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GRAIN YIELD OF MAIZE INOCULATED WITH DIAZOTROPHIC BACTERIA WITH THE APPLICATIONOF NITROGEN FERTILIZER[1]

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ABSTRACT - Increasing the crop yield of maize planted in the climate and soil conditions of the northeast region of Brazil can be accomplished with the use of biological inputs coupled with the application of lower doses of nitrogen (N) fertilizer. The objective of this work was to evaluate the yield of maize inoculated with two diazotrophic bacterial species under different N rates, during three consecutive years in an Inseptisol of Sergipe state, Brazil. The two bacterial species used were *Azospirillum brasilense* BR11005 (Sp245) and *Herbaspirillum seropedicae* BR11147 (ZAE94). N was applied in the form of urea at 0, 100, 200, and 300 kg ha^{-1}. Under field conditions and depending on the year, the crop yield was limited by the rainfall regime. The inoculation associated with the 100 kg ha^{-1} N treatment with BR11147 and BR11005, increased productivity by 1,230 kg ha^{-1} and 614 hg ha^{-1}, respectively. This corresponded to a 37% and 19% productivity increase, respectively, compared to the productivity of the uninoculated control. At zero N, inoculation increased grain yield of maize plants inoculated with BR11147 by 18%. Additionally, the N content of the index leaf inoculated with BR11005 increased by 10% over that of the uninoculated control.

Keywords: *Azospirillum brasilense. Herbaspirillum seropedicae. Zea mays.*

RENDIMENTO DE GRÃOS DE MILHO INOCULADO COM BACTÉRIAS DIAZOTRÓFICAS COM APLICAÇAO DE FERTILIZANTE NITROGENADO

RESUMO – Aumentar a produtividade de milho em condições de clima e solo do nordeste brasileiro podem ser atingida com a utilização de insumos biológicos acoplado a aplicação de menores doses de N-fertilizante. O objetivo deste trabalho foi avaliar o rendimento de grãos de milho inoculado com duas espécies de bactérias diazotróficas combinadas com doses de N-fertilizante durante três anos consecutivos em um Cambissolo no estado de Sergipe, Brasil. Duas espécies de bactérias foram utilizadas: *Azospirillum brasilense* estirpe BR11005 (Sp245) e *Herbaspirillum seropedicae* BR11147 (ZAE94) e as doses crescentes de ureia de 0, 100, 200 e 300 kg ha^{-1}. Sob condições de campo houve limitação do rendimento dependendo do ano agrícola e regime hídrico. A inoculação, associada a uma dose de 100 kg ha^{-1} N com as estirpes BR11147 e BR11005, aumentou a produtividade em 1.230 e 614 kg ha^{-1}, respectivamente; sendo 37% e 19% maior que o controle não inoculado. Na ausência do fertilizante também foi observado incremento da produtividade no milho inoculado com BR11147 de 18% e no teor de N na folha índice pela inoculação da BR11145 em 10% comparado ao controle.

Palavras-chave: *Azospirillum brasilense. Herbaspirillum seropedicae. Zea mays.*

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INTRODUCTION

The productivity of maize grown in semi-arid regions of northeastern Brazil is conditioned to the water regime associated with saline soils, which, however, have chemical characteristics such as medium to high levels of macro and microelements, thus being considered fertile soils. Another limiting factor in terms of maize productivity, is the limited use of technology, which results in an average productivity of 4,000 kg ha^{-1} in the northeastern region of Brazil, compared to the productivity of more than 8,000 kg ha^{-1} in areas with less environmental risk and with high application of agricultural inputs and irrigation, such as these in the central west and south of Brazil (CONAB, 2019). Owing to the rain conditions in this region, corn is planted in winter, which is the season with the highest and most regular rainfall, with the temperature not being a significant factor.

