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RESEARCH ARTICLE

# ASSOCIATION BETWEEN *AZOSPIRILLUM BRASILENSE* BACTERIA AND NITROGEN FERTILIZATION IN CORN PRODUCTION COMPONENTS

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**ABSTRACT**

The combined use of nitrogen (N) with bacteria *Azospirillum brasilense* can improve the components of corn production and contribute to sustainable agricultural practices. Thus, the aim of this study was to evaluate the application of *Azospirillum brasilense* associated with nitrogen fertilizer on corn production components. The experiment was set in a randomized block design, in a 5x4 factorial scheme, with five doses of *Azospirillum brasilense* (0, 100, 150, 200, 250 ml per 20 kg of seed), BR 11005 strain (Sp 245) and four doses of N (0, 50,100, 150 kg ha$^{-1}$) applied in the $V_6$phenological stage, using urea (45% N) as a source of N, with three replications. It was evaluated the number of rows per ear, number of kernels per row, ear diameter, thousand grain weight and grain yield. The use of *Azospirillum brasilense* strain BR 11005 (Sp 245) isolated or associated with N doses does not influence the productive characters of corn. The dose of 50 kg ha$^{-1}$ N in coverage is recommended to corn crops, as it promoted greater economic returns, regardless of the *Azospirillum brasilense* dose that was used.



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## INTRODUCTION

Corn (*Zea mays* L.) has great importance in the national and international scenarios. According to the National Supply Company - Conab (2016), in the 2015/16 harvest, the area cultivated corn crop and second crop in Brazil reached nearly 15,655,100 ha, with an average grain yield of 5107 kg ha$^{-1}$. Maize (*Zea mays* L.) has great importance in the national and international scenarios. According to the Companhia Nacional de Abastecimento- Conab (2016), in the 2015/16 harvest, the cultivated area with corn crop and second crop in Brazil reached nearly 15,655,100 ha, with an average grain yield of 5107 kg ha$^{-1}$.In the state of Minas Gerais area cultivated with corn crop and second crop represent 5.4% and 2.2% of the national crop, respectively. The Brazilian corn production is beneath the productive potential for this crop, given that Duete *et al*. (2009) reported that the productivity capacity is beyond 16,000 kg ha$^{-1}$. Thus, the national productivity is more than three times below the productive potential of this species. Several factors are attributed to the low Brazilian productivity, including the low consumption and the mishandling of nitrogen fertilizers (Andrade *et al*., 2014). In corn crops, the nitrogen (N) is one of the most limiting nutrients of grain yield (Malavolta, 2006; Fernandes *et al*., 2008). In addition, the soil N can be susceptible to leaching, volatilization, erosion (Alva *et al*., 2005), these losses increase even further the production cost. However, with the growing demand for sustainability in agricultural production systems, research has provided alternative ways of nitrogen fertilizer economy, such as biological nitrogen fixation (Bergamaschi, 2006), which consists of the use of microorganisms in order to fix N atmospheric. Several diazotrophs were isolated from maize crop, highlighting the *Azospirillum brasilense*, which can provide N for plants (Reis Júnior *et al*., 2008). For Araujo (2008), bacteria of the genus *Azospirillum* penetrate the plant roots; presents antagonism to pathogens; produces phytohormones; It is not very sensitive to temperature variations and occurs in all types of soil and climate. Among

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the plant hormones, *Azospirillum brasilense* bacteria can produce auxins, gibberellins, citocianinas under "in vitro" conditions (Masciarelli *et al*., 2013). Hungria (2011) when using *Azospirillum*, found increases of 26% in grain yield for corn, with supply of part of the nitrogen required by the plant for mineral fertilizer. Therefore, the combined use of N with *Azospirillum brasilense* bacteria can promote improvements in the corn production components and contribute to sustainable agricultural practices. Thus, the aim of this study was to evaluate the application of *Azospirillum brasilense* associated with nitrogen fertilizer on corn production components.

