRO and the inoculant was the commercial product Masterfix Gramínea® (strains - AbV5 and AbV6). Each plot consisted of 10 lines with 6 m long, spaced 0.5 m and the plot useful for harvest consisted by four core lines, excluding one meter from each end.

The planting of corn in the year 2011/12 was applied by 18 kg ha<sup>-1</sup> for Mg and 24 kg ha<sup>-1</sup> S in the form of magnesium sulphate (9% Mg and 12% S); 120 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as triple superphosphate; 50 kg ha<sup>-1</sup> K in the form of potassium chloride (KCl) and 50 kg ha<sup>-1</sup> of N in the form of urea (44% N), except for the treatment with doses of zero N. The fertilization of covering held in stage of development V4 consisted of the application of 100 kg ha<sup>-1</sup> K as potassium chloride and N dose required to complete the dose of each treatment. In the stage V8, foliar fertilization was performed with 40 g ha<sup>-1</sup> molybdenum; 4.0 g ha<sup>-1</sup> cobalt; 300 g ha<sup>-1</sup> manganese and 147 g ha<sup>-1</sup> of sulfur, 400 g ha<sup>-1</sup> boron and 2 kg ha<sup>-1</sup> zinc through ground.

While the planting of corn in the year 2012/13 was applied 120 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as triple superphosphate 50 kg ha<sup>-1</sup> K in the form of potassium chloride and 50 kg ha<sup>-1</sup> of N as urea, except in the treatments with doses of zero N. The fertilization of covering held in stage of development V4 consisted of the application of 100 kg ha<sup>-1</sup> K<sub>2</sub>O as potassium chloride and N dose required to complete the dose of each treatment. Fertilization was performed 300 g ha<sup>-1</sup> of boron with tetrahydrated sodium octaborate (20% B) via soil and 2 kg ha<sup>-1</sup> zinc with zinc oxide (76% Zn) via foliar.

In stage V7 we performed a weeds control using backpack

**Table 1.** Chlorophyll content A, B and Total related to N doses, in the absence and presence of bacterium *Azospirillum brasilense*, corn stage V8 in 2011/12 and 2012/13 crops, Brazil.

Bacterium	N doses									
	2011/12					2012/13				
	0	50	100	150	200	0	50	100	150	200
	kg ha <sup>-1</sup>									
	<b>Chlorophyll A</b>									
Absence	33.6 <sup>Aa</sup>	34.3 <sup>Aa</sup>	35.9 <sup>Aa</sup>	36.0 <sup>Aa</sup>	36.2 <sup>Aa</sup>	35.3 <sup>Aa</sup>	38.2 <sup>Aans</sup>	38.7 <sup>Aa</sup>	38.0 <sup>Aa</sup>	38.9 <sup>Aa</sup>
Presence	35.2 <sup>Aa</sup>	34.8 <sup>Aa</sup>	36.4 <sup>Aa</sup>	36.0 <sup>Aa</sup>	36.8 <sup>Aa</sup>	35.7 <sup>Aa</sup>	40.0 <sup>Aa</sup>	39.2 <sup>Aa</sup>	39.1 <sup>Aa</sup>	39.2 <sup>Aa</sup>
CV %			4.55					4.57		
	<b>Chlorophyll B</b>									
Absence	14.5 <sup>Aa</sup>	15.8 <sup>Aa</sup>	17.8 <sup>Aa</sup>	18.3 <sup>Aa</sup>	19.3 <sup>Aa</sup>	14.4 <sup>Aa</sup>	19.3 <sup>Aa*</sup>	20.7 <sup>Aa</sup>	20.4 <sup>Aa</sup>	21.4 <sup>Aa</sup>
Presence	16.1 <sup>Aa</sup>	16.5 <sup>Aa</sup>	19.0 <sup>Aa</sup>	18.7 <sup>Aa</sup>	20.3 <sup>Aa</sup>	14.9 <sup>Aa</sup>	22.7 <sup>Ba</sup>	21.5 <sup>Aa</sup>	20.8 <sup>Aa</sup>	21.5 <sup>Aa</sup>
CV %			13.61					12.85		
	<b>Tot<sup>a</sup>l chlorophyll</b>									
Absence	48.2 <sup>Aa</sup>	50.1 <sup>Aa</sup>	53.7 <sup>Aa</sup>	54.3 <sup>Aa</sup>	55.6 <sup>Aa</sup>	49.8 <sup>Aa</sup>	57.6 <sup>Aa</sup>	59.5 <sup>Aa</sup>	58.4 <sup>Aa</sup>	60.4 <sup>Aa</sup>
Presence	51.3 <sup>Aa</sup>	51.2 <sup>Aa</sup>	55.4 <sup>Aa</sup>	54.8 <sup>Aa</sup>	57.1 <sup>Aa</sup>	50.7 <sup>Aa</sup>	62.8 <sup>Ba</sup>	60.7 <sup>Aa</sup>	59.9 <sup>Aa</sup>	60.7 <sup>Aa</sup>
CV %			7.45					7.12		