The nitrogen (N) fertilization of maize in Brazil is linked to productivity expectations and the region's favorable climate and it is higher during the rainy season with yields greater than 10 Mg ha^{-1}. The application of 1% N is recommended, with up to 200 kg of N fertilizer applied, owing to fertilizer formulation and expected losses (CANTARELLA; DUARTE, 2004). An increase in the efficiency of N use can be accomplished in different ways, such as spreading the fertilization, using new formulations where N availability is controlled with, e.g., volatilization inhibitors (CANTARELLA et al., 2008), and, more recently, by applying plant growth-promoting bacteria such as those of the *Azospirillum* genus (SZILAGYI-ZECCHIN; MARRIEL; SILVA, 2017), which are associated with N fertilization.

As of 2010, products containing selected strains of diazotrophic bacteria of the *Azospirillum brasilense* species started being recommended for application on corn (HUNGRIA et al., 2010). This species is known to affect plant growth, which can be attributed to the production of different growth regulator classes (CASSÁN; DIÁZ-ZORITA, 2016). Additionally, the *Azospirillum* genus produces the largest amount of indole compounds in the auxin group among the different genera of studied bacteria (CASSAN; VANDELEYDEN; SPAEPEN, 2014), in addition to contributing to biological N fixation (FBN) (SALAMONE; DÖBEREINER, 1996).

Alves et al. (2015) selected strains of diazotrophic bacterial species for maize application. Among the 21 *Herbaspirillum* genus strains that were tested, the application of *H. seropedicae* BR11147 (also described as strain ZAE94), resulted in higher productivity both in the greenhouse and in the field compared with treatments in which increased N doses were applied and the contribution of FBN was quantified (ALVES et al., 2015; BREDA; ALVES; REIS, 2016).

Azospirillum brasilense and *H. seropedicae* strains can colonize cereal roots (BASHAN; BASHAN, 2010; MONTEIRO et al., 2012), promote a better use of N fertilizer applied in the form of urea (MARTINS et al., 2018), and contribute to root growth that can be more tolerant of drought conditions (CASSAN; VANDELEYDEN; SPAEPEN, 2014); strains of these two species can be used together in co-inoculation treatments (DARTORA et al., 2006).

The inoculation of plants with *A. brasilense* has been tested in different regions of Brazil with varying water regimes, however little is known about plants' response to inoculation with *H. seropedicae*. The hypothesis tested in this study was that corn planted in northeastern Brazil, with its characteristic climatic conditions, may respond differently in terms of productivity under increasing N fertilizer applications, depending on the bacterial species used for inoculation. The objective of this work was to evaluate the maize grain yield and leaf N content of plants inoculated with two diazotrophic bacterial species and subjected to four doses of N during three consecutive years in a Inceptisol in Sergipe, northeastern Brazil.

MATERIAL AND METHODS

The experiment was carried out in the 2015–2017 period at the Pedro Arle Experimental Field of Embrapa Tabuleiros Costeiros, located in the municipality of Frei Paulo, State of Sergipe, Brazil (10° 36' 8.114" S, 37° 38' 11.558" W) during the rainy season (Figure 1). The soil was classified as an Inceptisol (USDA) and a Cambisolo Háplico Eutrófico (Brazilian classification). The three experimental crops were planted in the same area.

The soil sample was collected from horizon A that was 0–20 cm thick and the following results were obtained: organic matter, 25.1 g dm^{-3}; pH (H2O), 6.0; P (Mehlich-1), 2.9 mg dm^{-3}; K, 220.8 mg dm^{-3}; Ca, 9.6 $cmol_c$ dm^{-3}; Mg, 4.2 $cmol_c$ dm^{-3}; (H + Al), 0.0; Al, 0.02 $cmol_c$ dm^{-3}. The analysis was performed according to Silva et al. (2009). The experiments were installed in randomized blocks in a 4 × 3 factorial scheme with four replications. The factors were N fertilizer (in the form of urea) applied at doses of 0, 100, 200, and 300 kg N ha^{-1} and inoculation or the absence of it with *A. brasilense* and *H. seropedicae*, totaling 12 plots per block. The entire plot consisted of six lines that were 5 m long and spaced by 0.80 m in 2015 and by 0.70 m in 2016 and 2017. The useful plot harvested consisted of four lines that were 4.2 m long and the area of the useful plot was 2.8 m × 4.2 m = 11.76 m^2. Leaf samples were collected in the useful plot. The experimental crops were harvested 148, 174, and 188 days after planting in 2015, 2016, and 2017, respectively. Irrigation was not implemented in any of the years.