## MATERIALS AND METHODS

The experiment was conducted in 2011/12 crop year, in the municipality of Itutinga – MG, at Milanez Farm, located at 21º23'S latitude, 44º39'W longitude with an average altitude of 958 m in a soil classified as distroferric red latosol - LVdf (Embrapa, 2013), with clay texture and the following textural values: Clay: 400 g kg$^{-1}$; Silt: 290 g kg$^{-1}$; Sand: 310 g kg$^{-1}$. The chemical composition of the soil is presented in Table 1. The climate is Cwa, according to Köppen classification, with average annual temperature of 19.3ºC and annual rainfall of 1,530 mm (Dantas *et al*., 2007).Climatic data during the experiment were collected at the meteorological station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras - UFLA and are presented in Figure 1. The experiment was set in a randomized block design, in a 5x4 factorial scheme, with five doses of *Azospirillum brasilense* (0, 100, 150, 200, 250 ml per 20 kg of seed), BR 11005 strain (Sp 245) and four doses of N (0, 50,100, 150 kg ha$^{-1}$) applied in the V6 phenological stage, using urea (45% N) as a source of N, with three replications. Each plot consisted of four sowing lines of 5m long, spaced 0.80 m, and 16m$^2$ of area for each plot (5 m x 3.20 m).The two central rows were considered the useful area. The sowing was performed in November 2011, using plant density of 4 to 5 plants per meter and the fertilization consisted of 350 kg ha$^{-1}$ of N-$P_2O_5$-$K_2O$ (08-24-12) applied via groove. *Azospirillum brasilense* bacteria were inoculated in the seed, using the inoculant Gelfix®. The application of N doses were performed by hand .It was used the UFLA hybrid (not commercial cultivar). Cultural practices (control of weeds, pests and diseases) before and after sowing were recommended for the region and for the hybrid, and according to crop needs. When the culture reached the stage of physiological maturity, it was evaluated the number of rows per ear, number of kernels per row, ear diameter .Five ears per plot were used to measure the mentioned variables. The thousand grain weight was determined as BRASIL (2009) recommendations. The yield was measured after the grain harvest in the entire plot area, the ears were mechanically threshed and the production turned into kg ha$^{-1}$ of grain, standardized to 13% moisture. After variance analysis (F test) and in case of significance ($p \leq 0.05$), the variation sources were subjected to polynomial regression analysis. The statistical analysis was performed with the aid of SISVAR® statistical software (Ferreira, 2011).

## RESULTS AND DISCUSSION

There was no significant effect on the evaluated traits for the variation source doses of *Azospirillum brasilense* (Table 2). Results are similar to those highlighted by Campos *et al*. (2000) and Godoy *et al*. (2011). The authors, working with an inoculant composed of *Azospirillum brasilense*, did not observe improvements in corn agronomic traits. It was found that levels of N significantly influenced the other studied variables, except the thousand grain weight (Table 2). These results are similar to those obtained by Andrade *et al*. (2014), who also observed influences of this source of variation in agronomic characteristics of corn.Regarding Azo doses x N doses, there were no statistical differences for the evaluated characters (Table 2). Thus, the variations of these factors were coincident. Souza (2014) working with and without *Azospirillum brasilense* bacteria and levels of N in corn, also found that the interaction of the factors was not significant for any of the evaluated parameters, confirming the results obtained in this study. Hungria (2011) using the *Azospirillum* noted increases of 31 and 26% in grain yield in wheat and corn crops, respectively, but with part of the required nitrogen by the plant supplied through mineral fertilizer.Currently there are *Azospirillum brasilense* based inoculants, commercialized in Brazil with recommendation for corn and wheat crops. Such products have the AbV5 and AbV6 strains. Thus, probably no significant effect is due to the product used, because of the inefficiency of the BR 11005 (SP 245) strain to promote improvements in plants, such as the N supply and plant hormone production. Additionally, Didonet *et al*. (2000) reported that for the inoculation with *Azospirillum* bacteria be effective, they must be capable of compete with native diazotrophs and soil microflora. Thus, it may have also occurred competition between *Azospirillum* bacteria with native species. N doses significantly influenced the number of rows per ear (Figure 2A), number of kernels per row (Figure 2B), ear diameter (Figure 2C) and grain yield (Figure 2D) of corn plants. For these parameters, the results were adjusted to the quadratic regression model.