\* = Significant ( $p < 0.05$ ); ns = Not significant. Means followed by the same capital letter comparing presence and absence in the dose evaluated in the column and minuscule line in comparing the two years in the same dose, do not differ by Tukey test ( $p < 0.05$ ).

sprayer with spray, volume of 350 L ha<sup>-1</sup>. Herbicides used were: Atrazine (400 g L<sup>-1</sup>) dose of 4.0 L ha<sup>-1</sup> and tembotrione (420 g L<sup>-1</sup>) at a dose of 0.24 L ha<sup>-1</sup> with Aureo adjuvant (methyl ester soybean oil - 720 g L<sup>-1</sup>) at a dose of 0.35 L ha<sup>-1</sup>.

Plants were harvested in May 2012 and April 2013, with the grain moisture corrected to 13% for productivity calculation. In the two years (2011/12 and 2012/13) we evaluated:

1. The chlorophyll content (A, B and total) on the stage V8 using chlorophyll ClorofiLOG® model CFL1030.
2. The leaf N in stage V8, using the last fully developed leaf in the stadium R1 and R3 (spike leaf in the reproductive phase), according to the methodology proposed by Embrapa (2009).
3. The spike length and diameter, which were measured with a graduated ruler (cm) and pachymeter (mm), weight of thousand grains, percentage of burning grains from a sample of 250 g grain from of each plot.
4. Fresh and dry weight will be evaluated in handy when the plant reaches flowering.
5. Productivity, obtained from the weighing of grain harvested in the useful portion, adjusted the humidity to 13%.

The results were analyzed for normality and homogeneity of data through tests Lilliefors, Cochran and Bartlett. The results were submitted to variance analysis, and applied the F test for significance and means compared by Tukey test ( $p < 0.05$ ) for the variable inoculation using Sisvar software version 4.0, being made a regression analysis to dose Sigmaplot using the 2010 version.

## RESULTS AND DISCUSSION

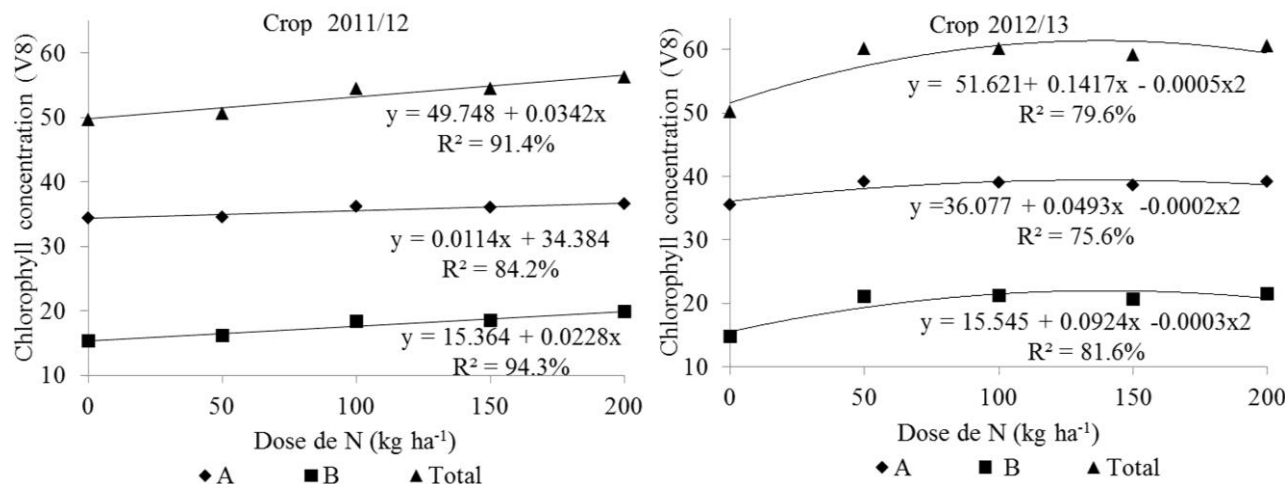
Analyzing the effect of increasing doses of N on the parameters evaluated in 2011/2012 and 2012/2013, it was observed that the value was significantly higher in