The N fertilizer was divided into two applications; the first was applied in the planting stage and the other in the V4 stage (four leaves). Thirty percent of N was used in the first and 70% in the second application, divided as follows: 100 kg ha^{-1} (30/70); 200 kg N ha^{-1} (60/140); 300 kg N ha^{-1} (90/210). The source of N used was urea. The N doses used were based on the work of Sobral, Anjos and Carvalho (2015). In addition, 90 kg ha^{-1} of P_20_5 was used in the form of triple superphosphate for phosphorus fertilization and was applied to the planting furrow in all treatments. No other nutrients were required according to soil analysis soil analysis. The maize hybrids used were 2B-433 in 2015, 2B433PW in 2016, and 2B647PW in 2017 (Dow Agrosciences), selected for their precociousness and adaptation to the region; based on these hybrid characteristics it was assumed that the inoculation results were not influenced by the use of different genotypes each year. Each pit received two seeds; after thinning, the stand was reduced to one seed.

A) B)

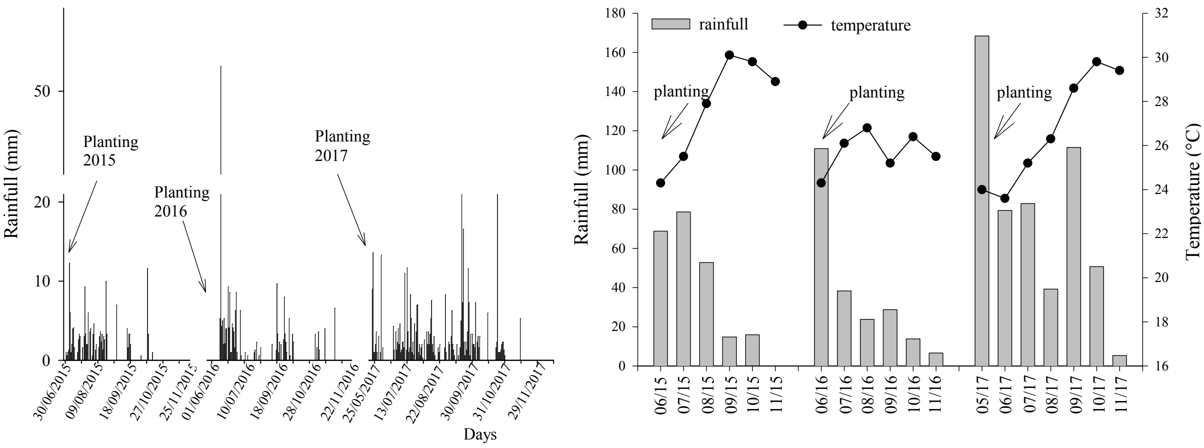


Figure 1. Daily rainfall (mm) (A) and weekly and average monthly temperature (B) of the three years of maize cultivation (2015, 2016, and 2017). The data were obtained from the Embrapa Tabuleiros Costeiros Experimental Station, city of Frei Paulo, State of Sergipe, Brazil.