The number number of rows per ear (14 units) (Figure 2A) and diameter of ears (45.73 mm) (Figure 2C) were observed at the dose of 150 kg ha$^{-1}$ of N, that is a higher dose than the one verified by Andrade *et al*. (2014). The authors noted that applications of 100 kg ha$^{-1}$ provided greater means in this parameter. For the ear diameter, Heinrichs *et al*. (2003) found no significant differences due to N doses, so the results differ from those obtained in this study. Probably, these differences are related to soil and climate conditions of each experiment, given that the efficiency in the use of fertilizers is highly influenced by soil and climate conditions of each region, so it is necessary to define the fertilizer for each specific region (Petter *et al*. 2012). For the number of kernels per row (Figure 2B) and grain yield (Figure 2D), the maximum number was 35 units and 3927 kg ha$^{-1}$, respectively, at a dose of 100 kg ha$^{-1}$.These results are the same as those obtained by Andrade *et al*. (2014), which also found higher grain yield with dose of 100 kg ha$^{-1}$.The highest yield is approximately 64% higher than the control (2509 kg ha$^{-1}$). However, these figures are below the corn average yield (5978 kg ha$^{-1}$) for the State of Minas Gerais (Conab, 2012). This may be related to the low rainfall in February and April 2012 (Figure 1).The highest estimated corn yield was obtained with the application of 113 kg ha$^{-1}$ of nitrogen, resulting in a grain yield of 4003 kg ha$^{-1}$.

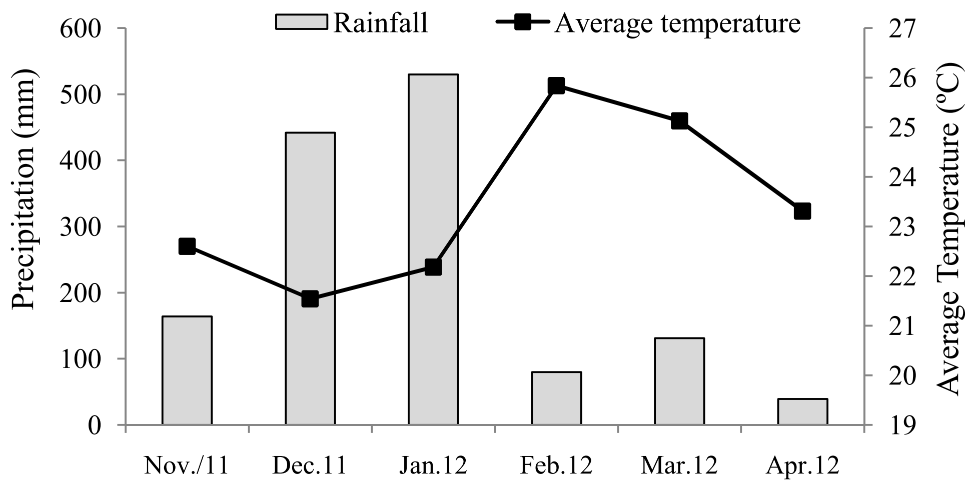


**Figure 1. Monthly averages of rainfall and air temperature in Itutinga - MG in 2011/12 crop year, during the experiment. Source: National Institute of Meteorology (INMET)**