the presence of bacterium *Azospirillum brasilense* only in the dose of 50 kg ha<sup>-1</sup> in the year 2012/13 (Table 1), showing that the higher N absorption increased the chlorophyll content in the leaf, which implies a higher rate of photosynthesis, thus in greater carbon accumulation in the plant. This biomass accumulation, associated to increased absorption of water and nutrients, increases the translocation and accumulation of nutrients to the grains, which demonstrates the possibility of using these bacteria as promoters of plants growth.

Similar results were observed in other studies with seed inoculation by bacterium *Azospirillum brasilense*, which had higher chlorophyll content and growth in cane sugar (Pereira et al., 2013) and corn (Kappes et al., 2013). However, for corn results are still divergent, as Moraes (2012) has made the seed inoculation with the same bacteria observing no changes in leaf chlorophyll content.

Increasing N levels in the presence and absence of *Azospirillum* promoted linear response of chlorophyll content A, B and total in 2011/12 harvest. In the next harvest an increase in chlorophyll contents A, B and total was observed according to increasing doses of N, up to doses of 122, 153 and 142 kg ha<sup>-1</sup> N, respectively, providing quadratic increase in leaf chlorophyll content (Figure 1). Above these doses, there was reduction in chlorophyll content, due to the plant no longer respond to the increased supply of N.

According to Rambo et al. (2004) the relative chlorophyll content in the leaf has been considered the best indication of the N level and according to Malavolta



**Figure 1.** Contents of chlorophyll (A, B and Total) due to increasing doses of N in the stage V8 of corn coverage, Brazil, 2011/12 and 2012/13 seasons.

et al. (1997) that is due to its practicality and economy of the determination by chlorophyll meters. Furthermore, the determination of the relative content of chlorophyll has been used to predict the need for N fertilization in coverage in various crops, among the corn (Argenta et al., 2004).

With respect to N uptake by the corn crop, the maximum absorption occurs in two distinct periods, in the stages of vegetative and reproductive development or formation of the corn ear, while the lowest absorption rates occur in the period between the issuance of the tassel and the beginning of the formation of the ear (Olness and Benoit, 1992).

For foliar N content in the V8 stage, R1 and R3 in the years 2011/12 and 2012/2013 there was no significant increase in values was applied when the evaluated doses, however, in general, all observed values of this parameter were within the sufficiency range (28 to 35 g kg<sup>-1</sup> of N), considered suitable for corn (Malavolta et al., 1997).

Similar results were found by Gôes et al. (2013) and Kappes et al. (2013) for corn and by Pereira et al. (2013) for cane sugar. However this study also points to the specificity between bacteria and genotypes, since this response was not found in other varieties evaluated. That was also highlighted by Dotto et al. (2010) who found no significant difference in leaf N content due to N levels or inoculation. However, leaf N content in the hybrid AS 1540 was higher if compared to hybrid 1570, demonstrating the greater efficiency in AS 1540 N absorption. These results point to the need for further research involving the bacteria-genotype relationship, since the different materials have particular genetic characteristics and, therefore, the interaction with the bacteria can occur differently.