The bacteria used were *H. seropedicae* strain Hs-BR11417 (= ZAE94, isolated from rice roots) and *A. brasilense* strain Ab-BR11005 (= Sp245, isolated from wheat roots), both acquired from the Johanna Döbereiner Biological Resource Center [CRB-JD (Portuguese acronym)]. The peat inoculant was prepared as described in Breda et al. (2019). The strain was initially grown in a plate with JNFb solid culture medium (BALDANI et al., 2014), with the aim of verifying its purity. To obtain the inoculant, pure colonies were inoculated in a DYGS culture medium (BALDANI et al., 2014) and multiplied for 24 h at 30 ºC on a shaking table at 175 rpm. After growth, 75 mL of the cell suspension containing 10^9 cells mL^{-1} was mixed with 175 g of ground peat, neutralized, sterilized, and homogenized. The seeds were covered with a 10% starch solution and applied to peat in the proportion of 250 g of peat per 10 kg of seed. Each seed received a dose of 10^6 cells mL^{-1}.

Leaf samples were collected at the beginning of flowering from the leaf opposite of the ear (described in this publication as the index leaf) according to Silva et al. (2014). Five leaves were collected from each row and mixed to obtain a sample representative of a plot. The leaves were cleaned with water-soaked cotton, dried in an air-circulating oven, and ground in a Wiley mill. The N content was determined by the Kjeldahl method (BREMNER; MULVANEY, 1978). At the end of the experiment, the ears were harvested and threshed and the seeds were weighed to determine the grain yield, which was adjusted to 13% moisture.

The data were analyzed for normality using the Shapiro-Wilk test. Cochran's and Bartlett's homogeneity of variance tests were performed using the SAEG 8.0 program (EUCLYDES, 1983). With these, the necessary assumptions for the evaluation of data by parametric tests were met. After the normality and homogeneity tests, the data were subjected to analysis of variance and when the treatments were significant, they were subjected to mean or regression tests. The mean differences were assessed with a t-test and the significance level was set at 5%.

RESULTS AND DISCUSSION

Maize productivity, in response to nitrogen application, irrespective of the presence or absence of an inoculation treatment, differed between the three years and was modulated by significant water regime differences (Figure 1). (Table 1) shows the F values of the analysis of variance verified in the three experiments conducted with the same experimental design. There was a difference among the doses for all variables, except in 2016 for grain yield. However, in the same year there was a difference among doses and strains in terms of the N content. The table also shows that the interaction between the N fertilizer dose and the inoculation factors was significant, thus indicating that there is a better combination between the factors.

The experiments were carried out in the three agricultural years with a total precipitation of 234, 214, and 482 mm, respectively, during the harvest cycle (Figure 1A, 1B); however, differences in the distribution of rainfall throughout the cycle were observed. Rainfall was higher in 2017 and lower in 2015 and 2016 (Figure 1A). In 2016, rainfall decreased sharply after the sowing date, mainly in the period of planting and between 8 and 9 weeks after planting (Figure 1B). This water deficit reduced the crop yield and, consequently, the response to the treatments applied.

Table 1. F values and coefficient of variation (CV) of each analysis of variance of the experiments performed in 2015, 2016, and 2017.

Variation	DF	2015		2016		2017	
		Prod.	N-leaf	Prod.	N-leaf	Prod.	N-leaf
Blocs	3	0.0037	0.0291	0.0299	0.6144	0.2953	0.0302
Doses	3	0.0002***	0.0000***	0.2600 ns	0.0023**	0.0000***	0.0000***
Inocul.	2	0.1710ns	0.4663 ns	0.5111 ns	0.0138**	0.3829 ns	0.5014 ns
D x I	6	0.0306*	0.0461*	0.3685 ns	0.0426*	0.0482*	0.0350*
Error	33						
CV (%)		18.83	11.47	17.14	4.45	13.70	9.49