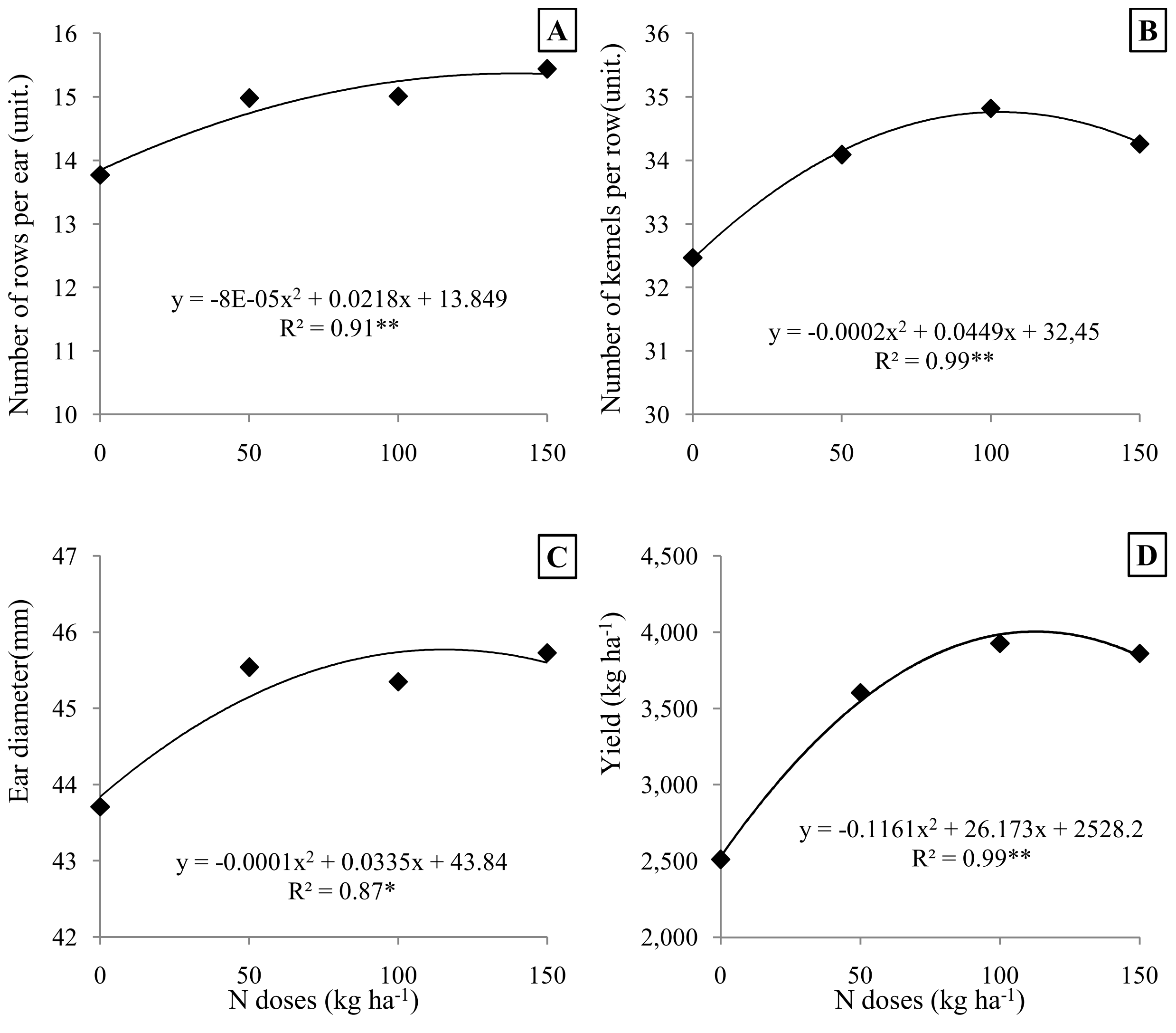


**Figure 1. Number of rows per ear (A) number of kernels per row (B), ear diameter (C) and grain yield (D) of corn plants based on N doses, in 2011/12 crop year. Itutinga – MG, Brazil**

**Table 1. Chemical composition of distroferric red latosol (0-0.20 m) prior to the experiment.Itutinga - MG, in 2011/12 crop year**

| CropYear | pH | $Ca^{2+}$ | $Mg^{2+}$ | $Al^{3+}$ | $H^{+}+Al^{3+}$ | SB | CEC | P | K | OM | V |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $H_2O$ | ---------------------- $cmol_c\ dm^{-3}$ ---------------------- | | | | | | -- mg $dm^{-3}$ -- | | $dag/kg^{-1}$ | % |
| 2011/12 | 5.2 | 1.6 | 0.5 | 2.0 | 39.0 | 51.0 | 6.0 | 1.8 | 96.0 | 38.0 | 51.0 |