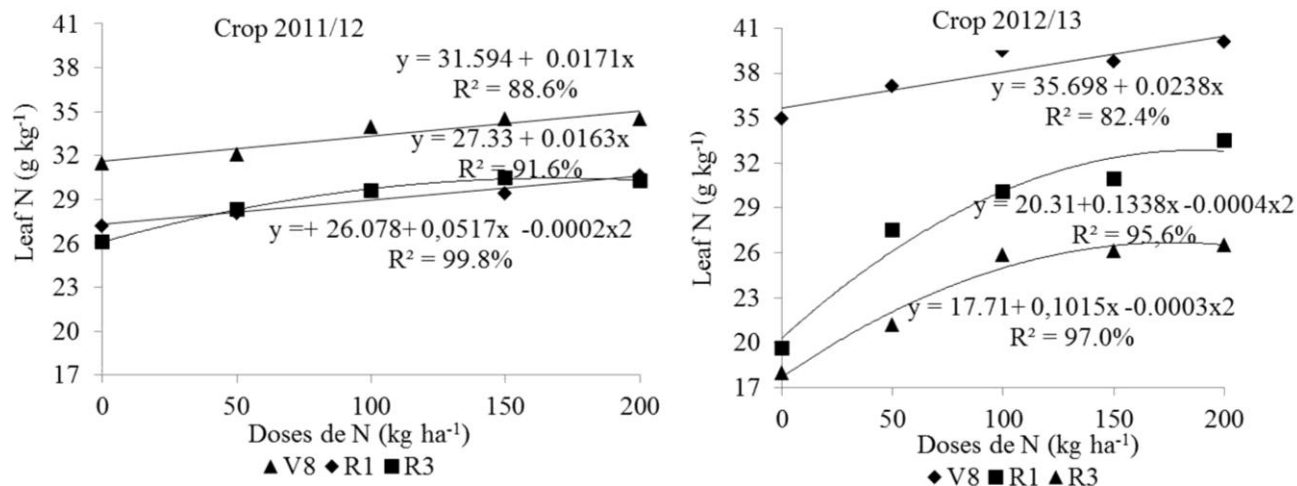
In the growth stages R1 and R3 there were no

significant interactions among the different doses of N and the presence or absence of *A. brasilense* inoculation. Through regression analysis shown in Figure 2 of 2011/12 season, there is a linear increase due to the doses applied, because the higher the amount of N applied, the greater the foliar N content, for stages V8 and R1. In stage V8 an absorption increase of this element is verified because the roots have a strong development (Fancelli, 2010). For each 1.0 kg ha<sup>-1</sup> of N applied, there is an increase of 0.0171 g kg<sup>-1</sup> of foliar N to stage V8, with good predictive capacity of the 88.57% model.

In the R1 stage, with the maximum dose of 200 kg N has 30.98 g kg<sup>-1</sup> of N leaf, which every 1 kg of N applied, the increase in leaf N content in the R1 stage is 0.0163 with predictive ability of the model 91.62%. For stage R3 there was an increase in foliar N content as doses went up to 129 kg ha<sup>-1</sup> N, where the foliar content was of 29.41 g kg<sup>-1</sup>. Goês et al. (2013) found a quadratic response of foliar N content by applying increasing doses of urea in corn.

Through the regression analysis shown in Figure 2 of 2012/13 crop, it is observed that according to the equation obtained in stage V8, with the highest dose applied of N in this experiment (200 kg ha<sup>-1</sup>), it would be possible to achieve maximum N content in leaves (40.41 g kg<sup>-1</sup>). For Cruz et al. (2011) there is a major impact of the N metabolism in the growth and yield of corn, because this nutrient establishes and maintains the photosynthetic capacity. Therefore, in order to achieve high yields one has to keep this activity during seed formation and grain filling.

Analyzing the average values of agronomic parameters evaluated in 2011/2012, it was observed that there was an increase in plant height and the height of ear insertion depending on the dose of 200 kg ha<sup>-1</sup> of N in the



**Figure 2.** Content of foliar N due to increasing doses of N, in stages V8, R1 and R3 of corn crop harvests in 2011/12 and 2012/13, Brazil.

presence of the bacterium, while the same was not true for the year 2012/2013. For a stem diameter there was no influence of doses of N or seed inoculum evaluated in the two years (2011/2012 and 2012/2013) (Table 2). These results show that high doses of N associated with seed inoculation with *Azospirillum* alter the physiological response of maize, demonstrating the importance of N in plant metabolism because it is essential to the biosynthesis of amino acids, proteins, chlorophyll, hormones (Rambo et al., 2004).