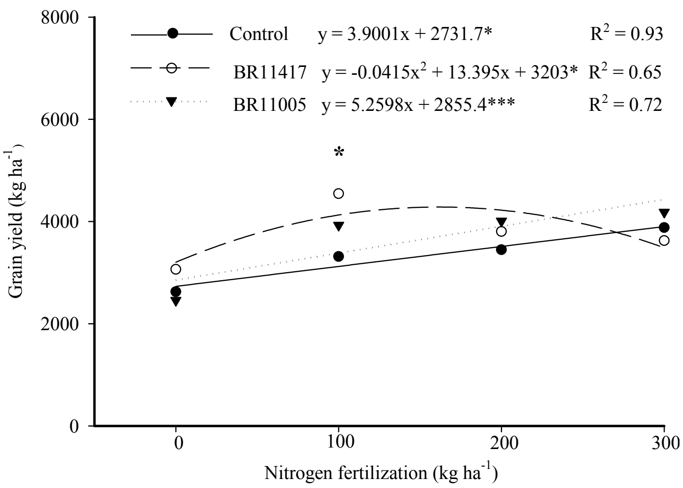
DF: degree of freedom; Prod: grain yield; N: nitrogen content in the leaf; D: nitrogen fertilization dose; I: inoculation; CV: coefficient of variation. *: F-test ($P \leq 0.05$); **: F-test ($P \leq 0.01$); ***: F-test ($P \leq 0.001$); ns: not significant by the F-test.

In 2015, the rainfall volume was always reduced, amounting to less than 40 mm per month (Figure 1A and 1B). In Brazil, corn has an estimated water requirement of 380–550 mm and the distribution of rainfall interferes with growth (ALBUQUERQUE, 2010). There was a difference among the fertilizer application response curves depending on the inoculation; the curve was quadratic for Hs-BR11417 and linear for Ab-BR11005 and the control treatment, in terms of grain yield (Figure 2A). The inoculation was advantageous only at the dose of 100 kg N ha^{-1}, even with the severe water reduction (Figure 1). At this dose (100 kg N ha^{-1}), the grain yields of the crops inoculated with Hs-BR11147 and Ab-BR11005 were 1,230 kg ha^{-1} (37%) and 614 kg ha^{-1} (19%), respectively, higher than that of the uninoculated control. At higher doses, the grain yield was not affected by the application of the two strains in any of the agricultural years (Figure 2A, 3A, and 4A). In 2015, there was a response associated with N in the lowest dose, but there was no response in the absence of the fertilizer as is expected in rainy years, as was observed in 2017 (Figure 4A). There was no difference among the treatments in terms of the N content of the index leaf in 2015; all three models adjusted their linear response to the fertilizer application, showing that there was absorption of the applied N, which was not used in the formation of grains (Figure 2B).

The response to inoculation was variable and dependent on the environment and the plant/strain genotype and applied dose interaction (ARAÚJO et al., 2013; QUADROS et al., 2014; SZILAGYI-ZECCHIN; MARRIEL; SILVA, 2017). The factors that control this interaction may be related to the response of the cultivar to the production of auxins and other hormones produced by growth-promoting bacteria. For example, a study has shown that *A. brasiliense* can produce up to eight times more auxins than *H. seropedicae* (RADWAN; MOHAMED; REIS, 2004); the authors of this study observed that the form and amount of N can also alter the production of auxins by the two species used as inoculants.

In 2016, there was no influence of inoculation or the dose of N applied on the productivity gain and no regression model could be adjusted to the data obtained (Figure 3A, 3B). This lack of response is directly linked to the severe water deficit suffered by the plants in this agricultural year (Figure 1). At the dose of 100 kg ha^{-1} of N fertilizer, the Ab-BR11005 inoculation increased the N content in the index leaf in relation to the control treatment and the Hs-BR11417 inoculation (Figure 3B). This result can be partially explained by the auxins that this species produces, which result in the greatest root development (COHEN et al., 2015) and a more efficient absorption of the available N in the soil, which, however, are not reflected in higher grain yields owing to hydric stress. The lower deficit phase coincided with the beginning of flowering and grain filling, increasing the leaf N content of plants inoculated with Ab-Sp11005. It is necessary to have an adequate water supply close to the cropping/ maize spike, as the amount of water used at this stage has a direct effect on the yield (BERGAMASCHI et al., 2004). Water deficiency, prior to the emission of anthers, can result in a 50% reduction in grain yield and full flowering and can cause a 20% to 50% drop in periods of 2 and 8 days of stress, respectively (PEGORARE et al., 2009). In 2016, the two-week rain deficiency during the anther emission and flowering phases, caused a reduction of more than 50% in productivity and, consequently, in the response to the applied treatments.