H + Al: potential acidity; SB: sum of bases; CEC: cátion Exchange capacity at pH 7,0; MO: organic matter; V: base saturation

**Table 2. Analysis of variance and mean values for the number of rows per ear (NRE), number of kernels per row (NKR), ear diameter (ED) thousand grain weight (TGW) and grain yield (GY) in corn based on the Azos and N doses in the 2011/12 crop year. Itutinga - MG, Brazil**

| Variation sources | GL | ANOVA (MS)[1] | | | | |
|---|---|---|---|---|---|---|
| | | NRE | NKR | RD | TGW | GY |
| | | - - - - -cm - - - - - | | unidade | - -g - - | kg $ha^{-1}$ |
| Block | 2 | 1.20 | 63.10 | 6.35 | 4.06 | 17380744.44 |
| Azos Dose (A) | 4 | $0.46^{ns}$ | $2.94^{ns}$ | $2.48^{ns}$ | $10.00^{ns}$ | $279076.74^{ns}$ |
| N dose (N) | 3 | 7.71** | 15.32* | $12.95^{*}$ | $2.17^{ns}$ | $6515174.19^{*}$ |
| A x N | 12 | $1.25^{ns}$ | $7.34^{ns}$ | $6.19^{ns}$ | $2.40^{ns}$ | $668586.90^{ns}$ |
| Error | 38 | 0.76 | 5.48 | 5.60 | 3.68 | 396870.29 |
| Média Geral | - | 14.80 | 33.91 | 45.08 | 286.60 | 3475.40 |
| CV (%) | - | 5.93 | 6.91 | 5.25 | 6.69 | 18.13 |
| Factor | | | | Means | | |
| Azos Dose(mL) | | | | | | |
| 0 | | 14.83 | 34.36 | 44.91 | 273.30 | 3345.15 |
| 100 | | 14.60 | 33.40 | 45.06 | 296.60 | 3581.68 |
| 150 | | 14.60 | 34.05 | 44.46 | 286.60 | 3432.56 |
| 200 | | 14.96 | 33.38 | 45.68 | 283.30 | 3334.60 |
| 250 | | 15.01 | 34.37 | 45.31 | 293.30 | 3683.02 |

[1] ** and * significant at 1 and 5% of probability, according to F test, respectivelly. ns – not significant; MS – Mean Squares; DF – degree of freedom; CV – coefficient of variation.

**Table 3. Cost ratio as a function of N dose and financial return on the investment Milanez Farm, Itutinga - MG, Brazil**

| N dose (kg $ha^{-1}$) | Yield (kg $ha^{-1}$)[1] | Income (R$ $ha^{-1}$)[2] | Investmentwith N (R$)[3] | Return (R$ $ha^{-1}$) |
|---|---|---|---|---|
| 0 | 2.509 | 961.78 | - | 1080.48 |
| 50 | 3.604 | 1381.53 | 152.5 | 1229.03 |
| 100 | 3.927 | 1505.35 | 305.0 | 1200.35 |
| 150 | 3.861 | 1480.05 | 457.5 | 1022.55 |

[1] Yield Means observed, regardless of *Azospirillum brasilense* dose. [2] price bag of corn (60 kg), commercialized in Itutinga, MG, R$ 23.00. Survey conducted on 05/10/12.[3] Average prices, regardless of *Azospirillum brasilense* dose.

It is known that N is a constituent of the chlorophyll molecule, thereby the nitrogen doses tend to influence the photosynthetic process and increase the production of photoassimilates by plants. As observed in this study, the dose of 100 kg $ha^{-1}$of N is responsible for the higher grain yield. However, when evaluating the financial return the dose of 50 kg $ha^{-1}$ of N, resulted in a gain of R$ 1,229.03 $ha^{-1}$. Therefore, the lower the dose, the more economically viable becomes the corn crop.

### Conclusion

The use of *Azospirillum brasilense* strain BR 11005 (Sp 245) isolated or associated with N doses does not influence the productive characters of corn. The dose of 50 kg $ha^{-1}$ N in coverage is recommended to corn crops, as it promoted greater economic returns, regardless of the *Azospirillum brasilense* dose that was used.

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