For plant height parameter Lana et al. (2012) found no significant difference according to the inoculation with *Azospirillum* spp. or N fertilization. With regard to the ear insertion height, similar results were observed by Goês et al. (2013), who found a quadratic response to this insertion height as a function of N doses, both as urea and ammonium sulphate applied to corn in winter cover.

Evaluating N fertilization and inoculation with *A. brasilense* and *Herbaspirillum seropedicae* in corn Dartora et al. (2013) obtained 15% increase in stem diameter in the treatments inoculated with respect to the control treatment, while Dotto et al. (2010) observed divergent results because they did not find significant effect of dose or inoculation on the stem diameter.

Among evidenced average values of fresh and dry weight of the plant (leaves + stem) it was observed that these indices have increased linear response to N rates evaluated in the presence and absence of *Azospirillum* (Figure 3).

In 2011/2012 in the presence of *Azospirillum*, fresh weight increase was of 1.08 g kg<sup>-1</sup> of N, therefore, higher than in its absence when the gain was of only 0.67 g kg<sup>-1</sup> of N. In the dry mass, the increase was of 178 mg kg<sup>-1</sup> N. These differences are due to the ability of these bacteria to synthesize plant hormones that once available to plants, stimulate the growth of fine roots of plants, thus

increasing their ability absorption of water and nutrients (Oliveira et al., 2009).

The plant dry matter is important because it sets the amount of carbohydrates to be translocated to the grain, so it is directly related to the size of grains as well as the production of the plant. According to Sangoi et al. (2005) the dry weight response in the presence of *Azospirillum* may be related to the ability of the bacterium to provide part of the fixed N<sub>2</sub> to the plant, because N is absorbed in large amounts by corn. As a constituent of amino acids, proteins, chlorophyll, its greater availability for culture can promote increases in carbohydrate accumulation by the plant.

In 2011/2012 season ear length was not affected by the application of increasing doses of N or seed inoculation, while the diameter increased linearly with increased N doses up to 200 kg ha<sup>-1</sup> (Figure 3). Seed inoculation increased the diameter in 23%. That difference is probably due to the improved root system generated by *Azospirillum*, increasing nutrient uptake by the plant, resulting in larger ear diameter.

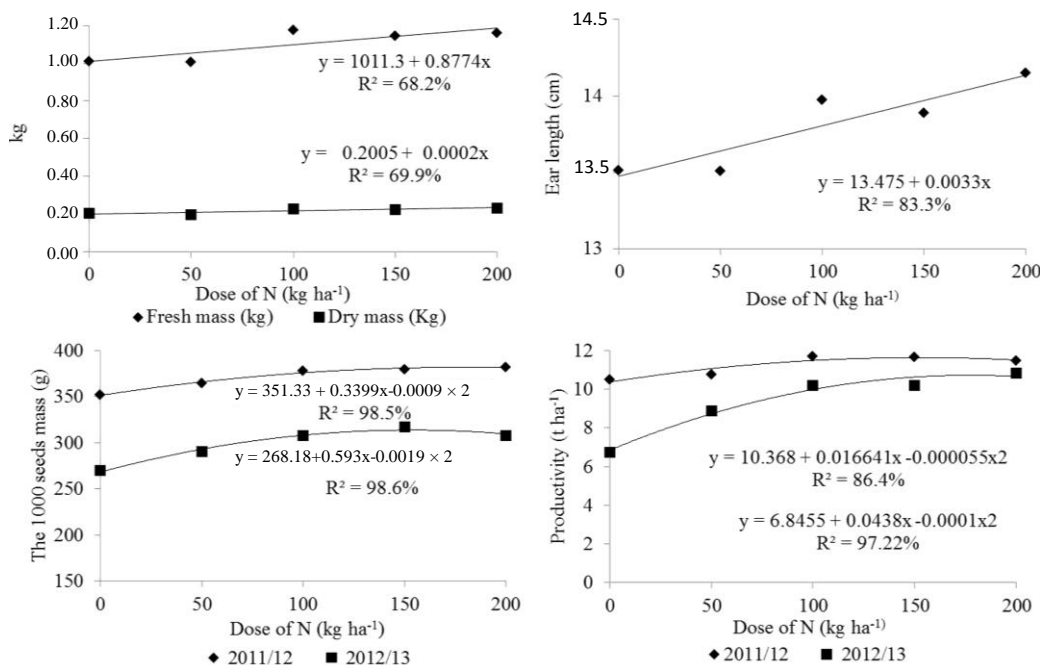
Goês et al. (2013) evaluated sources and doses of N in winter cover, and Okumura et al. (2013) evaluated doses of urea treated with urease inhibitor. They have found respectively quadratic and linear adjustment of the ear diameter according to the increasing doses of N in corn.