A)



B)

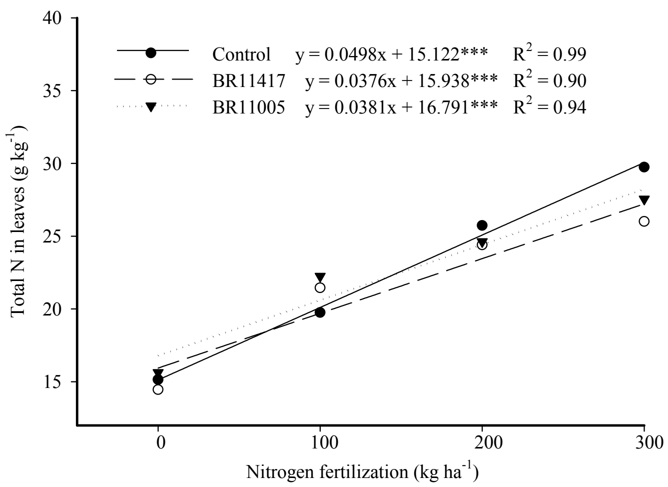


Figure 2. Regression equations related to grain yield (A) and total nitrogen (N) in the index leaf (B) of maize plants (cultivar 2B-433) that were either inoculated or not with Ab-BR11005 and Hs-BR11417 in the 2015 agricultural year. * Different by t-test ($P < 0.05$) ($n = 4$).

In 2017, rainfall increased and was distributed throughout the growth cycle; owing to this, the 2B647PW cultivar responded to the application of Ab-BR11005, reaching its maximum yield with the application of 100 kg N ha^{-1} (Figure 4A). During this year, the application of Hs-BR11417 resulted in a linear adjustment model while that of the Ab-BR11005 and the control treatment resulted in quadratic models regarding grain yield at different N doses; these results were opposite to those achieved in 2015. Without the addition of urea, inoculation with Hs-BR11147 and Ab-BR11005 increased the yield by 1,496 kg ha^{-1} (18%) and 504.50 kg ha^{-1} (6%), respectively, compared to the control. With the application of 100 kg ha^{-1} of urea, only Ab-BR11005 differed from the control, producing 1,124 kg ha^{-1} (+ 10%). The N content of the leaf presented a quadratic model of response to the N fertilizer in the three inoculation treatments, however there was no difference among them in each applied dose (Figure 4B).

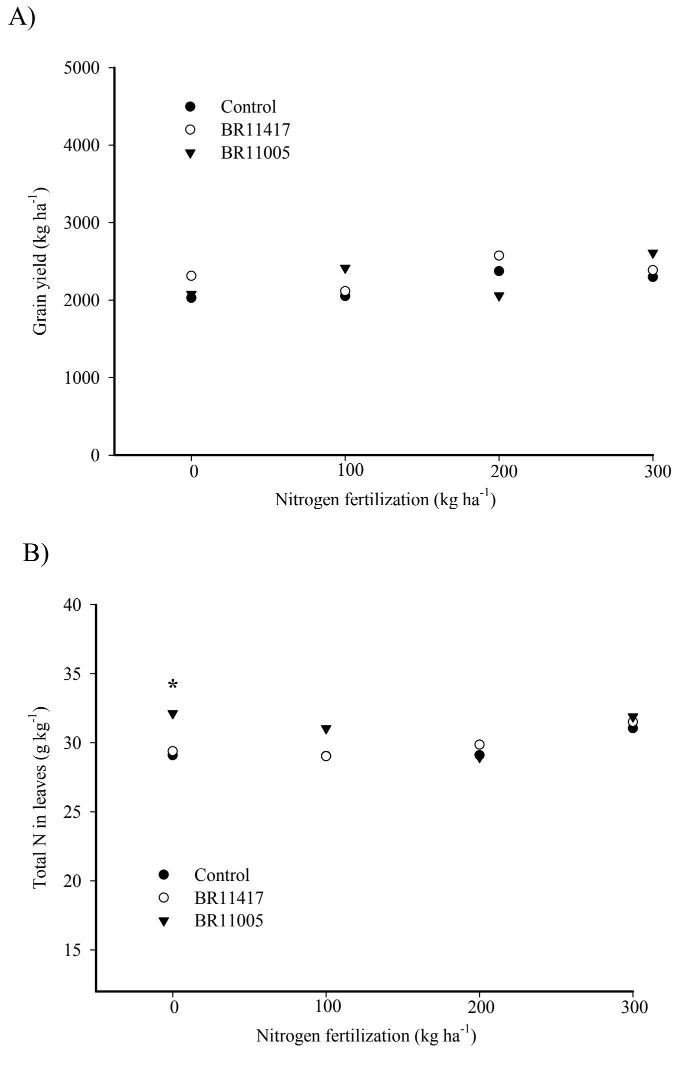
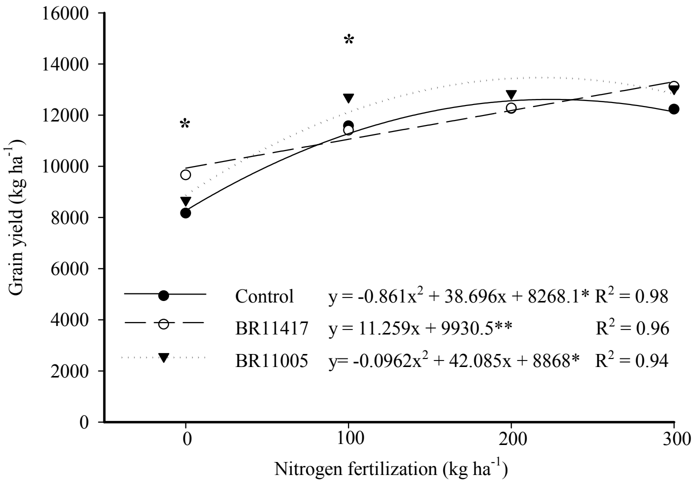


Figure 3. Grain productivity (A) and total nitrogen (N) content in the index leaf (B) of maize plants (cultivar 2B433PW) either inoculated or not with Ab-BR11005 and Hs-BR11417 in the year 2016. * Different by t-test ($P < 0.05$); there was not an adjustment model for the regression analysis in this agricultural year ($n = 4$).

Crops inoculation with *A. brasilense* is well-described in the literature and its effects of modifying the root architecture attenuate the effects of water stress as observed in maize (COHEN et al., 2009; CREUS; SUELDO; BARASSI, 2004). As noted, depending on the agricultural year, there was a reduction in grain production from 12,000 to 4,000 kg ha^{-1} (2017 to 2015) at the dose 100 kg N ha^{-1}. At this dose, the inoculation with Ab-BR11005 increased corn yield in two of the three cultivars tested (Figure 2A, 3A, and 4A).

A)



B)