In 2011/2012 and 2012/2013 the incidence of rotted grains in this study was not significantly influenced by the application of different doses of N, as they are normally resulting from the decay of eyes due to fungi present in the production field (*Fusarium* spp., *Gibberella zeae*), which occurs mainly when the grains remain in the field after physiological maturity until harvest (Marcos, 2005). However, if we compare only the averages in the dose of 200 kg ha<sup>-1</sup> N, the percentage of rotten grains is 44% lower in the presence of *Azospirillum* (Figure 3).

**Table 2.** Plant height, ear height, stem diameter, fresh and dry matter (leaves + stems) depending on N doses, with and without seed inoculation with *Azospirillum brasilense* in 2011/12 and 2012/13 seasons, Brazil.

Bacterium	N doses									
	2011/12					2012/13				
	0	50	100	150	200	0	50	100	150	200
<b>Plant height (m)</b>										
Absence	2.08 <sup>Aa</sup>	2.10 <sup>Aa</sup>	2.16 <sup>Aa</sup>	2.12 <sup>Aa</sup>	2.09 <sup>Ba*</sup>	2.05 <sup>Aa</sup>	2.12 <sup>Aa</sup>	2.17 <sup>Aa</sup>	2.13 <sup>Aa</sup>	2.18 <sup>Aa</sup>
Presence	2.07 <sup>Aa</sup>	2.09 <sup>Aa</sup>	2.16 <sup>Aa</sup>	2.14 <sup>Aa</sup>	2.17 <sup>Aa</sup>	2.06 <sup>Aa</sup>	2.13 <sup>Aa</sup>	2.19 <sup>Aa</sup>	2.16 <sup>Aa</sup>	2.22 <sup>Aa</sup>
CV %			3.45					2.95		
<b>Ear insertion height (m)</b>										
Absence	1.20 <sup>Aa</sup>	1.22 <sup>Aa</sup>	1.23 <sup>Aa</sup>	1.22 <sup>Aa</sup>	1.18 <sup>Ba</sup>	1.09 <sup>Aa</sup>	1.12 <sup>Aa</sup>	1.15 <sup>Aa</sup>	1.14 <sup>Aa</sup>	1.16 <sup>Aa</sup>
Presence	1.19 <sup>Aa</sup>	1.20 <sup>Aa</sup>	1.24 <sup>Aa</sup>	1.21 <sup>Aa</sup>	1.25 <sup>Aa</sup>	1.09 <sup>Aa</sup>	1.14 <sup>Aa</sup>	1.18 <sup>Aa</sup>	1.15 <sup>Aa</sup>	1.21 <sup>Aa</sup>
CV %			4.50					5.07		
<b>Stem diameter (mm)</b>										
Absence	21.20 <sup>Aa</sup>	21.4 <sup>Aa</sup>	21.8 <sup>Aa</sup>	23.2 <sup>Aa</sup>	2.8 <sup>Aans</sup>	20.18 <sup>Aa</sup>	21.6 <sup>Aa</sup>	22.8 <sup>Aa</sup>	22.4 <sup>Aa</sup>	22.9 <sup>Aa</sup>
Presence	21.10 <sup>Aa</sup>	21.5 <sup>Aa</sup>	21.6 <sup>Aa</sup>	22.5 <sup>Aa</sup>	22.3 <sup>Aa</sup>	20.78 <sup>Aa</sup>	21.9 <sup>Aa</sup>	23.9 <sup>Aa</sup>	22.8 <sup>Aa</sup>	24.0 <sup>Aa</sup>
CV %			17.64					11.75		

\* = Significant (p <0.05); ns = Not significant. Means followed by the same capital letter comparing presence and absence in the dose evaluated in the column and minuscule line in comparing the two years in the same dose, do not differ by Tukey test (p <0.05).