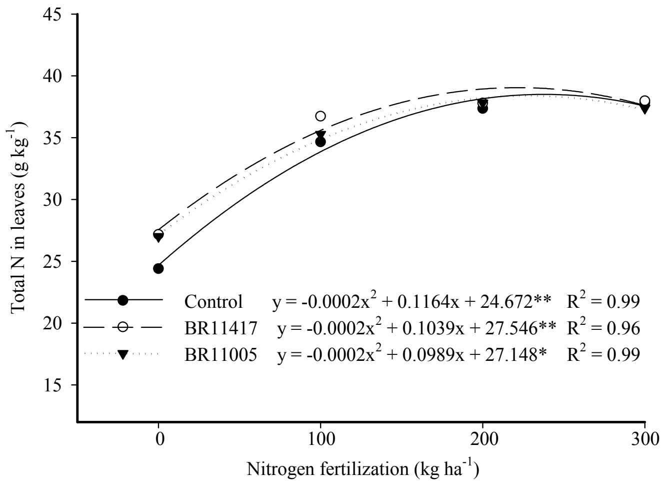


Figure 4. Regression equations for grain yield (A) and total nitrogen (N) in the index leaf (B) of maize plants (cultivar 2B647PW) either inoculated or not with Ab-BR11005 and Hs-BR11417 in the agricultural year of 2017. * Different by t-test ($P < 0.05$) ($n = 4$).

The plant's response to water stress is complex. Factors affecting this response include environmental conditions (including the soil), the plant's genotype and developmental stage, and the intensity and duration of the stress. These characteristics can induce morphological, physiological, and biochemical changes, thereby modifying ion absorption and stomatal closure, as is the case with abscisic acid (ABA) and the production of osmotolerant substances, among other effects (COHEN et al., 2015). Among the two species/ strains used, the most studied is Ab-BR11005, which has a good performance in terms of the growth of different plants subjected to water stress (BASHAN; BASHAN, 2010; BULEGON; GUIMARÃES; LAURETH, 2016). *Herbaspirillum seropedicae* has been studied less in terms of the agronomic efficiency of its application under field conditions (ALVES et al., 2015), while few studies have compared the two diazotrophic species (MARTINS et al., 2018; BREDA et al., 2019). The comparative results of the two diazotrophic bacterial species tested showed that the evaluated Hs-BR11417 strain had greater response versatility, as it acted efficiently both in the presence and in the absence of N in the soil in the context of the climate conditions of the state of Rio de Janeiro, for three consecutive years (BREDA et al., 2019). However, *A. brasilense* was more responsive compared to *H. seropedicae* when applied in an experiment in the Cerrado of Bahia State (MARTINS et al., 2018). The results described here also showed an alternation of the positive effects of inoculation, which were only observed in the absence of N fertilizer in 2017 with Hs-BR11417, and at the dose of 100 kg N ha^{-1} in 2017 and 2015 with Ab -BR11005.

The search for alternatives that facilitate the grain yield increase of cereals such as maize, the basis of human and animal food in the northeast region of Brazil, coupled with the use of lower doses of N fertilizer, should be prioritized. The application of inoculants containing selected diazotrophic bacteria has been implemented in Brazil since 2010 and in more than 3.5 million ha in South America (CASSÁN; DIÁZ-ZORITA, 2016). However, in order to cope with the consequences of water stress effectively, changes should be implemented in terms of the manner with which N fertilizer is applied, inoculation, and the choice of the best planting time. Such changes can result in productivity gains (in the case of our study this gain was more than 1,000 kg of grains ha^{-1}) and lower N fertilizer expenditure (savings of 200 kg ha^{-1} and 300 kg ha^{-1} of N-fertilizer) in the form of urea, the loss of which through volatilization is high, thereby affecting negatively greenhouse gas emissions. Increasing the efficiency of the use of N is of vital importance and can be improved with the right combination of N application, seed, and a cheap product containing growth-promoting bacteria selected for the crop of interest, such as maize.

CONCLUSION

Corn inoculation with *A. brasilense* BR11005 increased the grain yield when fertilized with 100 kg ha^{-1} of N. Corn inoculation with *H. seropedicae* BR11147 increased the yield both in the absence of the N-fertilizer and in the 100 kg N ha^{-1} dose of urea, in years when the water deficit did not limit productivity. Out of the two strains, Hs-BR11417 showed greater versatility response as it promoted growth in the absence and in the presence of the N fertilizer applied in the lowest dose. Therefore, this strain may be a viable inoculant alternative with higher adaptability in the northeast region of Sergipe.

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