**Figure 3.** Fresh and dry mass (kg), ear length (cm), weight of 1000 grains (g) and yield (Mg ha<sup>-1</sup>) as a function of increasing doses of N, in corn, 2011/12 and 2012/13 crops, in Uberlândia, Brazil.

As for the thousands grain weight in 2011/2012 harvest a smaller grain size was observed in the presence of *Azospirillum* and absence of N since the weight of a thousand grains was smaller. However, with increasing levels of N, the increment in the presence of the bacterium was of 200 mg kg<sup>-1</sup> of fertilizer applied to corn,

whereas in the absence of the bacterium this increase was of 100 mg kg<sup>-1</sup>, that is, twice when the fertilizer N was associated to inoculation (Figure 3). In 2012/2013 there was no significant difference for this parameter, depending on the absence or presence of *A. brasilense*. According to Borrás and Otegui (2001) the production

component was the one less affected by changes in management and fertilization practices.

When studying the management of N in coverage in maize grown under no-tillage Souza and Soratto (2006) did not observe any significant effect on the culture grain mass in conformity with what was observed in this study. Kappes et al. (2013) also obtained similar results by testing application methods and *Azospirillum* doses in corn, also finding no significant difference in the thousands grain weight. In this study corn hybrids used had a high productive potential and responded to high doses of N.

In 2011/2012 there was increased productivity to the highest dose tested (200 kg ha<sup>-1</sup>) in the presence of *Azospirillum* in which the estimated yield was of 12.0 Mg ha<sup>-1</sup>. This may be associated to *Azospirillum* which increases the amount of fine roots, enhancing the absorption and accumulation of nutrients by plants (Bashan and de-Bashan, 2010). In the absence of the bacterium, when N dose was increased from 0 to 200 kg ha<sup>-1</sup> there was an increase of 3% (314 kg) in grain production, while in presence of the bacterium the increase was of 16% (1630 kg) (Figure 3).

In 2012/2013 there was significant interaction among the N doses applied because corn yield is strongly influenced by the availability of N in soil, as evidenced by other studies (Santos et al., 2010), but there was no significant effect between seed yield with inoculation (9.31 Mg ha<sup>-1</sup>) and without inoculation (9.43 Mg ha<sup>-1</sup>), what gives the results obtained by Dotto et al. (2010). Despite that, around 160 bags per hectare were obtained. In 2012/13 quadratic response of grain yield was found as a function of N doses, with maximum productivity (13.1 Mg ha<sup>-1</sup>) achieved in the application of 200 kg ha<sup>-1</sup> of N. The corn production was 60.9% higher with increasing N doses (Figure 3), if compared to the control.

Bartchechen et al. (2010) found in a study of commercial inoculant based on *A. brasilense* associated to different doses of N that inoculation yielded superior results on maize productivity in relation to the witness, but there were no differences in productivity with isolated or associated inoculation to N topdressing. Morais (2012) found quadratic response of grain yield in tests with high technology in corn as a function of N doses, being the maximum productivity (10 to 14 Mg ha<sup>-1</sup>) achieved in the application of 260 kg ha<sup>-1</sup> of N.

## Conclusion

The addition of N fertilizers promotes further development of corn plants, increases the levels of chlorophyll and nutrients, plant biomass, ear diameter, thousands grain weight and productivity, which has greater effect in the presence of *A. brasilense*. The inoculation of maize seeds with *A. brasilense* improves absorption efficiency of N. The productivity of maize grains responds to the

dose of 200 kg ha<sup>-1</sup> of N in the presence of *A. brasilense*. The inoculation with *A. brasilense* does not replace the use of N fertilizers, but improves plant response to fertilization, especially in high doses. Corn production and chlorophyll levels A, B and total were significant with increasing N doses. In 2012/13 corn seed inoculation with *A. brasilense* did not provoke significant differences in productivity, plant height, stem diameter, thousands grain weight, number of ears and content of chlorophyll A, B and total.

## Conflict of Interest

The authors have not declared any conflict of interest.

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